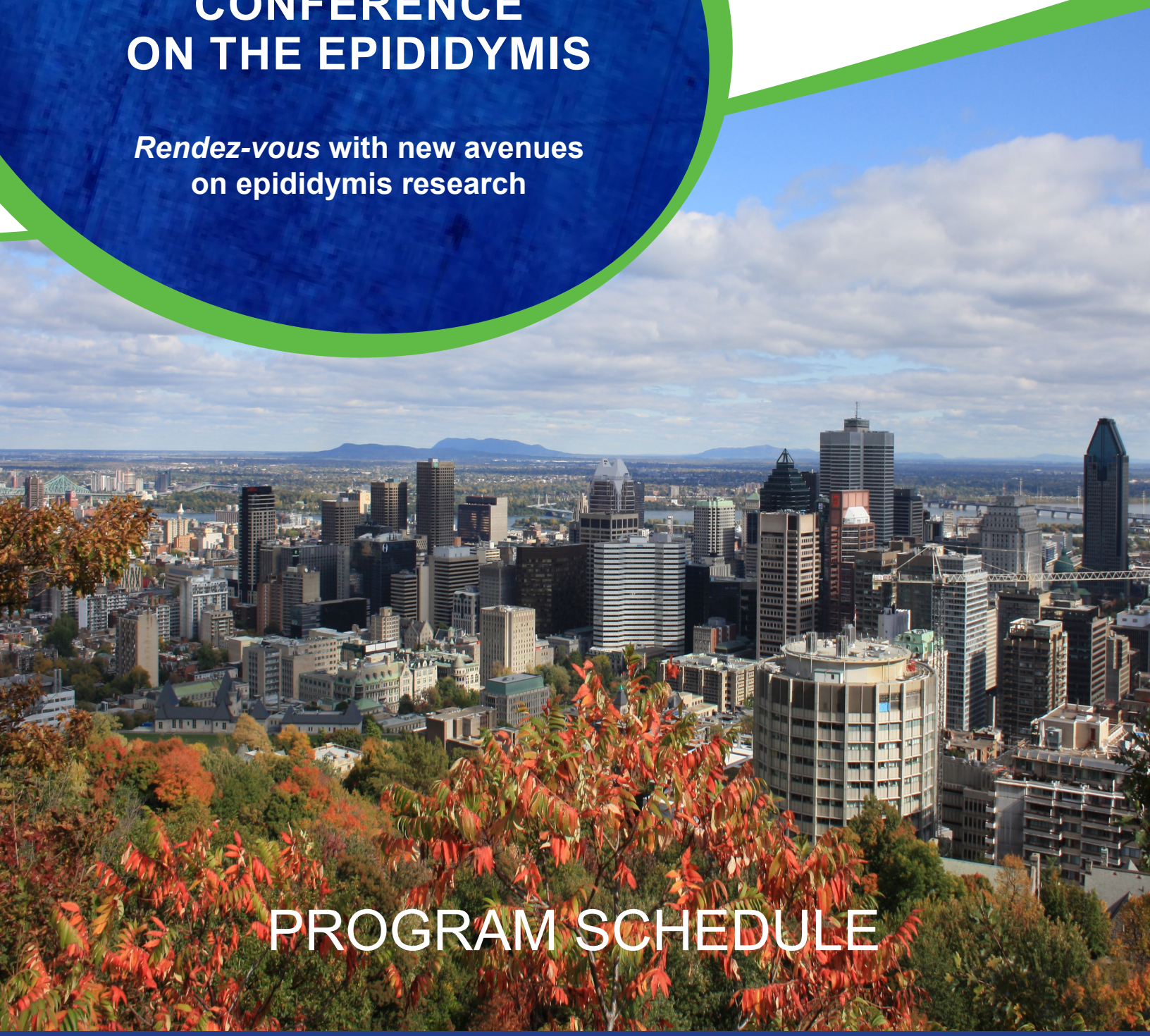
7TH INTERNATIONAL CONFERENCE ON THE EPIDIDYMIS

***Rendez-vous* with new avenues on epididymis research**



PROGRAM SCHEDULE

September 2018

20-23

Montreal
Canada

EPIDIDYMIS 7

Cover page:
Montréal skyline from the Mount Royal © Tourisme Montréal

Table of Contents

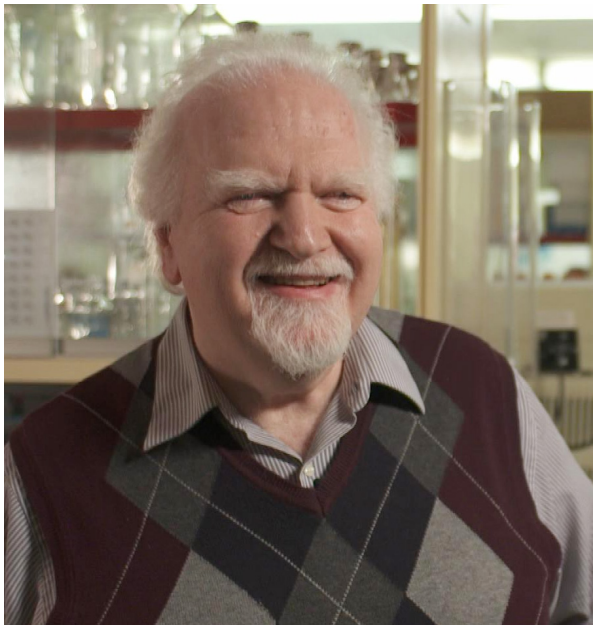
Welcome

It is an honor to welcome colleagues from around the world to the 7th International Conference on the Epididymis that is being held in Montreal from September 20 to 23, 2018.

The program reflects the cutting edge research that has been done on the epididymis, ranging from novel molecular studies on different types of RNAs to physiological, pharmacological and clinical aspects of our favorite tissue.

Leaders researching every facet of "the tissue" will be gathering in a fun and exciting city during the time of year when we are saying goodbye to summer and welcoming a wonderfully colourful fall season. Montreal is an easy city to navigate with an extensive metro/bus network. It is the site of four thriving universities and has been ranked in the QS Best Student Cities as number one in 2017. The meeting site is situated between "le vieux Montréal" and the thriving downtown core. Montreal boasts over 5000 restaurants from each corner of the globe, with some of the best chefs in the world.

We look forward to seeing you and sharing the remarkable advances being made in our understanding of the epididymis.



—Bernard Robaire, Ph.D.
Chair, Scientific program Committee

Conference Committees

Thank you to our Committee members for organizing a meeting with world renowned experts from North and South America, Europe, Australia and Asia for exchange of ideas and a perfect opportunity for establishment of new collaborations to advance the field of epididymal biology, reproduction, and fertility.

SCIENTIFIC PROGRAM COMMITTEE

The role of the conference Program Committee is to make sure that the conference presentations and the proceedings are of the highest possible quality. This includes suggesting plenary speakers, reviewing abstracts of contributed talks, and reviewing papers submitted to the proceedings. The members of the Program Committee for the 7th International Conference on the Epididymis are:

Maria C. Avellar, Brazil
Sylvie Breton, USA
Trevor Cooper, Germany
Gail Cornwall, USA
Patricia Cuasnicú, Argentina
Joël Drevet, France
Barry Hinton, USA
Bernard Robaire, Canada (Chair and Conference Organizer)
Yongliang Zhang, China

LOCAL ARRANGEMENTS COMMITTEE

The following persons are taking care of the local organization. They are the ones who end up doing most of the work and they deserve our warmest thanks.

Clémence Belleannée
Elise Boivin-Ford
Daniel Cyr
Aimee Katen
Puttaswamy Manjunath
Anaïs Noblanc
Cristian O'Flaherty
Bernard Robaire (Chair)
Robert Sullivan

Artists' Row © Canadian Tourism Commission

Our Sponsors

A special thank you to our sponsors!

We are proud to acknowledge the following organizations for their generous support of the 7th International Conference on the Epididymis

MCI
Male Contraceptive Initiative

CIHR IRSC
Institute of Human Development, Child and Youth Health
Institut du développement et de la santé des enfants et des adolescents

A special thank you to our sponsors!

We are proud to acknowledge the following organizations for their generous support of the 7th International Conference on the Epididymis

International Society of Andrology

INRS UNIVERSITÉ DE RECHERCHE

SSR Society for the Study of Reproduction

McGill Faculty of Medicine

American Society of Andrology

RQR Réseau Québécois en reproduction

Axe Reproduction, santé de la mère et de l'enfant (RSME)

Centre universitaire de santé McGill
McGill University Health Centre

Centre de la reproduction
Reproductive Centre

Our Sponsors

A special thank you to our sponsors!

We are proud to acknowledge the following organizations for their generous support of the 7th International Conference on the Epididymis

Institut de recherche
Centre universitaire de santé McGill

Research Institute
McGill University Health Centre

CRRD
Centre for Research in Reproduction and Development
Centre de recherche en reproduction et développement

McGill | Research and Innovation

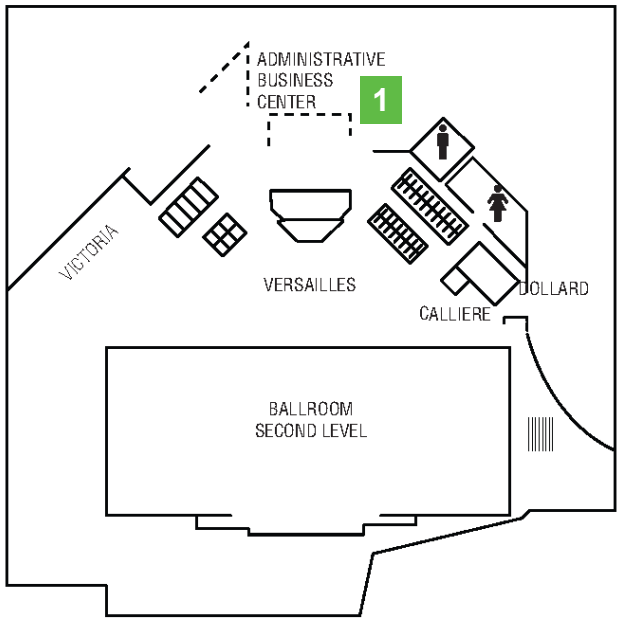
Centre de recherche en reproduction, développement et santé intergénérationnelle

Children Health Research
at the Montreal Children's Hospital
of the McGill University Health Centre

Université de Montréal

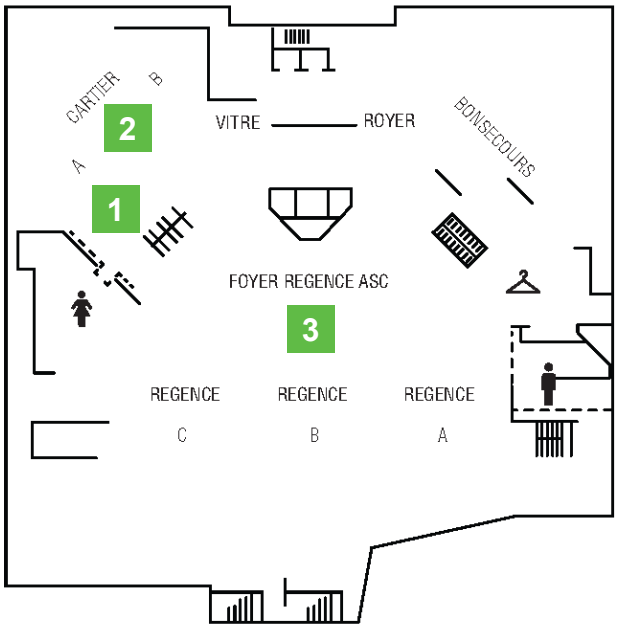
FLUXION

Plaza Centre-Ville



C1 1st Floor Below Lobby

1	Business Centre



C 2nd Floor Below Lobby

1	Registration / Information Desk
2	Conference Room Cartier AB
3	Cocktail / Meals / Poster Boards

General Information

MEETING VENUE

PLAZA CENTRE-VILLE
777 Boulevard Robert-Bourassa
Montreal, Quebec, Canada H3C 3Z7
Tel: (514) 879-0718
Fax: (514) 879-0907
info@plazacentreville.com
www.plazacentreville.com

Plaza Centre-Ville (formerly Delta Centre-Ville) is centrally located between the historical Old Montréal and the vibrant downtown Montréal – moments from some of the area's most notable sites including the Old Port and Mount Royal. It is at short walking distance from the Square Victoria metro station (Viger exit).

REGISTRATION / CONFERENCE ROOMS

The registration, conference rooms, poster viewing, breakfasts, health breaks,and lunches will take place on the C floor, 2nd floor below lobby.

- The registration and information desk will be located in the Foyer, next to Cartier AB.
- All the plenary sessions will be held in Cartier AB.
- The breakfasts, health breaks and lunches will be in the Foyer, next to Cartier AB.

Cartier AB

POSTERS

Posters will be exhibited in the Foyer of the 2nd floor below lobby throughout the conference. Please refer to page 10 for detailed instructions on poster set up and presentation.

COCKTAIL RECEPTION

The welcome cocktail reception will be held in the Foyer of the 2nd floor below lobby on Thursday at 17:30. Enjoy cocktails and canapés while meeting colleagues, catching up with friends and engaging in discussions about the epididymis. The singer and pianist will provide the perfect backdrop for a beautiful event.

TRANSPORTATION

Detailed information on Montreal's public transportation system can be found at http://www.stm.info. Maps of the entire network are available for free in the metro stations, where you can also buy a weekly pass, called CAM hebdo, giving unlimited access to the STM network (metro and bus) from Monday to Sunday.

If you prefer to bike to the conference you can use Montreal's bike sharing system, BIXI. You can buy a 1-day access, 3-day access or one-way trip. Trips of 30 minutes or less have no additional fees if you purchased short-term access; usage fees apply for longer trips. For more information visit https://bixi.com/.

PARKING

If you come by car, paid parking at Plaza Centre-Ville is available 7 days a week, 24 hours a day, for $5 to $13 with coupon. Please see Elise Boivin-Ford for parking coupons.

INTERNET ACCESS

For the convenience of our guests, Plaza Centre-Ville offers complimentary Wi-Fi throughout the public spaces of the centre. Please see Elise Boivin-Ford for detailed information on how to connect your laptop to the wireless network.

ADMINISTRATIVE BUSINESS CENTRE

The Administrative Business Centre is located on the C1 floor, 1st floor below lobby.

ATTIRE

Business casual. Reminder: meeting room temperatures and personal comfort ranges vary widely. Because meeting rooms may seem cold, you may want to dress in layers or bring a sweater or a jacket.

CLIMATE IN SEPTEMBER

September marks the beginning of autumn in Montreal; this is a great time to visit Montreal. With temperatures gradually starting to fall, the city experiences an average temperature of about 16 degrees Celsius (61°F) with highs of about 23°C (73°F) and lows of 9°Celsius (48°F).

TOURIST INFORMATION

Montreal official tourist site:
http://www.tourisme-montreal.org/

Quebec's Government official tourist site:
http://www.bonjourquebec.com/


Old Port of Montréal © Tourisme Montréal

Instructions

INSTRUCTIONS FOR SPEAKERS

- Plenary talks are 25 minutes plus 5 minutes for questions and discussion.
- Please make sure that you do not exceed your time. Focus on the essential of your message. Given the short time allowed to each speaker, it is generally not possible to give the full details of your work.
- You should concentrate on providing a clear explanation of your main results and their significance.
- If you use a Macintosh computer, save your presentation in a PC format.
- Send your presentation to Elise at elise.boivin-ford@mcgill.ca by **Friday, September 14**. Please name the file attachment(s) as follows: Presenter's Last Name_Plenary. Only PowerPoint presentations will be accommodated.

INSTRUCTIONS FOR POSTER PRESENTERS

- All poster presenters will be expected to present a 3-minute summary of their project during a poster session. You are limited to two slides maximum, without animations. There will be 2 minutes allotted for questions; your formal poster session presentation will allow attendees to ask you questions; remember this Poster Flash Talk presentation is in addition to your regular Poster Session presentation.
- If you use a Macintosh computer, save your presentation in a PC format.
- Send your presentation to Elise at elise.boivin-ford@mcgill.ca by **Friday, September 14**. Please name the file attachment(s) as follows: Presenter's Last Name_Poster. Only PowerPoint presentations will be accommodated.

INSTRUCTIONS FOR SESSION CHAIRS

As Session Chair, your responsibilities include:

- Arriving in the session room at least 10 minutes in advance of the session start time.
- Introducing presenters by name, affiliation, and title of presentation at the start of each presentation.
- Monitoring presentation time. Each plenary speaker has been allotted a total of 30 minutes for presentation, Q&A and discussion, and poster presenters have 3 minutes each. In order for the sessions to run smoothly, it is essential to adhere to these time limits. This is particularly important for groups of poster presenters; in order for all presenters to have equal time. We suggest that you make it very clear to each presenter before the session begins that you will adhere strictly to the time limit.

EQUIPMENT

The conference room (Cartier AB) will be equipped with a PC computer and a projector for displaying computer output.

Thank you all in advance for your consideration of your fellow presenters and attendees!

Magic Comedy Dinner at L'Auberge Saint-Gabriel

WHEN: Saturday – September 22, 2018

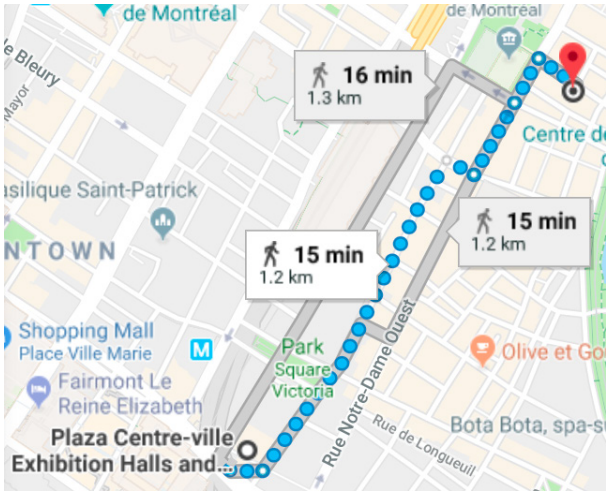
WHERE: Auberge Saint-Gabriel, Truteau Hall (ground floor), 426 Saint-Gabriel Street

WHEN: 19:00 - 22:30

AN OCCASION TO REMEMBER Join us for a New French cuisine experience in a chic & restored historic inn - the Auberge Saint-Gabriel. During the dinner, you will be entertained by a talented comedian and magician, Martin Rozon, the Grand prize winner of the On Stage category at Magie Montreal. Martin also won the Golden Nose award for being the Revelation of the year at Grand Rire festival in Quebec, and was nominated for Newcomer of the Year award at the Gala Les Olivier, and Best comedy performance at l'ADISQ. Come and enjoy an entertaining private dinner party with colleagues from around the world. The Auberge is conveniently located in Old Montreal, a 15 minute walk or one metro station from the meeting venue.


Martin Rozon

DIRECTIONS (from Place Plaza Centre-Ville)



Walking (15 minutes)

- Head southeast on boulevard Robert-Bourassa
- Turn left toward rue Saint-Jacques
- Turn left onto rue Saint-Jacques
- Turn right onto rue Saint-Sulpice
- Turn left onto rue Notre-Dame Ouest
- Continue onto rue Notre-Dame Est
- Turn right onto rue Saint-Gabriel

By metro (18 minutes)

- Walk northbound on Robert-Bourassa
- Turn right on Viger
- Take Orange line direction Montmorency to station Champ-de-Mars
- Walk 676m to 426, rue St-Gabriel
- Head toward rue Gosford
- Turn right on Gosford
- Turn right on Notre-Dame
- Turn left on Saint-Gabriel
- Walk to your final destination (426, rue St-Gabriel)

Scientic Program

7th International Conference on the Epididymis

Rendez-vous with New Avenues on Epididymis Research

September 20-23, 2018
Montreal, Quebec, Canada

Thursday, September 20

17:30-17:40 Welcoming comments/opening ceremony

17:40-17:50 A celebration of Mike Bedford's contributions – Patricia Cuasnicú (Argentina)

17:50-18:50 Marie-Claire Orgebin-Crist Lecture - Louis Hermo (Canada): Dark side of the epididymis: Tails of sperm maturation

Friday, September 21

07:30-08:30 Continental Breakfast / gathering

Session 1 • Development of the Wolffian duct and the epididymis

Chairs: Dr. Terry Turner (USA) and Pr. Winnie Shum (China)

08:30-09:00 Pradeep Tanwar (Australia): Wnts during Wolffian duct development

09:00-09:30 Barry Hinton (USA): Cell movements are major drivers of Wolffian duct morphogenesis

09:30-10:00 Clémence Belleannée (Canada): Primary cilia: biosensors of the epididymis

Session 2 • Physiology of the epididymis

Chairs: Pr. Marie-Claire Orgebin-Crist (USA) and Ms. Mary Gregory (Canada)

10:00-10:30 Gail Cornwall (USA): Functional amyloids in the epididymis and their role in host defense

10:30-10:45 Health break

10:45-11:15 Mike Bedford Lecture - Patricia Cuasnicú (Argentina): Multiple knockout of CRISP proteins as contraceptive targets: Effects on epididymal function and fertility

11:15-12:15 Poster Flash Talks - Posters 1-10 (Chair: Dr. Cristian O'Flaherty; Canada)

12:15-13:15 Lunch (buffet) and poster viewing

13:15-13:45 Daniel Cyr (Canada): Molecular regulation of cell-cell interactions in the epididymis

13:45-14:15 Sylvie Breton (USA): Intercellular crosstalk in the epididymis: Regulation of epididymal luminal acidification by purinergic signaling and its modulation by CFTR

Session 3 • Epididymal sperm maturation-contraception

Chairs: Dr. E. Mitchell Eddy (USA) and Dr. Wilma De Grava Kempinas (Brazil)

14:15-14:45 Pablo Visconti (USA): Signaling events occurring in spermatozoa during epididymal maturation

14:45-15:00 Health break

15:00-16:00 Poster Flash Talks - Posters 11-20 (Chair: Pr. George L. Gerton; USA)

16:00-16:30 Masahito Ikawa (Japan): Sperm calcineurin inhibition prevents mouse fertility with implications for male contraception

16:30-17:00 Fabrice Saez (France): Effects of diet/cholesterol overload on sperm maturation and capacitation

17:00-17:30 Cristian O'Flaherty (Canada): The Role of Peroxiredoxins in Epididymal Maturation

Saturday, September 22

07:30-08:30 Continental Breakfast / gathering

Session 4 • Non-coding RNAs in the epididymis

Chairs: Pr. Yonglian Zhang (China) and Dr. Aimee Lee Katen (Canada)

08:30-09:00 Petra Sipila (Finland): Regulation of epididymal gene expression: impact on sperm maturation

09:00-09:30 Brett Nixon (Australia): Non-coding RNAs in the epididymis

09:30-10:00 Chen Chu (USA): Small RNA dynamics in the epididymis and oxidative stress

10:00-10:30 Colin Conine (USA): Small RNAs acquired during epididymal maturation transmit epigenetic information to offspringt

10:30-11:00 Health break

Session 5 • Infections of and defense by the epididymis

Chairs: Dr. Maria Christina W. Avellar (Brazil) and Dr. Britta Klein (Germany)

11:00-11:30 Maria Christina W. Avellar (Brazil): Innate immunity, inflammatory mediators and steroid receptor signaling in epididymal development and function

11:30-12:30 Poster Flash Talks - Posters 21-31 (Chair: Pr. Barbara Hales; Canada)

12:30-13:30 Lunch (buffet) and poster viewing

13:30-14:00 Yonglian Zhang (PRC): DICER1 regulates antibacterial function of epididymis by modulating transcription of multiple β-defensins via a microRNA independent manner

14:00-14:30 Andreas Meinhardt (Germany): Epididymitis

14:30-15:30 Poster Flash Talks - Posters 32-41 (Chair: Dr. Daniel G. Cyr; Canada)

15:30-16:00 Health break

Scientic Program

Session 6 • Immune system and the epididymis

Chairs: Pr. Joël R. Drevet (France) and Dr. Flavia Lorena Carvelli (Argentina)

16:00-16:30 Mark Hedger (Australia): Activin/follistatin in the epididymis

16:30-17:00 Rachel Guiton (France): Epididymal immunity: lymph vessels and new cells unravelled

Social Event

19:00-22:30 Magic Comedy Dinner at L'Auberge Saint-Gabriel
Truteau Hall (ground floor), 426 Saint-Gabriel Street

Sunday, September 23

07:30-08:30 Continental Breakfast / gathering

Session 7 • Toxicants and epididymal function

Chairs: Pr. Rex A. Hess (USA) and Dr. Anaïs Noblanc (Canada)

08:30-09:00 Wilma Kempinas (Brazil): Effects of glucocorticoids on the epididymis

09:00-09:30 Mariana Machado-Neves (Brazil): Heavy metals and the epididymis

Session 8 • The human epididymis and clinical aspects of epididymal dysfunction

Chairs: Pr. Andreas Meinhardt (Germany) and Dr. Clémence Belleannée (Canada)

09:30-10:00 Ann Harris (USA): Gene regulation in human epididymal cell

10:00-10:30 Health break

10:30-11:30 Poster Flash Talks - Posters 42-52 (Chair: Dr. Puttaswamy Manjunath; Canada)

11:30-12:00 Robert Sullivan (Canada): Revisiting the structure/functions of the human epididymis

12:00-12:30 Plans for Epididymis 8 and Closing remarks

Marie-Claire and Mike;
Epididymis VI, Shanghai, October 2014
Cooper TG. Asian J. Androl. 2018;20:420-4

Speaker Biographies

Maria Christina W. Avellar, Ph.D.

Maria Christina W. Avellar received her B.Sc. in Biomedical Sciences (1985) and M.Sc. in Pharmacology (1988) from Federal University of São Paulo (UNIFESP-EPM), and her Ph.D. in Sciences (1992) from State University of São Paulo (USP), São Paulo, Brazil. After a Postdoctoral Fellowship training at the Laboratories for Reproductive Biology, UNC-Chapel Hill (NC, USA), she returned in 1996 to UNIFESP-EPM where she has remained and is currently a tenured Associate Professor in the Department of Pharmacology, Laboratory . Dr. Avellar´s research interests focus on reproductive biology, male reproductive tract, cell and molecular biology of epididymal development and function, steroid/nuclear receptor action, innate immunity and inflammation.

Session 5, Saturday, September 22

Clémence Belleannée, Ph.D.

Clémence Belleannée is an assistant professor in the Department of Obstetrics, Gynecology, and Reproduction at Université Laval, Quebec, Canada, since 2014. She holds a Fonds de Recherche en Santé du Quebec- Junior 1 salary award as well as operating grants from the Canadian Institutes of Health Research (CIHR), Natural Sciences and Engineering Research Council of Canada (NSERC) and the Sick Kids Foundation (SKF). She published 25 articles. Her laboratory investigates the means by which epididymal epithelial cells intercommunicate to control sperm environment and fertilizing abilities. She recently revealed the cell-lineage specificy of primary cilia during epididymal postnatal development and currently investigates the role of these cell organelles in epididymis homeostasis.

Session 1, Friday, September 21

Sylvie Breton, Ph.D.

Sylvie Breton, PhD is Professor of Medicine at Harvard Medical School. Her laboratory is part of the Program in Membrane Biology/Center for Systems Biology at the Massachusetts General Hospital (MGH). Dr. Breton received her Ph.D. in Biophysics from the University of Montreal, Canada, and she conducted postdoctoral studies on kidney biology at Harvard Medical School, Boston, MA. In 1996, she joined the Faculty of the Department of Medicine at MGH and Harvard Medical School, where she developed a multidisciplinary research program in epithelial cell biology. Dr. Breton's laboratory examines the regulation of epithelial cells in organs of the urogenital tract, including the kidney and epididymis. She examines how these cells receive cues from their environment and communicate with neighboring cells to modify their function. She trained several scientists, who now occupy leadership positions at different institutions throughout the world. She and her colleagues have published over 100 papers since 1993. Dr. Breton is the recipient of several awards, including the MGH Claflin Distinguished Scholar award in 1997, the American Physiological Society Renal Section investigator Award in 2005, the MGH Joseph Martin Basic Research Prize award in 2008, and the 2012 Research Award of the Society for the Study of Reproduction. She is a Charles and Ann Sanders Research Scholar at MGH. She is a board member of the American Journal of Physiology: Cell Physiology. Dr. Breton has been a member of the American Society of Andrology (ASA) since 2003, she presented the ASA Women in Andrology Lecture in 2011, she was recently elected Secretary of the ASA Council, and she was Co-Chair of the Program Committee for the ASA 2017 meeting.

Session 2, Friday, September 21

Speaker Biographies

Chen Chu, Ph.D.

Chen Chu, Ph.D. is a postdoctoral research fellow at Dana-Farber Cancer Institute, Harvard Medical School. During the years (2010-2017) at Dr. Yonglian Zhang's lab as a graduate student and research associate, his work was centered on exploring the small non-coding RNAs in the epididymis. Dr. Chu was the director of the research project on studying the rsRNA-28S in the mouse sperm cells from National Natural Science Foundation of China. He also presented at the Epididymis VI in 2014. Dr. Chu received his Ph.D. in Biochemistry and Molecular Biology from Chinese Academy of Sciences (CAS), an M.M. and a B.S. in Pharmacy from Shanghai Jiao Tong University (SJTU). He has coauthored 22 research papers and was the recipient of a Di Ao Scholarship (2015), a Pfizer Scholarship (2012) and an excellent graduate of SJTU award (2010).

Session 4, Saturday, September 22

Colin Conine, Ph.D.

Colin Conine's research is focused on the functions of small RNAs as a carrier of nongenetic information in sperm. He completed his Ph.D. in the laboratory of Craig Mello at University of Massachusetts Medical School. There he worked on the function of endogenous small RNA pathways regulating thermotolerant male fertility in C. elegans. Dr. Conine is currently a Helen Hay Whitney Postdoctoral Fellow in Oliver Rando's lab where he demonstrated that sperm transmitted small RNAs in mice are able to regulate embryonic development. In the process he found that small RNAs are shipped from the epididymis to maturing sperm via exosomes, establishing a novel soma-to-germline transfer of epigenetic information in mammals. He uses a combination of assisted-reproduction techniques paired with injection of RNAs or genetic ablation of small RNAs in the male germline, followed by single embryo RNA- sequencing to determine the effect of sperm small RNAs on embryonic development.

Session 4, Saturday, September 22

Gail A. Cornwall, Ph.D.

Dr. Cornwall received her Ph.D. in reproductive biology from The Johns Hopkins Bloomberg School of Public Health followed by a postdoctoral fellowship at Vanderbilt University School of Medicine. She currently is Professor of Cell Biology and Biochemistry at the Texas Tech University Health Sciences Center. Dr. Cornwall has served as President of the American Society of Andrology and on several Editorial Boards including that for Biology of Reproduction and Andrology. Dr. Cornwall's research interests focus on epididymal function with an emphasis on the CRES subgroup of cystatins including their functional roles as amyloid in sperm maturation and protection and mechanisms that mediate amyloidogenesis.

Session 2, Friday, September 21

Patricia S. Cuasnicú, Ph.D.

Patricia S. Cuasnicu (Ph.D, University of Buenos Aires) did her post-doctoral training at Cornell Medical Center (USA), and for the past 35 years, she has been serving as director of a research group in Argentina focused on the molecular mechanisms involved in epididymal maturation, fertilization and male contraception. She served for 14 years as consultant of WHO and CONRAD, and for the past 15 years as Chair of the International Liaison Committee of ASA. She was Associate Editor of the Journal of Andrology

and now serves as Associate Editor of "Andrology". She has participated as Program Co-Chair of Epididymis V (2010), as Chair of International Congress of Andrology (ICA 2013) and as member of the Program Organizing Committee of several ASA and Epididymis (VI, VII) meetings. She has 85 publications, 300 meeting presentations, and was invited as simposist or plenary lecturer in numerous meetings including the Gordon Research Conference, Spermatology, Epididymis, ICA, EAA, ASA and SSR.

Session 2, Friday, September 21

Daniel G. Cyr, Ph.D.

Dr. Cyr is a Professor in Toxicology and Pharmacology at INRS-Institut Armand-Frappier. He received his BSc (Hons) in Biological Sciences and MSc in Toxicology from Concordia University in Montreal. He then attended the University of Manitoba where he received his PhD in Endocrinology. He subsequently undertook postdoctoral training at McGill in Andrology. He worked in the Canadian Public Service as a biologist at Health Canada and as a senior scientist with the Department of Fisheries and Oceans. He joined INRS in 1995 where he now holds the rank of Professor. He has served on several Boards of director for reproduction and toxicology-related societies and research networks, and is currently member of editorial boards of several journals. He was awarded the Veylein Henderson Award (2004) from the Canadian Society of Toxicology (STC) for his research contributions as a young scientist and The Gabriel Plaa Award of Distinction (2014), for his contributions to toxicology. He is a member of the 'Cercle d'excellence' of the Université du Quebec and currently holds the Canada research chair in reproductive toxicology. His research has been consistently funded by NSERC and CIHR. He has published over 150 articles, including 17 book chapters, primarily dealing with male reproduction, toxicology and cell-cell interactions in the epididymis.

Session 2, Friday, September 21

Wilma De Grava Kempinas, Ph.D.

Wilma De Grava Kempinas is a Senior Reproductive Toxicologist and Full Professor of Embryology at the Institute of Biosciences of Botucatu, São Paulo State University, Brazil. She got her PhD in Cell Biology at the School of Medicine of the University of São Paulo and post-doc at the United States Environmental Protection Agency. Her research on reproductive and developmental biology and toxicology has led to more than 100 scientific papers and book chapters. Dr. Kempinas belongs to the editorial board of international journals on toxicology and animal reproduction, and is acting as one of the Associate Editors of the Journal "Reproductive Toxicology".

Session 7, Sunday, September 23

Rachel Guiton, Ph.D.

Rachel Guiton holds a PhD in Immunology. During her PhD training she studied an anti-Toxoplasma gondii vaccine strategy based on the use of dendritic cells as inducers of the anti-parasite response. Then she joined the Centre d'Immunologie de Marseille-Luminy (CIML, Marseille, France) as a post-doctoral fellow to work on the murine cytomegalovirus (MCMV) and its interactions with dendritic cells and Natural Killer (NK) cells. In 2012 she was recruited as an assistant professor at the Génétique, Reproduction et Développement laboratory (GReD, Clermont-Ferrand, France) in Pr Joël Drevet/Dr Fabrice

Speaker Biographies

Saez's team. She currently teaches Immunology at the Clermont-Auvergne University and focuses her research on the murine epididymal immunity. She is especially interested in the cells and mechanisms involved in the immune balance that takes place in the epididymis, between responses against genital tract ascending pathogens and setting and maintenance of tolerance toward sperm cells.

Session 6, Saturday, September 22

Ann Harris, Ph.D.

Ann Harris obtained her PhD from the University of London in Human Genetics in 1981 and went on to do postdoctoral studies at the Imperial Cancer Research Fund. After holding faculty positions at the University of London and Oxford, she moved to the US to take up a position at Northwestern University in 2005. She is currently the Leonard C. Hanna Professor and Vice-Chair for Research in the Department of Genetics and Genome Sciences, and co-Director of the Cystic Fibrosis Research and Development Program at Case Western Reserve University. She has served on numerous grant review panels in the USA, the UK, Europe and Hong Kong. Her research is currently funded by both the NIH and the Cystic Fibrosis Foundation; she has published over 180 peer-reviewed articles. Her research career has centered on molecular genetics of epithelial diseases primarily focusing on cystic fibrosis. She has made many contributions to epididymis-related research including the development of primary cultures of immature human epididymis epithelial cells and adult human epididymis epithelial cells from caput, corpus and cauda regions. The adult human cells are being used to advance our understanding of the human epididymis by using state of the art protocols of functional genomics.

Session 8, Sunday, September 23

Mark P. Hedger, Ph.D.

Professor Mark Hedger has worked in the field of men's reproductive health since completing his PhD at Monash University in 1984. He is currently a Senior Principal Research Fellow at the Hudson Institute of Medical Research and Professor in the Department of Molecular and Translational Sciences at Monash University. He has published more than 150 scientific papers and scholarly reports in the fields of male reproductive tract biology, activin biology and inflammatory disease. Professor Hedger is a member of several Australian and international scientific societies and was President of the Society for Reproductive Biology (2009 -2012). He is a Fellow of the Society for Reproductive Biology and a Fellow of the Society for the Study of Reproduction (USA).

Session 6, Saturday, September 22

Louis Hermo, Ph.D.

Dr. Louis Hermo graduated from Loyola College in Montreal QC with a BSc in 1967. In 1969, he graduated from St. Joseph's teacher's college in Montreal QC. He spent his graduate degrees (1969-1975) in the Department of Anatomy and Cell Biology at McGill University with Dr. Y. Clermont obtaining both an MSc and PhD. In 1976-1977 Dr. Hermo did a post doc degree with Dr. A. Rambourg (1976-1977) at the Centre Nuclear in Saclay, Paris. After his post doc he was hired as an assistant professor, and then went on to become an Associate and eventually full professor in the Dept. of Anatomy and Cell biology, McGill University. Dr. Hermo's research topics are morphological in nature and include studies on spermatogonial renewal, endocytosis in the male tract, cytoplasmic droplets of sperm and a long lasting investigation of the 3D structure and functions of the Golgi apparatus of different tis-

sues and organs of the rat. Dr. Hermo was the recipient of the Leo Yaffe award for outstanding teacher in Faculty of Science 2000 in McGill University and won the J.C.B Grant Award in 2007 from Canadian Association for Anatomy Neurobiology and Cell Biology for his teaching and research. In 2017, he became an Emeritus Professor.

Plenary Lecture, Thursday, September 20

Barry T. Hinton, Ph.D.

Barry T. Hinton is a Professor in the Department of Cell Biology, University of Virginia. Dr. Hinton received a B.Sc in Pharmacology and Biochemistry at the University of East London, completed his Ph.D. under the mentorship of Dr. Brian P. Setchell at The Babraham Institute, Cambridge, postdoc with Dr. Stuart Howards in the Department of Urology, University of Virginia, and then moved to the Department of Cell Biology. His initial research was on the analysis of epididymal luminal fluid, mechanisms by which the luminal fluid microenvironment is formed, and regulation of initial segment function by testicular lumicrine factors. Currently, Dr. Hinton's research focuses on Wolffian duct morphogenesis. Dr. Hinton has published over 100 papers/reviews/book chapters, received numerous awards including the Distinguished Andrologist Award (ASA), and is the Director of Section 3 of the Frontiers in Reproduction Course.

Session 1, Friday, September 21

Masahito Ikawa, Ph.D.

Dr. Masahito Ikawa is Distinguished Professor of the Research Institute for Microbial Diseases and Director of the Animal Resource Center for Infectious Diseases at Osaka University. His doctoral dissertation at Osaka University involved the application of green fluorescent protein (GFP) as a marker in transgenic mice, and he was the first to generate "green mice" expressing GFP ubiquitously. He performed postdoctoral studies at the Salk Institute in San Diego and used lentiviral vectors for transgenesis and gene therapy studies. As a Research Associate, Associate Professor, and Professor at Osaka University, he has been studying male and female fertility through gene-manipulated mice. In these capacities, Dr. Ikawa runs an active transgenic technology program in his laboratory, Institute, and Center that includes the use of CRISPR/Cas9 to produce knockout and knockin mice to study male and female infertility.

Session 3, Friday, September 21

Mariana Machado-Neves, Ph.D.

Mariana Neves is currently Professor of Histology and Embryology at the Federal University of Viçosa in Brazil. She earned her Bachelor of Veterinary Medicine at Federal University of Minas Gerais (UFMG), Belo Horizonte, in 2000, and her Ph.D. in Animal Science from the UFMG in 2008. In her thesis work, she studied the cryoprotection effectiveness of low-density lipoproteins on canine spermatozoa. Since 2010, she has been working on male reproductive toxicology, focusing on the effects of heavy metals in epididymis histophysiology, sperm quality, and fertility in vivo. Her latest research interest is to evaluate the negative impact of arsenic compounds in male fertility.

Session 7, Sunday, September 23

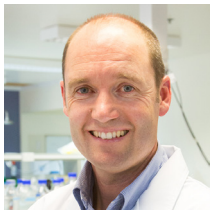
Andreas Meinhardt, Ph.D.

Prof. Andreas Meinhardt studied Human Biology at Philipps University in Marburg. After his postdoctoral stay at Monash University in Melbourne, Australia, he returned to Marburg. He was

appointed as professor in the Department of Anatomy and Cell Biology at Justus-Liebig-University Giessen, Germany in 2001. His research interest includes reproductive immunology with a focus on the understanding of infection and inflammation of the epididymis and testis as a cause of infertility. Andreas holds two Honorary Professorships with Monash University. He has served in the councils of the German Society of Andrology (2005-20012), European Academy of Andrology (2002-2010) and is the current President of the International Society of Andrology.

Session 5, Saturday, September 22

Brett Nixon, Ph.D.



Brett Nixon obtained his PhD form the University of Newcastle and Cooperative Research Centre for Vertebrate Biocontrol (awarded 1999) working on in the field of immunocontraceptive development, before undertaking post-doctoral studies at Emory University, GA, USA (1999 – 2000) where he investigated the mechanistic basis of sperm-egg interactions. Brett returned to the University of Newcastle in 2001 where he is an ARC Future Fellow and co-Director of the Priority Research Centre for Reproductive Science. Brett's current research lies at the basic biology / clinical andrology interface, with a focus on how spermatozoa acquire the functional competence to engage in fertilization during epididymal transit, and how this process becomes so dramatically disrupted in cases of male infertility.

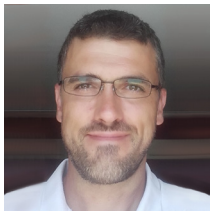
Session 4, Saturday, September 22

Cristian O'Flaherty, DVM, Ph.D.

Dr. Cristian O'Flaherty is an associate professor in the Department of Surgery (Urology Division), Faculty of Medicine, McGill University and the Research Institute of the McGill University Health Centre (MUHC) in Montreal, Québec, Canada. He earned a DVM and a Ph.D. in Animal Reproduction degrees from University of Buenos Aires, Argentina. During his postdoctoral training at McGill University, he characterized new phosphorylation events associated with human sperm capacitation and performed studies on the impact of chemotherapy on sperm chromatin structure of cancer survivors. He is dedicated to studying the biology of male reproduction. He has been studying sperm function in many animal models and humans, including the role of reactive oxygen species and dehydrogenases during bovine sperm capacitation and strategies to improve cryopreservation protocols in domestic animals based on antioxidant supplementation. His research program aims to elucidate the molecular mechanisms necessary for the production and functioning of healthy spermatozoa. His current research focuses on the redox regulation and antioxidant protection during epididymal maturation and capacitation of the spermatozoon by the family of antioxidant enzymes called peroxiredoxins.

Session 3, Friday, September 21

Fabrice Saez, Ph.D.



Fabrice Saez completed his PhD in reproductive biology in 2000 in Clermont-Ferrand (France). He studied the antioxidant capacities of sub-micron organelles of the prostate secretions, the prostasomes, present in human semen. He then joined Robert Sullivan's group in Québec (Québec, Canada) for a two-year postdoctoral position, supported by a grant from the Lalor Foundation. During this period, he studied local gene regulations by the epididymal renin-angiotensin system, in the monkey epididymis and its dysregulations after vasectomy. Since 2003, he is an assistant professor at the "Génétique, Reproduction et Développement" laboratory (GReD, Clermont-Ferrand, France), where he joined Pr Joël Drevet's team. He became the co-leader of the team on January 2017. He is

an animal physiology teacher at the Clermont Auvergne University and his research is centered on the impact of cholesterol-induced dyslipidemia on the epididymal function and its consequences on sperm fertilizing ability, mainly capacitation.

Session 3, Friday, September 21

Petra Sipilä, Ph.D.

Petra Sipilä did her PhD at the Department of Biomedicine, University of Turku, Finland with prof. Matti Poutanen. The PhD work concentrated in generating tools for epididymal research, for example she generated an immortalized epididymal epithelial cell line that is still used by several research groups working in the field. After completing the PhD she joined Prof. Andrew McMahon's lab as a post-doctoral Fellow at Molecular and Cellular Biology, Harvard University, to study the development of kidney. She then returned to Prof. Poutanen's lab to continue her studies on regulation of epididymal gene expression and sperm maturation. Currently she works as Vice director of Turku Center for Disease modeling (TCDM) at the University of Turku. Her research work focuses on male reproduction, using genetically modified mouse models. Her expertise area is the androgen and RNAi mediated regulation of segment specific gene expression and how it affects epididymal sperm maturation.

Session 4, Saturday, September 22

Robert Sullivan, Ph.D.

After completing is post-doctoral training on preimplantation embryo development at the Ludwig Institute for Cancer Research (Montreal, Canada) in 1987, Dr Sullivan became Associated Professor of Obstetrics gynecology at Université de Montréal. From 1987 to 1992 he was laboratory director of an academic program of Assisted Medical Reproduction. In 1995 he moved to Université Laval where he was promoted to the rank of full professor in 2002. He directed the Reproduction, women and youth health division at the CHU de Québec-Université Laval Research Center from 2008 to 2018. Over the years he has trained more than 65 graduate and post-doctoral students, pronounced 75 conferences as invited speaker on different international platforms and published more than 125 articles in international journals. During the last 30 years, he contributed to the understanding of epididymal physiology and function in sperm maturation and involvement in the pathophysiology of male infertility.

Session 8, Sunday, September 23

Pradeep Tanwar, Ph.D.

Associate Professor Pradeep Tanwar is a NHMRC Career Development Fellow at the University of Newcastle, NSW, Australia. Dr Tanwar was originally trained in Veterinary Medicine, and then obtained his PhD in Reproductive Biology. The major focus of his research is on elucidating the molecular players involved in the organogenesis and oncogenesis of reproductive tract organs. His group is using genetically modified mouse models, patient-derived xenograft models, and primary human tissue samples to define the molecular and cellular events involved in these processes and then using this knowledge to develop targeted therapies to improve clinical outcomes in human patients.

Session 1, Friday, September 21

Pablo E. Visconti, Ph.D.

As an undergraduate, Pablo Visconti studied Chemistry at the University of Buenos Aires in Argentina. He completed his Biological Chemistry Ph.D. dissertation in 1991 in the same University on the topic of hamster sperm capacitation. His Ph.D. adviser was Dr. Jorge Tezon. After

finishing his Ph.D., he had postdoctoral training in Dr. Gregory Kopf's laboratory at the University of Pennsylvania in Philadelphia. During this period, he published work that associated capacitation with the increase in tyrosine phosphorylation and the role of cAMP and Protein Kinase A in regulating this signaling process. He became Assistant Professor at the University of Massachusetts in 2003 and received tenure
in 2011. At present, he continues his research on the molecular basis of sperm capacitation. At the translational level, he is interested in methods to induce fertilizing capacity in sperm otherwise sterile. He has recently showed that a short treatment with Ca^{2+} ionophores can up-regulate hyperactive motility in mouse sperm and render sperm from sterile models fertile. He is also interested in using testis-specific protein kinases as targets for male contraception.

Session 3, Friday, September 21

Yong-Lian Zhang, Ph.D.

After graduated from the Fu-Dan University, Pr. Yong-Lian Zhang, academician of the Chinese Academy of Sciences, has been working in the Shanghai Institute of Biochemistry & Cell Biology (Chinese Academy of Sciences). She took part in radiobiology and the molecular mechanisms for androgen regulation of eukaryotic gene transcription. Since 1998, she shifted her research interests on studying the molecular mechanisms of the sperm maturation in epididymis. She constructed a human epididymis cDNA library. Except novel genes cloning, the functions of the 12 newly discovered genes have been identified by anti-sense RNA, RNAi, Crispr Cas-9 and conditional KO approaches. She observed that more than 50 β-defensins are highly abundant in the epididymis which not only maintain an antimicrobial environment for the epididymis lumen but also pay various contributions for sperm gaining its motility. In the meantime, she has been also paying close attention on the roles of the small RNAs in the epithelial cells and sperm in the epididymis as well.

Session 5, Saturday, September 22

SESSION 2 - Friday, September 21 from 11:15-12:15

List of abstracts

SESSION 3 - Friday, September 21 from 15:00-16:00

SESSION 5 - Saturday, September 22 from 11:00-12:00

List of abstracts

SESSION 5 - Saturday, September 22 from 14:30-15:30

SESSION 8 - Sunday, September 23 from 10:30-11:30

Transcriptomic landscape of rat epididymis during postnatal development

Bao-Li Zhang[1,2*S], Bao-Ying Liu[1,2], Shuo Shi[1], Winnie Shum[1]

[1] School of Life Science and Technology, ShanghaiTech University, Shanghai, China
[2] Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai, China

The epididymis in adult is an essential organ for sperm maturation. Epididymis is not completely differentiated until it goes through a series of pre- and post-pubertal developmental processes. Accumulating evidence suggests life intervenes such as epigenetic changes and environmental insults during early age can lead to pathological reproduction in adulthood. However, the normal physiology and pathophysiology of epididymis during postnatal ages are still largely unexplored. To begin to examine the development of the epididymis during early and pubertal paternal age, we performed deep RNA sequencing on RNA extracts. A total of 260 Gb of RNA-seq raw data were generated from 28 whole epididymis from eight developmental stages (from 1-week to 12-weeks-old including immature, growth, peri-pubertal and adulthood phases). We identified over 37000 transcripts from these raw data. We used two bioinformatics tools, WGCNA (Weighted Gene Co-Expression Network Analysis) and STC (Series Test of Clusters), to analyze the differentially expressed transcripts and the dynamic trends of Gene Ontology (GO) and KEGG pathways in the various developmental stages. Both tools revealed five common categories of the transcripts and pathway trends, whereas STC revealed two more categories of these transcripts. Using quantitative mass spectrometry, we identified more than 6900 proteins from the same developmental stages of rat epididymis. Combined analyses of transcriptomic and proteomic data are underway in order to reveal coordinated regulation of transcription and translation. Further analysis and investigations are still in progress. Our study provides a comprehensive and systemic resource for subsequent mechanistic studies and the identification of core factors of postnatal development of epididymis as well as their physiological implications.

Development and Characterization of Epididymal Organoids from the Rat Epididymis

Laurie Pinel[1*S] and Daniel G. Cyr[1]

[1] INRS-Institut Armand-Frappier, Université du Québec, Laval, QC, Canada

The epididymal epithelium is a pseudostratified epithelium comprised of various cell types, including principal cells which line the lumen of the epididymis, and basal cells located at the base of the epithelium. Studies from our laboratory have shown that purified basal cells share common properties with adult stem cells. Furthermore, two dimensional culture of these cells shows that they differentiate *in vitro* under the regulation of FGF and dihydrotestosterone (DHT). However, culture of basal cells under such conditions is limited and cannot maintain long-term differentiation of cells. The objective of the present study is to develop a three-dimensional culture protocol of epididymal progenitor cells, in order to induce their differentiation into functional organoids and to understand the regulation of epididymal progenitor cell differentiation. Epididymal cells were first dispersed enzymatically and cultured in suspended drops of Matrigel. The cells initially formed three-dimensional spheroid structures which differentiated morphologically with time into larger organoid structures. To determine whether or not epididymal progenitor cells were present in the basal cell fraction, we isolated basal cells from the epididymis using integrin-α6 (ITG6) and magnetic separation of ITG6+ basal cells. Three-dimensional cell cultures were established from both the basal cell and non-basal cell fraction containing other epididymal cell types. Spheroid structures were rapidly formed in both cell enriched fractions. These early spheroid structures stained positively for KRT5 and connexin43, suggesting that they were of basal cell origin. The structures in the non-basal cell fractions grew more rapidly and were larger than those in the basal cell fraction in a commercial medium used for prostate progenitor cells. However, the use of a defined DMEM medium allowed the formation of a significantly higher number of spheres in the basal cell fraction as compared to the non-basal cell fraction. The addition of DHT and/or FGF to the culture medium indicated that for the first 7 days of culture, the cells were generally unresponsive to DHT, but these became highly sensitive to DHT afterwards, resulting in rapid growth of epididymal organoids. The addition of the recombinant protein Noggin, an inhibitor of the BMP pathway, resulted in a decrease in number of organoids in the basal cell fraction. Differentiation of organoids indicated that early spheroid structures were both PCNA and KRT5 positive but, as organoids developed, the cells at the periphery of the organoids maintained KRT5 staining while those of the inner cell mass did not express KRT5 but stained positively for Aquaporin 9, a marker of principal cells. These results suggest that epididymal organoids reflect some of the characteristics of normal epididymal differentiation and represent a novel model for understanding mechanisms of differentiation and effects of toxicants on the epididymal development.

Supported by CIHR and CRC.

CRISP (Cysteine Rich Secretory Proteins) as novel regulators of epididymal epithelium differentiation

Guillermo Carvajal[1*S], Nicolás Brukman[1], Mariana Weigel-Muñoz[1], María Agustina Battistone[2], Vanesa Guazzone[3], Livia Lustig[3], Sylvie Breton[2], Patricia Cuasnicú[1]

[1] Instituto de Biología y Medicina Experimental (IBYME-CONICET), CABA, Argentina
[2] Massachusetts General Hospital, Harvard Medical School, USA
[3] Instituto de Investigaciones Biomédicas (INBIOMED-UBA-CONICET), CABA, Argentina

Epididymal CRISP1 and CRISP4 associate with the sperm surface during maturation and are key mediators of fertilization. Whereas single knockout (KO) males for these molecules showed *in vitro* sperm fertilizing defects but normal fertility, all double KO (DKO) animals for these proteins exhibited impaired *in vivo* fertilization and fertility. In addition, one third of DKO showed bigger testes and epididymides not observed in single KO. Based on this, in the present work we investigated the mechanisms underlining this DKO phenotype. Histological studies of DKO testes and epididymides showed that whereas mice with normal tissues (Group 1) were not different from controls, those with bigger organs (Group 2) had clear histological defects as well as an abnormal presence of immune cells in the interstitium and lumen. RT-qPCR for different immunomodulator molecules revealed higher levels of *Il-6* and *Il-10* and a downregulation of *Tgf*-β in DKO from Group 2 not observed in Group 1. Interestingly, immunofluorescence experiments using specific markers for each of the different epididymal epithelial cells revealed fewer and shorter basal cell projections in the initial segment known as axiopodia, defects in principal cells and clear cells with an immature phenotype in both groups. Accompanying these epithelial changes, males from both groups also exhibited an increase in intraluminal pH. Altogether, these observations support the relevance of CRISP proteins for male fertility through their involvement in epididymal epithelium differentiation and luminal acidification which are critical for sperm maturation and storage.

Three-dimensional reconstruction of the epididymal duct focusing on the transit between neighboring segments

Thorben Hau[1*S], Daniela Beyer[1], Andrea Mietens[1], Ralf Middendorff[1]

[1] Institute of Anatomy and Cell Biology, Justus-Liebig-University, Giessen, Germany

The epididymis ensures proper maturation, transport and storage of sperm released by the testis. Epididymal structure is defined by the division into the three regions caput, corpus and cauda. These parts can be further subdivided into segments which are separated from each other through connective tissue septa (CTS). In this context an animal model of luminal ascending infection is of particular interest: ascent of bacteria was found to be restricted to the last segment three days after infection and bacteria were not present in the upstream segment (Stammler et al., 2015). Information about the three-dimensional (3D) organization of the epididymal duct in the context of segmentation, however, is barely available.

To investigate the folding of the epididymal duct at the exact location where it passes the CTS (from one segment to the other), the epididymis was reconstructed based on histological sections and new imaging tools.

The created 3D reconstruction demonstrated a clear separation of neighboring segments and differences in duct folding. Transit from one segment to the other was shown to take place in a broader area of dense connective tissue. When using the 3D dataset of histological sections, a specific folding pattern of the epididymal duct could be revealed in the intersegmental part consisting of a central U- or Ω-shape almost completing a 180-degree turn (x-y plane). Then, the duct leaves the x-y plane, both in the caput and cauda direction, in a wider loop.

Our findings of the duct folding within the intersegmental parts might help to understand the differences among single epididymal segments.

Primary cilia: cell antennas and signalling hubs in the epididymis

Laura Girardet[1*S], Agathe Bernet[1], Christian Roy[1], Clémence Belleannée[1]

[1] Université Laval, Centre de Recherche du CHU de Québec, Québec, Québec, Canada

General context: The epididymis is the organ of the male reproductive system responsible for the acquisition of motility and fertilizing power of spermatozoa. In our laboratory, we have shown that epithelial cells of the epididymis present primary cilia (PC). PC are organelles on the surface of the cells playing an essential role in the development and homeostasis of the organs. In humans, the dysfunction of these signalling antennas leads to multisystem pathologies commonly referred as ciliopathies that are associated with a broad spectrum of clinical features, including male infertility.
Objective: Our goal is to further develop the role of primary cilia as a mediator of the hedgehog (Hh) pathway, in the control of spermatozoid maturation.
Methods: Complementary approaches will be performed with *in vitro* studies in immortalized Distal Caput 2 (DC2) epididymal epithelial cells and *in vivo* studies will be done in a conditional KO mouse model (cKO) which lack the Arl13b GTPase exclusively in keratin 5-positive basal cells. To study the Hh pathway we will use ligands Ihh and Shh, an antagonist, cyclopamine, and ciliobrevinD also an inhibitor of the elongation of PC.
Results: Our first results demonstrate the presence of Hh signaling in DC2 cells *via* PC. Indeed, we observed when using agonists of Hh pathway, a significant increase in its mediators Gli1 and Patch1. Futhermore, we found a significant decrease of those mediators in the presence of both antagonists, cyclopamine and ciliobrevinD.
Expected outcome: In our project, using cKO mice lacking their PC component, we seek to determine the contribution of basal cell PC in the control of the epididymis gene expression, homeostasis and epithelium organization.
Our research will explore the potential role of ciliary components for the diagnosis and treatment of male infertility, which is still unexplained in 30% of cases.

Castration results in increased procathepsin D secretion mediated by prosaposin oligomers in adult rat epididymis

Lorena Carvelli[1,3]*, Carolina Aguilera[1,3], Leila Zyla[1], Louis Hermo[2], Carlos R. Morales[2], Miguel A. Sosa[1,3]

[1] Laboratorio de Biología y Fisiología Celular, IHEM-CONICET, Universidad Nacional de Cuyo, Mendoza, Argentina
[2] Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada
[3] Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina

The epididymis is implicated in sperm maturation by remodeling the sperm plasma membrane throughout lysosomal proteins secreted by the epididymal epithelium. CatD is a lysosomal hydrolase which is secreted by principal cells into the epididymal lumen, and this secretion could be associated with PSAP. PSAP is nonenzymic protein that is trafficked in a monomeric form (70kDa) to the lysosomes or secreted as oligomers (250 kDa). Here, we analyzed cathepsin D (catD) and prosaposin (PSAP) expression in the cauda epididymis of intact rats and rats subjected to castration followed or not by testosterone replacement. Immunoblotting showed an increase in the expression and secretion of catD due to castration in all epididymal regions, which was reversed by testosterone injection. Immunohistochemistry showed a supranuclear localization of catD in the principal cells, and the reaction was more intense in castrated animals, consistent with the immunoblots. Co-immunoprecipitation demonstrated that secreted catD is associated in part to PSAP and this interaction increased by castration (Figure 1). From this, we analysed oligomeric status of PSAP in epididymal fluid. By immunoblotting, we observed that castration increased oligomeric PSAP (250kDa) in the fluid at the expense of monomeric form (70 kDa) (Figure 2). Like catD, PSAP showed a supranuclear staining in the principal cells that spread over the entire apical cytoplasm after castration. Our results suggest that PSAP oligomers associate to catD due to castration, inducing its secretion. In conclusion, testosterone may regulate the secretion of catD and PSAP creating an optimal environment for the sperm maturation.

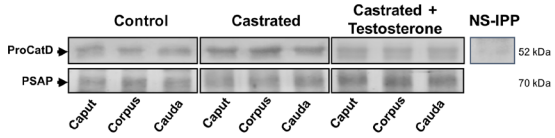


Figure 1. Co-immunoprecipitation of cathepsin D with PSAP. Epididymal fluid from control, castrated or castrated with hormone replacement rats were mixed with beads preincubated with anti-PSAP antibody. Cathepsin D was detected from the precipitates by immunoblot. NS-IPP: beads preincubated with buffer alone.

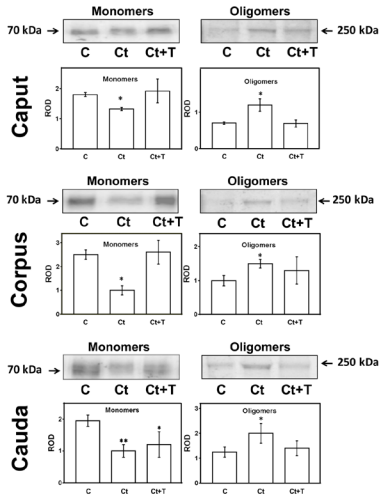


Figure 2. Detection of oligomeric form of PSAP in epididymal fluid. Proteins from epididymal fluid were run on SDS-gel under reducing (monomers) or non-reducing (oligomers) conditions and electrotransferred onto nitrocellulose membrane. The figure shows representative immunoblots of PSAP from each epididymal region and the corresponding quantification of the bands, as indicated. Bars represent the means of relative optical density (ROD) ± SE from three independent experiments. (*) and (**) significant differences from the control ($p < 0.05$ and $p < 0.01$, respectively). C: Control; Ct: Castrated; Ct+T: Castrated with testosterone replacement.

Role of prosaposin and cathepsin D in male reproduction

Lorena Carvelli[1,2]*, Cristian O'Flaherty[3], Richard Oko[4], Miguel A. Sosa[1,2], Carlos R. Morales[3]

[1] Laboratorio de Biología y Fisiología Celular, IHEM-CONICET, Universidad Nacional de Cuyo, Mendoza, Argentina
[2] Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina
[3] Faculty of Medicine, McGill University, Montreal, Quebec, Canada
[4] School of Medicine, Queen's University, Kingston, Ontario, Canada

In mammalian epididymis, luminal proteins interact with the surface of in-transit spermatozoa as a requirement for maturation and acquisition of gamete fertilization capacity. The major sulfoglycolipid of the sperm plasma membrane is a sulfogalactosylglycerolipid (SGG), substrate of Arylsulphatase A (ARSA). ARSA modifies SGG in the presence of a sphingolipid activator protein, termed saposin B (SapB). Both the precursor of SapB, Prosaposin (PSAP), and ARSA are secreted by the epididymal epithelium, and they interact with the sperm plasma membrane. Moreover, the intracellular processing of prosaposin into saposins occurs in an acidic pH, and requires the protease cathepsin D (CatD). Given that CatD is secreted by the epididymal epithelium and that the female genital tract has an acidic environment, we tested whether CatD processes PSAP at an acidic pH and whether the inhibition of PSAP/SapB results in decreased fertilization. Mice epididymal fluid and spermatozoa were collected with buffers, adjusted to different pHs (5.5-6.3-7.2), with and without the presence of Pepstatin A (CatD inhibitor) or antibodies raised against PSAP/SapB. Luminal epididymal proteins were subjected to immunoblotting. Sperm motility (by CASA) and *in vitro* fertilization (IVF) were also tested. Acidification did not alter motility parameters; however, PSAP processing by CatD was enhanced at pH 6.3 (Figure 1). Moreover, sperm motility and IVF were significantly decreased by a blockade of PSAP/SapB with a specific antibody (Figures 2 and 3). These results suggest that PSAP/SapB, and CatD, play an important role in modifying the sperm plasma membrane during sperm capacitation and fertilization.

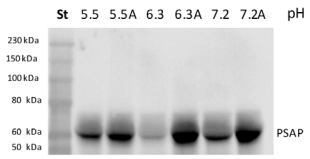


Figure 1. pH effect on PSAP processing in cauda epididymal fluid. The PSAP processing by CatD in epididymal fluids (adjusted to different pHs: 5.5, 6.3 and 7.2) was evaluated. Likewise, they were incubated in the presence (5.5A, 6.3A and 7.2A) or absence (5.5, 6.3 and 7.2) of a CatD specific inhibitor, Pepstatin A.

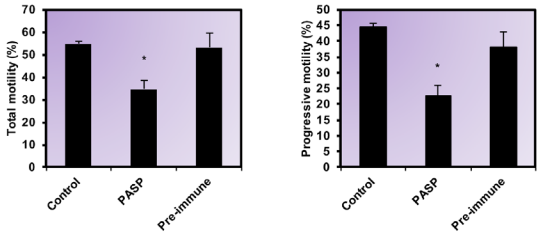


Figure 2. Effect of PSAP action on sperm motility. Cauda epididymal spermatozoa treated with pre-immune serum or antibodies against PSAP (which also recognize SapB) were analyzed by CASA. The graphs represent the average percentages of total motility and progressive motility resulting under the different conditions ± SE. * (P<0.05).

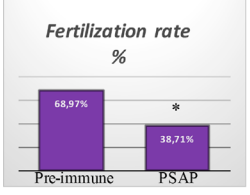


Figure 3. Effect of PSAP antibody in mouse IVF. Cauda epididymal spermatozoa treated with pre-immune serum or antibodies against PSAP (which also recognize SapB) were subjected to IVF. Bars represent the percentage means of fertilized oocytes obtained from three independent experiments.
* p value of 0,0227.

A new model to visualize emission-related contractions in the epididymis and beyond

Mathias Seidensticker[1#], Andrea Mietens[1#], Sabine Tasch[1], Betty Exintaris[2], Ralf Middendorff[1*]

[1] Institute of Anatomy and Cell Biology, Justus-Liebig University Giessen, Germany
[2] Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Melbourne, Australia
[#] equal contribution

Almost along the entire length of the epididymal duct, maturation of spermatozoa and their transport are the main tasks of the organ which are supported by spontaneous contractility in caput, corpus and proximal cauda regions. However, in the distal cauda, storage of sperm and the coordinated contribution to the emission phase of ejaculation is the priority. Alpha1-adrenergic effects were described to enhance transport function in the epididymis by increasing contractile frequency (Mewe et al. 2007). In contrast, phosphodiesterase 5 (PDE5) inhibitors (which enhance cGMP signalling) reduced contractile frequency and thereby had relaxing effects in the epididymal duct (Mewe et al. 2006, Mietens et al. 2012). In patients suffering from benign prostatic hyperplasia (BPH), the classical treatment with alpha1-adrenergic blockers is known to have negative side effects on ejaculation. PDE5 inhibitors have only recently been approved for BPH treatment. While they have no adverse effect on transport function in the epididymal duct (Mietens et al. 2012), no information is available on potential side effects in the distal cauda with its peculiar function during emission.

We therefore analysed the impact of alpha1-adrenergic as well as cGMP-related signalling on contractile function of the distal cauda in the epididymis and compared it to the excretory ducts (Kügler et al. 2018) of the prostatic gland and the seminal vesicle which all contribute to proper emission. For this, time-lapse imaging of isolated intact tissues was used to directly visualize contractile activity under near-physiological conditions.

Interestingly, all tissues investigated were devoid of spontaneous contractile activity which was in contrast to the spontaneous and regular contraction pattern in the remainder of the epididymal duct. Distinct contractions could be elicited by noradrenaline thus imitating alpha1-adrenergic signalling during emission. While pre-treatment with the alpha1-blocker tamsulosin did not show direct effects, it reduced or even prevented noradrenaline-induced contractions and the release of sperm or secretions in all tissues investigated. In contrast, pre-treatment with the PDE5 inhibitor tadalafil was without adverse effects regarding noradrenaline-induced contractions, neither in the distal cauda of the epididymis, nor in the prostatic ducts and the seminal vesicle.

Our imaging approach could visualize interference of alpha1-receptor blockers with emission-related contractions in the contributing structures while no effects were observed with the PDE5 inhibitor tadalafil. This *ex vivo* model thus represents a new test system to screen for ejaculatory side effects.

Glucocorticoid receptor signaling regulates androgen-dependent Wolffian duct morphogenesis

Camilla M. Ribeiro[1*§], Daniel S. Thimoteo[1], Lucas G.A. Ferreira[1], Barry T. Hinton[2], Maria Christina W. Avellar[1]

[1] Department of Pharmacology, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, Brazil
[2] Department of Cell Biology, University of Virginia, School of Medicine, Charlottesville, Virginia, USA

Introduction and Aims: Glucocorticoids acting via glucocorticoid receptor (GR) are essential for growth and maturation of several fetal organs. Prenatal exposure to glucocorticoid excess due to maternal stress during pregnancy, administration of synthetic glucocorticoids to mothers at risk of preterm labour, and impaired cortisol metabolism within the decidua, placenta and/or fetus, can lead to long-term effects in programming the hypothalamic-pituitary-adrenal axis function, metabolism, and the reproductive performance of the male offspring. The mechanisms of fetal glucocorticoid programming are still scarcely known. It is not clear whether changes in GR signaling during fetal life indeed disrupt testosterone/androgen receptor (AR) signaling and lead to disorders of male reproductive tract morphogenesis that ultimately determine fertility dysfunction. In the present work, we tested the hypothesis that GR signaling acts collectively with androgens in the morphogenesis of the Wolffian duct (WD), which is the embryonic precursor of the epididymis. **Methods:** Fetuses were harvested from pregnant Wistar rats at embryonic days (e) 14.5-20.5 and processed for GR and AR immunohistochemistry. Isolated WDs were collected at e12.5-e20.5 for RT-qPCR studies. Additionally, WDs (e17.5) were cultured for up to 96 h on cell inserts floating in basal medium containing or not the following drugs: testosterone (T, 10 nM); hydroxyflutamide (OH-Flut, synthetic androgen receptor antagonist, 10 μM); dexamethasone (DEX, selective synthetic agonist for GR, 10 nM, and 100 nM) and RU486 (synthetic GR antagonist, 1 μM). The medium was replaced every 24 h. Temporal changes in WD gross morphology, nuclear GR and AR immunofluorescence, cell proliferation and apoptosis, and transcriptomic regulation of GR and AR downstream targets and morphogenes involved in mesenchymal-epithelial paracrine interactions in treated WDs were assessed. **Results:** WD morphological differentiation was accompanied by a parallel increase in fetal plasma corticosterone and testosterone levels, as well as changes in the spatio-temporal expression of key molecular components of the GR and AR signaling pathways in the developing duct. DEX promoted a time- and concentration-dependent elongation/coiling of cultured WDs. DEX-induced effects were abrogated by RU486, but not OH-Flut. The stimulatory effects of glucocorticoids on WD morphogenesis ex vivo were mediated by both GR nuclear translocation and changes in AR signaling. Glucocorticoid incubation changed the expression of a subset of morphogenes known to be involved in mesenchymal-epithelial cell interactions critical for WD development as well as regulated the ductal cell proliferation and apoptosis. **Conclusions:** Altogether, our data demonstrates GR functionality in WD morphogenesis. The cross talk between glucocorticoid and testosterone signaling in WD morphogenesis ex vivo also suggest that an interplay between these steroid hormones may be required for proper WD morphogenesis. Disruptions in the balance of these hormones and their cognate receptors may help the understanding of male reproductive disorders that have their origin during prenatal life.

Funding support: CAPES, CNPq/Science Without Borders, FAPESP (Brazil), NIH–NICHD. Research Ethics Committee approval: #4637290115, UNIFESP/EPM.

Lysosomal abnormalities in epithelial cells of the efferent ducts and epididymis in a combined Neuraminidase 3 and 4 deficient mouse model

Regiana Oliveira[1]*, Louis Hermo[1], Alexey V. Pshezhetsky[2], Carlos R. Morales[1]

[1] Department of Anatomy and Cell Biology, McGill University, Montréal, Quebec, Canada
[2] Division of Medical Genetics, Centre Hospitalière Universitaire Sainte-Justine, University of Montréal, Montréal, Quebec, Canada

Mammalian neuraminidases consist of a family of four enzymes (Neu1-4) responsible for the removal of sialic acids from glycoproteins and glycolipids. Sialic acids function as crucial recognition markers mediating a variety of biological phenomena. Plasma membrane- and intracellular-associated neuraminidases (Neu3 and Neu4) participate in many biological processes such as lysosomal catabolism. In this study a detailed morphological analysis of the deficiency of these enzymes was performed on the structural appearance of epithelial cells of the efferent ducts and epididymis of 3 month-old *neu3*$^{-/-}$ *neu4*$^{-/-}$ mice in comparison with wild type mice as visualized with the light and electron microscope. In the efferent ducts, nonciliated cells of wild type mice revealed small pale and dense lysosomes that were noted to be larger and more numerous in *neu3*$^{-/-}$ *neu4*$^{-/-}$ mice. In addition, these cells demonstrated a unique organelle that appeared as large elongated cylindrical-shaped structures with an irregular profile that contained empty looking spaces of different sizes, which at times lined up to form parallel rows. Dense lysosomes of small size appeared to have fused with these structures and released their contents. Such structures have not been reported in epithelial cells of the efferent ducts of other lysosomal storage disease models. Based on their unique shape and internal spaces, it is suggested that they represent abnormal mitochondria with the spaces remnants of cristae, whose membranes have undergone degeneration.This hypothesis could be envisioned as Neu4 has been reported to be in the matrix of mitochondria. However, it is also likely that they represent lysosomes whose internal contents have been degraded in an unusual manner due the absence of Neu3 and Neu4. In the epididymis, principal cells demonstrated region-specific lysosomal abnormalities. Principal cells of the initial segment revealed numerous small dense lysosomes as compared to wild type mice. In the cauda region, principal cells revealed huge vacuoles both apically and supranuclearly and an accumulation of lysosomes infranuclearly as stained with anti-prosaposin antibody. Throughout the epididymis, large basally located cells were evident in all epididymal regions, which were filled with huge dense lysosomes often with a lipidic nature. Occasionally, basal cells without lysosomal accumulations were also noted, thus confirming the existence of different classes of basally located cells. Those with large dense lysosomes may correspond to dendritic and/or F4/80 macrophages monitoring the abnormalities of the epithelium of *neu3*$^{-/-}$ *neu4*$^{-/-}$ mice. Halo cells were also noted some with lysosomal accumulations. In addition, in the initial segment only, large dilated intercellular spaces appeared at the base of the epithelium. They were filled with membranous profiles and suggested an increased movement of fluids between the epididymal lumen and the underlying interstitium. Capillaries under the basement membrane were also numerous and enlarged compared to wild type mice. The basement membrane was highly convoluted and thicker in size compared to wild type mice, with numerous vesicular profiles in its anastomotic spaces. The intertubular space between epididymal tubules was at times filled with immune cells. Thus the absence of Neu3 and Neu4 reveals major abnormalities in the epithelium and interstitium of *neu3*$^{-/-}$ *neu4*$^{-/-}$ mice.

Supported by CIHR and NERSC

Biodiversity of the mammalian epididymis

Christine Légaré[1]*, Ezéquiel Calvo[1], Robert Sullivan[1]

[1] Centre de recherche du CHU de Québec, Université Laval, Québec, Canada

The epididymis is the hallmark of vertebrate species practicing internal fertilization. In these phyla, sperm deposition is not always synchronized with ovulation. In order to increase the fertilizing window of a given ejaculate, the epididymis may be responsible of creating heterogeneity among sperm cells so that subpopulations of male gametes will be «fertile» at different time point following copulation. The epididymal function may thus vary from one mammalian species to the other depending on their reproductive strategies. In this study, human epididymal transcriptome previously described by our laboratory (Thimon *et al.*, 2007) was compared to the domestic cat (*Felix catus*), a species in which copulation induces ovulation assuring the synchronicity between sperm deposition with egg laying. The cat epididymis is relatively small, with numerous efferent ductules occupying a significant portion of the proximal epididymis. Affymetrix Gene Chip Feline Gene 1.0 ST Array was utilized to assess gene expression along the epididymis of three sexually mature toms. Principal Component Analysis revealed clear differences between caput, corpus and cauda epididymis; the corpus and cauda clustered closely by opposition to the caput who has a more distinct qualifiers profile. To compare gene expression between human and cat epididymis, transcripts with a differential expression (>2-fold change and a FDR < 0.05) were clustered in relation to their intensity profiles. Whereas 25% of the qualifiers were expressed in both human and feline microarrays, 57% and 18% were exclusive to human and feline species respectively. Cluster analysis demonstrated that transcript expression pattern along the human epididymis is more regionalized when compared to the cat datasets. Bioinformatics tools were used to identify differentially expressed genes in epididymis from the caput, corpus and cauda segments. As an example, in domestic cat, gene ontology annotation revealed the differentially expressed genes (DEGs) in caput/corpus were predominantly related to the cellular component terms of plasma membrane and exosomes. In contrast, DEGs biological significance revealed that exosomes terms characterized a more distal region of the human epididymis. In conclusions, the pattern of gene expression along the feline epididymis revealed a functional differences between the caput and the downstream segment of the epididymis by opposition to human; a species in which segmentation of gene expression is in accordance with the caput, corpus, and cauda segmentation of the excurrent duct. The physiological significance of these differences between human and feline epididymal transcriptome remains to be elucidated.

This work was supported by Natural sciences and engineering research council of Canada.

Pattern of protein expression in the epididymis of *Oligoryzomys nigripes* (Cricetidae, Sigmodontinae)

Mariana Machado-Neves[1]*, Tatiana Menezes[1], Eric Hill[2], Arlindo Moura[3], Sylvie Breton[2]

[1] Federal University of Viçosa, Viçosa, Minas Gerais, Brazil
[2] Harvard Medical School, Boston, Massachusetts, USA
[3] Federal University of Ceará, Fortaleza, Ceará, Brazil

In the epididymis, epithelial cells work in a concerted manner to create a luminal environment for sperm maturation, transport, and storage. However, the cell functions may be affected by anthropogenic factors, causing negative impacts on male fertility. In our study, we describe the pattern of protein expression in the epithelium and luminal fluid from epididymis of *Oligoryzomys nigripes*, a South American sigmodontine rodent whose reproductive biology has been little studied. Nine animals were captured from a preserved area of Atlantic Forest, where the exposure to anthropogenic influences is minimal. Epididymides were processed for histological analysis under light and epifluorescence microscopy, in which we used cell-specific markers aquaporin 9 (AQP9), vacuolar H^+-ATPase (V-ATPase), and cytokeratin 5 (KRT5). Other samples were assessed for protein expression using shotgun proteomics. Sections of epididymal duct were lined by a pseudostratified epithelium delimiting the lumen with sperm in the caput, corpus, proximal cauda, and distal cauda. Basically, we identified principal, clear and basal cells from the caput to distal cauda of the *O. nigripes* (Fig.1). Similarly to laboratory rodents, principal cells expressed AQP9 in their stereocilia. Basal cells, identified by KRT5 labeling, presented lateral body projections and a few axiopodia going toward the lumen (Fig. 2). Clear cells expressed V-ATPase in their sub-apical vesicles and microplicae, and showed different shapes along the duct. Shotgun proteomics detected 51 proteins from epididymal fluid. Most of them have been previously described in other species, indicating they are well conserved. Twenty-three proteins detected in *O. nigripes* were not described in epididymis from other South American sigmodontine rodents, confirming that the secretion pattern is species-specific. Our findings in *O. nigripes* from a protected area may help to create a baseline for studies investigating the effects of anthropogenic factors on functionality of the epididymal epithelium.

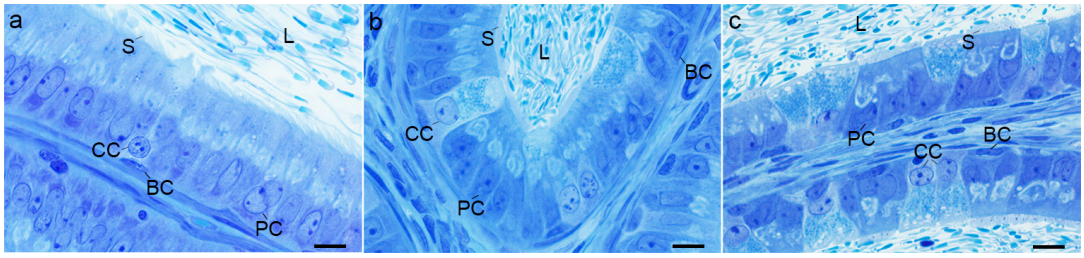


Fig. 1: Histological sections of epididymis in *Oligoryzomys nigripes* show principal cells (PC), basal cells (BC), and clear cells (CC) in the caput (a), corpus (b), and distal cauda (c). Note the presence of stereocilia (S) in the apical portion of principal cells. Sperm were observed in the lumen (L). Scale bars: 5 μm.

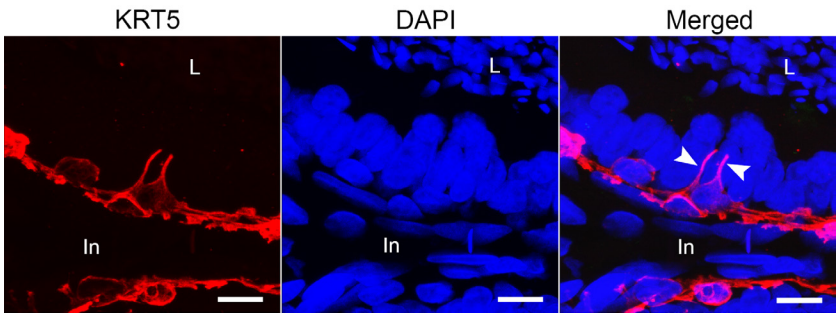


Fig. 2: Three-dimensional (3D) reconstruction of cauda epididymis sections of *Oligoryzomys nigripes*. Localization of KRT5 (red) as a marker for basal cells. We detected cytoplasmic projections going laterally into the basal epithelial portion, and a few projections (arrowhead) going toward the lumen (L). Nuclei and sperm were stained with DAPI (blue). Scale bar: 100 μm.

Impact of androgenic-anabolic steroids on the sperm quality of Wistar rats

Ramão Deus Junior[1,2,3], Ana Flávia Lozano[1,3*S], Thamiris Figueiredo[3], Cibele Borges[3], Josiane Rosa[3], Jorge Barros[1,3], Gabriel Leite[1,3], Wilma Kempinas[3]

[1] University of Campinas (UNICAMP), Campinas, São Paulo, Brazil
[2] Federal University of Grande Dourados (UFGD), Dourados, Mato Grosso do Sul, Brazil
[3] Department of Morphology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

Introduction: Anabolic-androgenic steroids (AAS) are synthetic medicines derived from testosterone and can be used to treat cancer, cachexia and hypogonadism. However, indiscriminate use of AAS became a growing problem for adults and young people seeking an esthetic improvement of their bodies. Oxymetholone, nandrolone and stanozolol are among the most used AAS for this purpose, but the effects on the male reproductive system are not well known. **Objective:** Thus, this study aimed to evaluate sperm quality in rats treated with oxymetholone, nandrolone and stanozolol. **Materials and Methods:** Male Wistar rats (n=7/group) were distributed into nine groups: three control groups: CO- oxymetholone control group, CN - nandrolone control group and CS - stanozolol control group, treated with saline (gavage) corn oil and saline (intramuscular injection), respectively. Two groups were treated by gavage with oxymetholone at different doses: O5 (5mg/Kg) e O10 (10mg/Kg), two groups were treated with nandrolone at different doses: N5 (5mg/Kg) and N10 (10mg/Kg) and two groups treated with stanozolol: S5 (5mg/Kg) and S10 (10mg/Kg). The last two groups were treated by intramuscular injection. After 10 weeks of treatment the rats were euthanized and the body and reproductive organ weights obtained. Sperm samples were used to evaluate sperm motility and to perform intrauterine artificial insemination. The results were compared using ANOVA ($p < 0.05$). **Results and Discussion:** The weights of the following organs were significantly different from control, depending on the experimental group: the weight of epididymis was reduced in all treated groups with nandrolone e stanozolol, the weights of testes were decreased in N5, S5 and S10. Seminal gland with fluid was decreased in O5, O10, N5, S5 and S10. Ventral prostate was decreased in N5, S5 e S10. Besides that, the weight of seminal gland without fluid was reduced in S5. Moreover, sperm motility and fertility were reduced in all treated groups with nandrolone and stanozolol. Rats treated with oxymetholone presented reduced sperm motility in O10. **Conclusions:** Exposure to these three AAS, in the present experimental conditions, compromised sperm quality, while fertility was committed in rats treated with nandrolone and stanozolol. These results, in rats, raise concern regarding the use of these AAS by men.

Murine Binder of SPerm (BSP) proteins engineered by CRISPR/Cas9 system are dispensable for male fertility

Marzieh Eskandari-Shahraki[1,2*S], Bruno Prud'homme[1], Puttaswamy Manjunath[1,2]

[1] Maisonneuve-Rosemont Hospital Research Centre, Montréal, Québec, Canada
[2] Department of Pharmacology and Physiology, Faculty of Medicine, University of Montréal, Montréal, Québec, Canada.

Sperm encounter various secreted proteins in the epididymis resulting in acquisition of motility and modifications in the plasma membrane lipid composition. A family of proteins named Binder of SPerm (BSP) are expressed exclusively in male mammalian reproductive tract. Despite differences in the number of forms and concentration, they share analogous function and conserved structure in all the mammals studied to date. Studies have indicated an ambivalent function for these proteins. These proteins interact with choline phospholipids (PC) on the sperm membrane in the male reproductive tract and prevent premature capacitation. However, in the female genital tract, BSP proteins promote capacitation by interacting with components present in this environment. Recently, two homologous epididymal BSP proteins were identified in mouse (BSPH1 and BSPH2) and one in human (BSPH1). In order to characterize and establish the role of these proteins on the fertility, the highly efficient CRISPR/Cas9 system was adopted to generate double knockout (*Bsph1/2*-DKO) mice. The DKO mice were assessed at mRNA (RT-PCR and qPCR) and protein (Mass spectrometry) levels. Trio mating was performed to assess the fertility capability of the DKO. Sperm Class Analyzer (SCA) was used to assess the perm parameters. Male and female DKO animals exhibited no differences in fertility compared to controls, with no differences in the litter size, motility and sperm count compared to wild-type. BSP null mice mated normally and were healthy. The DKO models were evaluated for sperm parameters using Sperm Class Analyzer and no differences were observed between DKO and wild type. Taken together, these data suggest that BSP proteins are not essential for sperm maturation and fertilization, at least in the mouse.

Supported by Canadian Institutes of Health Research (CIHR).

Effects of Leptin and Leptin Receptor Deficiency on Epididymal Sperm Number and Function in Mice

Christopher A. Pearl[1]*

[1] Department of Biomedical Sciences, Grand Valley State University, Allendale, MI, USA

Genetically modified mice lacking the leptin hormone (ob/ob) or leptin receptor (db/db) are reported to be subfertile and/or infertile, but there is limited information about epididymal function and the progression of infertility. These studies were designed to investigate epididymal sperm numbers and function in leptin and leptin-receptor-deficient obese mice. In study one, male ob/ob and db/db mice were compared to wild-type c57 mice at 8 and 16 weeks of age. Both ob/ob and db/db mice became obese, as expected, with body weights significantly higher than control mice. Paired testis weight was reduced in ob/ob mice but testis weight in db/db mice was similar to controls. Body weight increased from 8 to 16 weeks of age but testis weight was similar between age groups. The weight of the epididymal fat was also significantly higher than controls, but while body weight was approximately double control mice, epididymal fat weight was approximately ten times higher in ob/ob and db/db mice. Daily sperm production was significantly reduced in the ob/ob mice while sperm production in db/db mice was similar to controls. These data suggest that testicular sperm production is reduced in ob/ob mice which lack the leptin hormone, but normal sperm production may occur in db/db mice lacking leptin receptor. Total sperm per epididymis and sperm per milligram epididymis were similar between all three genotypes at eight weeks of age. In control mice, total sperm per epididymis was significantly higher at 16 weeks of age (35 ± 2.7 x 106) compared to 8 weeks of age (17.52 ± 0.88 x 106); sperm/mg epididymis showed a similar increase. Total epididymal sperm and sperm/mg epididymis did not increase with age in the ob/ob and db/db mice but were significantly reduced at 16 weeks compared to control mice. Sperm transit time in the epididymis was similar between all three genotypes at 8 weeks of age but was significantly reduced in both ob/ob (4.3 ± 0.7 days) and db/db (4.4 ± 0.9 days) mice compared to control mice (10.3 ± 0.7 days) at 16 weeks of age. The observation that epididymal sperm number and transit time was similar at 8 weeks of age, but reduced in ob/ob and db/db mice at 16 weeks suggests a progressive decline in fertility potential with age and/or increased obesity. Morphological assessments indicate that the tubule diameter and epithelial cell height is altered in the initial segment and cauda of ob/ob and db/db animals. Traditional leptin receptor deficient mice (db/db) have been reported to still express some forms of the leptin receptor. Therefore, in a second study, a leptin receptor knockout mouse reported to be lacking all forms of the receptor (db/lb; Pound Mouse) was compared with wild-type mice. Body weight and epididymal fat weight of Pound mice showed similar results at 8 and 16 weeks to those observed in ob/ob and db/db mice. Epididymal sperm numbers were similar between control and Pound mice at 8 weeks of age but were significantly reduced in pound mice at 16 weeks of age. This is consistent with the db/db model that a progressive decline in fertility potential occurs with age and/or increased obesity. Collectively, these data suggest that the subfertility/infertility observed in leptin hormone deficient mice can be attributed in part to reduced testicular sperm production, but also reduced epididymal sperm numbers and possibly impaired epididymal maturation, whereas the subfertility/infertility in leptin receptor deficient mice may be primarily a result of problems associated with the epididymis.

Decreased rat sperm quality after administration of sibutramine in the dark phase of light/dark cycle

Cibele Borges[1*], Gabriela Missassi[1], Raquel Silva[1], Patricia Silva[1], Janete Anselmo-Franci[2], Wilma Kempinas[1]

[1] Departments of Morphology, Institute of Biosciences of Botucatu, UNESP – Sao Paulo State University, Botucatu, São Paulo, Brazil
[2] Department of Morphology, Stomatology and Physiology, USP – University of Sao Paulo, Ribeirão Preto, São Paulo, Brazil

Sibutramine is a selective norepinephrine reuptake inhibitor used to lose weight, since it allows a longer action of this neurotransmitter in the synaptic cleft. Previous work showed that sibutramine treatment promoted effects on epididymal function, as reduction in sperm reserves and sperm transit time in the epididymal cauda when the drug was administered during the light phase of the light/dark (l/d) cycle. However, other works showed that sibutramine effects are increased if the exposure to the drug occurs during the dark period of the cycle. Since the influence of the l/d cycle on testing is a relevant factor for the analysis of toxicological results, this study aimed to evaluate the adverse effects on sperm parameters and epididymal histology of male rats exposed to sibutramine or vehicle in the dark phase of the l/d cycle. For this, adult male Wistar rats were treated with sibutramine (10 mg/kg/day) or vehicle for 15 days, during the dark period of the l/d cycle (12 h of white light/12 h dark, lights going off at 7:00 am). At the end of the treatment the animals were killed for blood and reproductive organs collection for the determination of serum hormonal levels, organ weights, sperm motility, sperm count and epididymal histopathology. The results were compared using Student´s t-test ($p<0.05$). Ethics Protocol number: 248. Sibutramine decreased final body and reproductive organ weights, as well as the testosterone levels. There was a reduction in the sperm concentration in the epididymis and in the progressive sperm motility. Moreover, sperm transit time was accelerated in both, caput/corpus and cauda of the epididymis. Histopathology also showed a decrease in sperm concentrations in the epididymis. In conclusion, the decrease in sperm quality promoted by sibutramine, in this experimental model, mediated by alterations on epididymal function, are enhanced when the exposure occurs during the dark phase of the l/d cycle, which corresponds to more active period of diurnal humans.

Ligation of caput epididymis modulates sperm signaling pathways in mice

Darya A. Tourzani[1*S], Maria Gracia Gervasi[1], Maria Agustina Battistone[2], Sylvie Breton[2], Pablo E. Visconti[1]

[1] Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA USA
[2] Massachusetts General Hospital- Harvard Medical School, Boston, MA USA

The dynamic fusion of the sperm with an oocyte first begins with a long cascade of steps, eventually leading to this final moment. Prior to this moment, sperm need to undergo two vital steps: maturation in the male epididymis and capacitation in the female reproductive tract. Once completing spermatogenesis, sperm enter into a region of the epididymis known as the caput, where they are characterized as immature sperm lacking the ability to be motile and fertilize. They proceed through a convoluted tubule within the epididymis ending in the most distal region known as the cauda. Once here, sperm have completed their epididymal maturation step gaining an important factor: motility. In mice, this journey takes approximately seven days to pass through the entire epididymis. Many of the molecular changes that attribute to this change in motility are still not fully understood. Due to the unique nature that sperm are translationally silent, they must rely upon post-translational modifications during sperm capacitation. Previously, our group has showed that O-GlcNAcylation may be amongst one of the post-translational modifications that attributes to differences between caput and cauda sperm. Immature caput sperm show high regulation via OGT (O-GlcNAc transferase), the enzyme that regulates this mechanism while mature cauda sperm show low levels. In a proposed relationship, the same proteins that are glycosylated on a serine residue may not be able to be phosphorylated via protein kinase A (PKA) in immature sperm. While mature cauda sperm, lacking the glycosylated proteins, are now able to be phosphorylated via PKA. However, the relationship between this post-translational modification and PKA during sperm maturation has not been studied in depth.
In 1931, it was shown that through the use of epididymal ligation in rabbits, caput sperm could mimic the ability to become motile. Since then others has repeated these ligations in rabbits, ferrets, hamsters and rats. Here, we show for the first time in mice the effects of epididymal ligation on immature caput sperm. Our results indicate that during a ligation period of seven days, immature caput sperm are able to gain motility. When assessing activation of signaling pathways related to sperm capacitation, there is a significant change in the proteins that are glycosylated in the ligated caput sperm. They show an increase in proteins phosphorylated via PKA, compared to mature cauda sperm. Our results show that an extended period of time in the caput epididymis is sufficient to achieve motility and initial molecular changes indicative of maturation. This suggests that the role of epididymal maturation is fundamental for fertilization but not motility.

Studying spermatic epigenetic profile during post-testicular maturation

Hong Chen[1*§], Christian Roy[1], Clémence Belleannée[1]

[1] Université Laval, Centre de Recherche du CHU de Québec, Québec, Québec, Canada

General context. Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technology which consists in injecting a spermatozoon (from the testis, epididymis or ejaculate) into an oocyte. This technic is applied more and more frequently to overcome infertility problems. However, its consequences on gamete epigenetic and birth outcomes are still questioned.

Problem and hypothesis. Since the spermatic genome is highly condensed, epigenetic modifications during post-testicular maturation have been considered highly limited. However, few studies have determined that spermatic epigenome can be modified at the post-testicular level in epididymis (e.g., DNA methylation and small non coding RNA) (Ariel et al, Nature Genetics, 1994; Sharma et al, Science, 2016). We hypothesize that DNA methylation changes may occur during the post-testicular maturation and these modifications could be transmitted during oocyte fertilization, modifying spermatic gene expression in embryonic development.

Objectives and methods. In this context, our objective is: To Map out the epigenetic profile of spermatozoa at different stages of maturation. Spermatozoa from the testis and epididymal regions (caput, corpus, and cauda) were isolated by flow cytometry from eight CAG/su9-DsRed2, Acr3-EGFP transgenic mice, in which spermatozoa display endogenous fluorescence. After DNA extraction, DNA methylation mapping was performed by reduced representation bisulfite sequencing (RRBS, Diagenode kit) on Illumina Hiseq 2000 platform in collaboration of Dr Arnaud Droit.

Results. The purification yield of testicular, caput, corpus and cauda epididymal spermatozoa was assessed between 90 and 98% of all cells analyzed. Furthermore, the statistical analysis of RRBS data indicated that dynamic methylation changes occur on spermatic genome from diverse epididymal regions. We identified 3245 and 1338 methylation changes between caput vs. testis and corpus vs. testis, respectively (q value < 0.01, percentage of methylation difference above 25 %). However only 91 methylation changes were identified between cauda vs. testis. Among these changes, 16 sites were methylated in the 3 different segments of the epididymis while non-methylated in the testis and are associated with different gene ontology pathways (e.g., apoptosis, metabolic process).

Conclusion. By combining biochemical and high throughput sequencing approaches on wild type and transgenic mice, we identified numerous changes in terms of methylation profile that occur in the maturing spermatozoa. Further investigations will be needed to validate these changes from wild type mice and human samples and to assess their potential effect on embryo and post-natal development. Ultimately, our study might help defining the way spermatozoa are selected in order to reduce epigenetically related congenital diseases in children born after ICSI.

A new approach to reduce thermal andoxidative stress during vitrification procedure

Mojtaba Dashtizad[1]*, Parinaz Kazemi[2], Samaneh Fayazi[1], Fatemeh Sarmadi[1], Pouria Tirgar Bahnamiri[3], Ehsan Hashemi[1], Shiva Molaeiyan[1]

[1] Embryo Biotechnology Laboratory (Embio Lab), Department of Animal Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
[2] Department of Biology, McGill University, Montreal, Quebec, Canada
[3] Department of Biological & Biomedical Engineering, McGill University, Montreal, Quebec, Canada

The most effective characteristic of vitrification is high cooling/warming rate, which helps to reduce ice crystal formation and cellular damage. In recent years, the ultra-rapid cooling rate has been achieved using small volumes of vitrification solution along with various cryodevices. However, the heat conductivity of vitrification solutions as an effective parameter on the cooling/warming rate has received less attention. In this context, the present study was conducted to determine whether suspended graphene can be used instead of sucrose to reach higher thermal conductivity while maintaining the efficiency of vitrification solution. In this regard, the optimal concentration of the graphene in vitrification (VS) and warming solutions (WS) were first determined by replacing sucrose with different amounts of graphene in one of the solutions at a time. In the second part of the experiment, to evaluate the cryoprotective efficiency of the graphene, optimum WS and VS were used together, replacing sucrose in both sets of solutions. Mouse embryos vitrified/warmed using either the control media (Sucrose solutions) or the new graphene-based pair of solutions and compared in terms of survival, hatching and implantation rate, cellular peroxide levels and expression level of genes related to thermotolerance (*Hspa1a*) oxidative stress (*Sod1, Sod2*) and apoptosis (*Trp53, Bax, Bcl2*). The optimum concentrations of 1.5, 2 and 4 M were first selected for vitrification, thawing and dilution solutions, respectively. According to our results, grapheneas a cryoprotectant could be as efficientas sucrose in terms of survival, hatching and implantation rate. The real-timeRT-PCR analysis also revealed a reduction in the expression of *Hspa1a, Sod1, Sod2* and *Trp53* transcripts in the graphene group compared to the sucrose group; the abundance of *Bax* and *Bcl2*, however, did not change. Furthermore, there was a lower cellular peroxide level in the graphene-based group compared with sucrose-based one. In conclusion, our findings highlight the importance of heat conductivity of vitrification solutions, demonstrating that graphene can act as a non-permeatingcryoprotectant as well as a heat transfer agent, leading to a more moderate expression of apoptosis, oxidativeand thermal stress-related genes.

Assessing the role of beta-defensin126 on sperm motility and maturation

Raheleh Aram[1*§], Peter Chan[2], Daniel G. Cyr[1]

[1] INRS-Institute Armand-Frappier, Laval, Quebec, Canada
[2] Department of Urology, Royal Victoria Hospital, McGill University, Montréal, Quebec, Canada

A crucial function of the epididymis is to secrete factors that contribute to the formation of the sperm surface glycocalyx, a structure that is important for sperm maturation and capacitation. β-defensins are antimicrobial peptides that expressed primarily in the epididymis and which contribute to the formation of the sperm glycocalyx. In macaque and bovine β-defensin126 (DEFB126), has been shown to be important for sperm maturation and capacitation. Mutations in the DEFB126 gene have been linked with subfertility and infertility in humans. In azoospermic men, sperm can be retrieved from testes for assisted reproductive technologies; however, these sperm are immature and immotile. *In vitro* sperm maturation may provide better quality sperm for intracytoplasmic sperm injection. The objective was to develop an in vitro sperm maturation assay using DEFB126. Immunofluorescence staining of DEFB126 in human sperm indicated that the proportion of DEFB126-positive sperm was significantly higher in motile sperm. Furthermore, the proportion of DEFB126-labeled sperm was positively correlated with both sperm motility and normal morphology. Additional studies indicated that the proportion of DEFB126-positive spermatozoa in fertile patients was significantly higher than in patients with varicocele, and in infertile patients (semen deficiencies). The proportion of DEFB126 positive sperm was correlated with circulating testosterone levels, suggesting that this gene may be partially regulated by androgens. To determine the role of DEFB126 on sperm motility, the DEFB126 gene was cloned and used to generate recombinant DEFB126 (rDEFB126) in H9C2 cells. Deletion mutations were created into two regions of the gene to generate mutated rDEFB126 proteins which have been linked to male infertility. Cell lines that stably express rDEFB126 and two mutated forms of the genes were generated. Immotile testicular human spermatozoa were incubated with cells expressing the different forms of rDEFB126. Full-length rDEFB126 increased motility of co-cultured spermatozoa by 15%. However, no increase in sperm motility was observed with the mutated forms of DEFB126. Immotile spermatozoa from the initial segment of adult rats were also incubated with cells expressing the different forms of rDEFB126. Sperm motility was increased in cell co-incubated with rDEFB126 but the mutated forms of the proteins failed to increase sperm motility. These data suggest that the DEFB126 effects on sperm motility may not be species-specific but rather that this protein exerts an effect that can be induced across different species. In conclusion, this results support the notion that DEFB126 is important in human sperm maturation and that DEFB126 may be useful for *in vitro* sperm maturation.

Supported by CIHR.

Profiling of epididymal small non-coding RNAs

Brett Nixon[1]*, Natalie Trigg[1], Andrew L. Eamens[1]

[1] Priority Research Centre for Reproductive Science, School of Environmental and Life Sciences, University of Newcastle; Hunter Medical Research Institute

The production of fertilization competent mammalian spermatozoa represents the culmination of an extraordinary sequence of cytological differentiation and functional maturation events. The latter of this coincides with passage through the epididymis, during which the transcriptionally and translationally inert spermatozoa are remodeled in such a way that they gain the potential for forward progressive motility and to participate in sperm-oocyte interactions. While a traditional focus for investigation of epididymal sperm maturation has been the contribution of fertility modulating proteins, more recent work has begun to uncover the importance of small non-protein-coding RNAs (sncRNA) as key regulators of this process. Here, we report on our use of deep sequencing technology to systematically survey the complete sncRNA profile of mouse spermatozoa at different stages of their epididymal maturation; providing the first evidence for substantial modification of the sperm sncRNA profile under normal physiological conditions. Further, we compare sperm sncRNA profiles with those of the epididymal epithelium and the epididymosomes they secrete to confirm that this form of soma-spermatozoa intercellular communication does account, at least in part, for the dynamic changes in the sperm-borne sncRNA profile. Collectively, these data identify the epididymis as an important site for establishment of the sperm epigenome with the potential implications extending to influence over the: (i) peri-conceptual environment of the female reproductive tract, (ii) inheritance of acquired characteristics, and/or (iii) altered developmental trajectory of the resulting offspring.

THIS ABSTRACT WAS SUBMITTED FOR AN INVITED SYMPOSIUM TALK

Testicular heat stress alters sperm miRNAs profile in bulls

Maíra Bianchi Rodrigues Alves*[1S], Rubens Paes de Arruda[1], Leonardo Batissaco[1], Shirley Andrea Florez-Rodriguez[1], Laura Nataly Garcia-Oliveros[1], Vitor Hugo Guilger Gonzaga[1], Sâmara Cristine Costa Pinto[1], Vinícius José Moreira Nogueira[1], Flavia dos Santos Almeida[1], Juliano Coelho da Silveira[2], Felipe Perecin[2], Eneiva Carla Carvalho Celeghini[1]

[1] School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil
[2] School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil

CRISPR/Cas9 mediated genome editing in mice and its application for the study of reproduction

Masahito Ikawa[1]*

[1] Research Institute for Microbial Diseases, Osaka University, Osaka, 565-0871, Japan

CRISPR/Cas9 system has opened the new era for reverse genetics. In 2013, we developed the efficient gene knockout system in mice by injecting the plasmids expressing humanized Cas9 (hCas9) and single guide RNA (sgRNA) into zygotes. Now it is replaced by electroporation of oocytes with CAS9/crRNA/tracrRNA ribonucleoprotein complex. To date, we have knocked out 170 testis abundant genes and analyzed the phenotypes *in vivo*. Whereas 105 of the KO mouse lines were fertile and did not show any drastic phenotypes, 2 KO mouse lines showed lethality. The remaining 63 KO mouse lines showed infertility or subfertility and propelled our *in vivo* study of reproduction [1-3].
Our approaches are highly relevant in this fiscally tight funding period and postgenomic age when large numbers of genomes are being analyzed for disease association, and will prevent unnecessary expenditures and duplications of effort by others.

1. Miyata H. *et al.*, Sperm calcineurin inhibition prevents mouse fertility with implications for male contraceptive. Science. 2015 Oct 23; 350(6259):442-5.
2. Miyata H. *et al.*, Genome engineering uncovers 54 evolutionarily conserved and testis-enriched genes that are not required for male fertility in mice. PNAS. 2016 Jul 12; 113(28):7704-10.
3. Kto K *et al.*, Structural and functional insights into IZUMO1 recognition by JUNO in mammalian fertilization. Nat Commun. 2016 Jul 15; 7:12198.

THIS ABSTRACT WAS SUBMITTED FOR AN INVITED SYMPOSIUM TALK

Identification of segment-specific gene expression patterns in epididymal clear cells, and extracellular mediators involved in the regulation of luminal acidification in the epididymis

Maria Agustina Battistone[1]*, Maria Merkulova [1], Maria A. Peralta[1], Yoo-Jin Park[1], Nicolas DaSilva[1], Dennis Brown[1], Sylvie Breton[1]

[1] Program in Membrane Biology, Nephrology Division, Massachusetts General Hospital (MGH) and Harvard Medical School. BOSTON, MA, USA

In the epididymis, elaborate communication networks between the different epithelial cell types (clear cells (CCs), principal cells (PCs) and basal cells) are important to establish a luminal environment that is essential for sperm maturation. CCs play a critical role in this process by secreting H^+ via the vacuolar H^+-ATPase (V-ATPase) located in their apical membrane, and acidifying the lumen. In this study, we used RNA sequencing to characterize the transcriptome of CCs, in caput, corpus and cauda, isolated by fluorescence-activated cell sorting from our transgenic mice that express EGFP only in CCs. Several genes were common to all CCs, but subsets of genes were exclusively expressed in CCs from each epididymal region, including cell-surface receptors, transcription factors, transporters, and secreted proteins. This database provides a new framework for dissecting the respective roles of CCs depending on their location in the different epididymal segments. Analysis of the most abundant transcripts revealed Ly6g5b (lymphocyte antigen 6 complex) and Npnt (nephronectin) in caput CCs; Sultd1 (sulfotransferase family 1D, member 1), Crhbp (corticotropin releasing hormone binding protein) and Chrd (chordin) in corpus CCs; and Slc7a15 (sodium-dependent neutral amino acid transporter B(0)AT2), S100a8 (calcium-binding protein A8) and Sptlc3 (serine palmitoyltransferase, long chain base subunit 3) in cauda CCs. In addition, we identified several ectonucleotidases (EctoNs), including ecto-5'-nucleotidase (NT5E), ectonucleoside triphosphate diphosphohydrolase (ENTPD:1, 4, 5, 6) alkaline phosphatase (ALPL2) and ectonucleotide pyrophosphatase phosphodiesterase (ENPP: 1, 2, 3, 4) in cauda CCs. Moreover, proteomic analysis of epithelial cell apical membranes (by LC/MS-MS) confirmed the expression of these EctoNs, and identified others, which are expressed in adjacent PCs. We showed that PCs secrete ATP, which is hydrolyzed into adenosine. We then focused on the roles of ATP and adenosine in the regulation of V-ATPase in CCs, and their modulation by luminal pH. There was an increase in luminal adenosine and a reduction in ATP levels after perfusion of the cauda *in vivo* at 7.8 *vs* the control pH of 6.6, suggesting an increase in ATP hydrolysis at alkaline pH. Perfusion of the cauda with NT5E and ENTPD inhibitors (AMPCP and POM-1) at pH 7.8 decreased adenosine levels (33 and 47% *vs* Ctr, $p<0.03$), but the ENPP (sc32826) and ALP inhibitor (tetramisole) did not. ENTPD and ENPP inhibitors produced a 3-fold increase in ATP levels *vs* Ctr ($p<0.05$). Immunofluorescence (IF) and cell fractionation followed by Western blot showed that all inhibitors prevented the usual alkaline pH-induced V-ATPase apical membrane accumulation in CCs. Perfusion of the cauda at pH 7.8 stimulated V-ATPase-dependent H^+ secretion, which restored the pH towards the control pH of 6.6. The EctoN inhibitors partially prevented this recovery (Ctr: 7.2 ± 0.05, AMPCP: 7.5 ± 0.03, POM-1: 7.6 ± 0.04, tetramisole: 7.6 ± 0.01, sc-32826: 7.6 ± 0.01, $p<0.01$). Altogether, our study reveals novel mechanisms by which CCs respond to luminal agonists via purinergic regulation, and provides new information for the identification of proteins that might be involved in the pathogenesis of male infertility. Conversely, some expression patterns suggest new targets to exploit in the development of novel methods for male contraception.

Microenvironment homeostatic regulation in the epididymis requires occludin-dependent cell-cell contacts for sperm maturation and male fertility

Da-Yuan Gao[1,2], Bao-Li Zhang[1,2], Bao-Ying Liu[1,2], Jia-Yin Gu[1], Xiao-Bo Xue[1], Qing Li[2], Yu-Chuan Zhou[2], Tegan Smith[3], Sylvie Breton[3], Jin-Song Li[2], Yong-Lian Zhang[2], Winnie Shum[1]*

[1] School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China
[2] State Key Laboratory of Cell Biology, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai 200031, China
[3] Center for Systems Biology, Program in Membrane Biology and Division of Nephrology, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, US

The blood-epididymis barrier is tightly maintained in order to protect the epididymal spermatozoa from the body contents. Tight junctions are the front line of the barrier. Occludin is a necessary component of the tight junction complex; however, its functional mechanism is not clearly elucidated. We found that occludin expression level is the highest in the distal caput and proximal corpus epididymides and that the cellular localization of this protein is not only restricted to the apical tight junctions but also the apical-lateral membranes of epithelial cells. It has been reported that male *occludin* knockout are overall normal but infertile. We found that *Occludin* mutant sperm failed to undergo *in vitro* fertilization but normal pubs were generated using ICSI method. The infertility of *occludin*-mutant males is due to defects in sperm motility and capacitation – the essential functions acquired during sperm maturation in the epididymis – but not spermatogenesis in the testis, which is normal in these mutant mice. We also revealed that the corpus epididymides of *occludin* knockout mice was often swollen, accompanied with extracellular matrix accumulation in the interstitial space. In the cauda epididymides, the tubule showed obvious morphological regression, in which the sperm counts were significantly lower compared to controls and many sperm were morphologically malformed. RNAseq and proteomics analyses revealed that metabolic pathways were the downregulated, whereas inflammation pathways were activated in the epididymis of mutant mice. Our analyses suggested that secretory pathways were disordered, including the V-ATPase-dependent acidification pathway. Further study confirmed that the number of clear cells was significantly decreased in the mutant epididymis. The mRNA of various V-ATPase isoforms and their expression regulator Foxi1 were dramatically downregulated, and the pH of the luminal fluid from vas deferens was significantly increased. Electron microscopic analysis revealed that the apical-lateral cell-cell contacts were obviously increased in the mutant epididymal principal cells compared to controls. Taken together, our results reveal that the male infertility caused by *occludin* deletion is associated with disorders of luminal microenvironment. Our results also reveal that occludin is essential for the proper cell-cell contacts by epididymal cells that maintains the luminal microenvironment homeostasis. Further investigations are underway to elucidate the molecular action of occludin for epididymal barrier integrity for sperm maturation and male fertility.

Nestin marks proliferating smooth muscle cells of the vasculature, but not of the epididymal duct, and is expressed in a hormone-dependent manner

Ansgar N. Reckmann[1], Claudia U. M. Tomczyk[1], Michail S. Davidoff[2], Tatyana V. Michurina[3,4,5], Stefan Arnhold[6], Dieter Müller[1], Andrea Mietens[1], Ralf Middendorff[1]*

[1] Institute of Anatomy and Cell Biology, Justus-Liebig-University Giessen, Giessen, Germany
[2] Institute of Anatomy and Experimental Morphology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[3] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA
[4] Center for Developmental Genetics and Department of Anesthesiology, Stony Brook University, Stony Brook, NY, USA
[5] Moscow Institute of Physics and Technology, Moscow, Russia
[6] Institute of Veterinary Anatomy, Histology and Embryology, Justus-Liebig-University Giessen, Giessen, Germany

Distinct smooth muscle cells (SMCs) of the vasculature that express the neuronal stem cell marker nestin are thought to be involved in remodeling and development. These nestin positive cells were described in various organs including the lung and the testis (Saboor *et al.*, 2016; Davidoff *et al.*, 2004) where they are regulated by hypoxia and testosterone deficiency, respectively. In the epididymis, no information on the occurrence and function of nestin is available.

We investigated epididymal tissues of adult nestin-GFP mice, rats after Leydig cell depletion via ethane dimethane sulfonate (EDS), rats and mice during postnatal development and human tissues. Using Clarity, a new histochemical method to render tissues translucent, allowed to demonstrate nestin-GFP positive cells within the three-dimensional vascular network. Moreover, nestin could be localized to proliferating vascular SMCs in the epididymis by colocalization with both smooth muscle actin and PCNA, and was clearly distinct from CD31-positive endothelial cells. In the human epididymis, the same nestin localization was found. Interestingly, nestin was not expressed in SMCs of the actual epididymal duct. During postnatal development of the mouse and rat, nestin expression was high, but found to be down-regulated towards adulthood when testosterone levels increase. When temporarily ablating Leydig cells (and thus testosterone production) with EDS in a rat model, nestin increases dramatically. Interestingly, the expression of androgen receptor in the epididymis was low under low testosterone conditions and increased until nestin reached normal low levels of adulthood.

Our study shows the expression of the neuronal stem cell marker nestin in the epididymis and localizes nestin exclusively to the vasculature of the organ. Our results provide new insights into the yet underestimated role of proliferating nestin-positive vascular SMCs during postnatal development as well as remodeling and repair of the epididymis.

The epididymis specific gene, Sperm associated antigen 11 (Spag11) as a possible marker for cancer

Sangita[1] and Suresh Yenugu[1*]

[1] Department of Animal Biology, University of Hyderabad, Hyderabad – 500034, India

Inflammation in post-traumatic stress disorder (PTSD): effects on the epididymis and sperm parameters

Geanne A. Freitas[1,2*S], Cristoforo Scavone[2], Maria Christina W. Avellar[1]

[1] Department of Pharmacology, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo-SP, Brazil
[2] Department of Pharmacology, Biomedical Sciences Institute, Universidade de São Paulo-SP, Brazil

Introduction and Aims: Stressfull and traumatic life events result in development of mood disorders, such as depression and anxiety spectrum disorders, which includes post-traumatic stress disorder (PTSD), a debilitating condition that follows trauma exposure. Several studies indicate a relationship between PTSD and its consequences on poorer health outcomes, which include systemically pro-inflammatory state and chronic diseases. How the neurobiological abnormalities in PTSD impact male reproductive function and fertility still remains largely unknown. The behavioral and neuroinflammatory changes that are found in PTSD can be reproduced by social isolation (SI) in mice. Here we evaluated the consequences of this protracted stress model on the epididymis and sperm parameters. **Methods:** Adult Swiss mice (90 days) were kept in groups (GH, control) or individually (socially isolated, SI) for 4 weeks. Total RNA was extracted from isolated pre-frontal cortex, hippocampus and epididymal regions (initial segment, caput, corpus and cauda) and used for RT-qPCR. Cryosections from brain (20 μm) and epididymis (10 μm) were used for immunofluorescence studies (IF) with antibodies against molecular markers for glial cells (microglia, IBA1; astrocyte, GFAP), F4/80-positive epididymal cells (dendritic cells and macrophages) and β-defensin SPAG11C. Positive and negative controls were performed. Image analyses were conducted by fluorescence and confocal microscopy. Testicular and epididymal sperm analysis were also performed. **Results:** IF revealed that both the number and reactivity of astrocytes and microglia were significantly increased in pre-frontal cortex and hippocampus of SI mice, confirming the expected neuroinflammatory profile induced in the central nervous system (CNS) by this stress condition. Its impact on the epididymis was revealed by the significant increase in the number of F4/80-positive cells immunostained along the entire tissue from SI mice when compared to controls. Although testicular sperm count and daily sperm production were similar in GH and SI mice, the stress condition led to an increase in the sperm count (caput/corpus and cauda region) as well as in the sperm transit time in epididymis. The motility of cauda epididymal spermatozoa was also reduced in SI mice, indicating that social isolation impaired both quantitative and qualitative sperm parameters. These changes occurred in the absence of changes in testis and epididymis relative tissue weight. Interestingly, SPAG11C was immunolocalized in most, but not all, microglia and astrocytes in the brain, as well as in F4/80-positive epididymal cells from GH and SI mice, indicating a potential role for this β-defensin in tissue homeostasis and immune responses during inflammatory conditions. **Conclusions:** Collectively our results support the hypothesis that a neuroinflammatory condition such as PTSD may be related to impaired epididymal function and male fertility, due to systemic inflammation. The data also highlight a role for β-defensins in the modulation of immune responses in the CNS and epididymis.

Financial support: CNPq, CAPES, FAPESP (Brazil).
Ethics Research Committee Approval: CEUA UNIFESP-EPM #7991170915/2015.

Immune tolerance in the murine epididymis: potential contribution of Transforming Growth Factor-beta isoforms and their receptors

Allison Voisin[1*S], Christelle Damon-Soubeyrand[1], Chantal Goubely[1], Fabrice Saez[1], Joël Drevet[1], Rachel Guiton[1]

[1] MePTI Team, GReD laboratory, INSERM U1103 - CNRS UMR6293 – Université Clermont Auvergne, Clermont-Ferrand, France

Up to 15% of male infertility has an immunological origin, either due to repetitive infections or to autoimmune responses mainly affecting testis and/or epididymis. Clinical observations and epidemiological data clearly contradict the idea that testis would confer immune protection to the whole male genital tract. Epididymis is key to fertility as it provides a protection for post-testicular spermatozoa against ascending pathogens as well as against the host immune system. Indeed sperm cells are produced at puberty, long after the setting of self-tolerance, and they are composed of unique proteins that cannot be recognized as self. They turn to be potential targets of the immune system, with the risk to induce autoantibodies and finally male infertility. Protection of spermatozoa from the immune system in the epididymis thus relies on the establishment and maintenance of peripheral tolerance. Recently, the Transforming growth factor-beta (TGF-β) signaling has been proven to be crucial for the establishment of epididymal tolerance to spermatozoa in a mouse model. Nevertheless, little is known about the presence of the TGF-β and dedicated receptors isoforms in the murine and human epididymis. Using qRT-PCR, ELISA and immunohistochemistry we found that the three existing TGF-β isoforms and their receptors are present in the murine epididymal epithelial and immune cells with significant differences between the caput, corpus and cauda regions. In parallel, we showed by immunohistochemistry the presence of TGF-β in the human epididymis.

Divergent immunoregulatory gene responses to unilateral or bilateral efferent duct ligation in the murine epididymis

Rukmali Wijayarathna[1*], Rosemary Genovese[1], Andreas Meinhardt[1,3], Barry Hinton[4], Mark Hedger[1,2]

[1] Hudson Institute of Medical Research, Clayton, Victoria, Australia
[2] Department of Molecular and Clinical Sciences, School of Clinical Sciences, Monash University, Clayton, Victoria, Australia
[3] Institute of Anatomy and Cell Biology, Justus-Liebig University, Giessen, Germany
[4] School of Medicine, University of Virginia, Charlottesville, Virginia, USA

The murine caput epididymis (segments 1-5) is immunologically unique, characterized by a complex intraepithelial immune cell network, and elevated immunoregulatory genes, such as activin A (*Inhba*) and B (*Inhbb*), and indolamine 2,3 dioxygenase-1 (*Ido1*). The role of testicular lumicrine factors in regulating caput epididymal immunoregulation was investigated using efferent duct ligation (EDL).

A group of 10-week-old C57/Bl6 mice underwent unilateral EDL. Efferent ducts were exposed, but not ligated, on the other side. Another group of mice had bilateral EDL surgery. Tissues were collected 7 days post-surgically.

Both unilateral and bilateral EDL caused pressure-induced testicular damage on the ligated side. Bilateral, but not unilateral EDL, resulted in increased testis weight and reduced epididymal weight. Unilateral and bilateral EDL caused reduced epithelial cell height and cell loss in the initial segment. The cauda contained fewer sperm, and many round cells, dying epithelial cells and eosinophilic, proteinaceous bodies.

Inhba and *Inhbb* were upregulated in the caput of bilateral EDL animals (2 and 6-fold, respectively), but not in unilateral EDL. *Ido1* showed a 60-fold upregulation in the bilateral EDL cohort. *Il1*β, *Il6*, *Il10* and *Tnf* expression analysis showed that *Ido1* upregulation was not associated with inflammation. Activin A protein was reduced in the epithelium, but was increased in the interstitium, in unilateral EDL.

Caput epididymal expression of *Inhba*, *Inhbb* and *Ido1* does not appear to be dependent upon testicular lumicrine factors. However, the evidence suggests that the testis influences these genes in the epididymis via a systemic, possibly endocrine or immunological, mechanism.

Region-specific innate antiviral responses of the human epididymis

James A. Browne[1]*, Shih-Hsing Leir[1], Scott Eggener[2], Ann Harris[1]

[1] Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH, USA.
[2] Section of Urology, University of Chicago Medical Center, Chicago, IL, USA.

Viral infections of the epididymis are associated with epididymitis, which damages the epithelium and impairs fertility. We showed previously that innate immune response genes were differentially expressed in the corpus and cauda region of the human epididymis in comparison to the caput. We investigate the antiviral defense response mechanisms of human epididymis epithelial (HEE) cells. Toll-like receptor (TLR) 3 and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) are enriched in HEE cells from the corpus and cauda region. These HEE cells show an enhanced response to antiviral ligands (poly(I:C) and HSV-60), as shown by increased IFN-β mRNA expression and IFN-β secretion. Nuclear translocation of phosphorylated p65 occurs after poly(I:C) exposure. In addition, paired box 2 (PAX2), which was implicated in regulating antiviral response pathways, is required for basal expression of the DNA sensor, Z-DNA binding protein (ZBP1) and type I interferon, in caput but not in cauda cells.

Regulation and functional aspects of innate immunity components during epididymal morphogenesis

Lucas G.A. Ferreira[1*S], Camilla M. Ribeiro[1], Barry T. Hinton[2], Maria Christina W. Avellar[1]

[1] Department of Pharmacology, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, Brazil
[2] Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, Virginia, USA

Introduction and Aims: Several components of host defense are expressed in the epithelium of postnatal and adult epididymis. Among them are the β-defensins, a family of multifunctional proteins with antimicrobial and immunomodulatory properties, which also have a role in sperm function and male fertility. We have previously detected a subset of β-defensin genes located within a gene cluster on chromosome 16 that is differentially expressed at mRNA level in the developing rat Wolffian duct (WD), the embryonic precursor of the epididymis. Next, we showed that one of these β-defensins, named sperm associated antigen 11 C (SPAG11C), was detected at both mRNA and protein levels between embryonic days (e) 12.5-20.5 and identified as an androgen-regulated mesenchymal factor with a role in epididymal morphogenesis. Transcripts for another β-defensin of this chromosome cluster, β-defensin 2 (*Defb2*), was only detected in WDs from e20.5 fetuses. Thus, the present study was designed to unveil the androgen regulation and potential role of DEFB2 in WD morphogenesis. Among other mechanisms, β-defensins can exert their functions by modulating Toll-like receptor (TLR) and chemokine receptor signaling pathways. For this reason, the expression levels of *Tlr4* and CC chemokine receptor 6 (*Ccr6*) mRNA were also evaluated in the developing WD. **Methods:** WDs were isolated from male Wistar rat fetuses at e17.5 and e20.5 and used for total RNA extraction. Expression levels of *Defb2*, *Tlr4* and *Ccr6* mRNA were assayed by RT-qPCR using ribosomal protein L19 (*Rpl19*) as reference gene. Androgen-dependency studies were performed using WD (e17.5) organotypic culture kept for up to 96 h in the absence (basal medium) or presence of testosterone (10 nM) and OH-flutamide (10 μM; androgen receptor antagonist). Tissues were collected at 72 h and used for RT-qPCR. To determine a functional response of the DEFB2 on WD morphogenesis, the effect of recombinant human DEFB2 (hDEFB2, 3 nM) was also studied on WD organotypic culture. Washout experiments were conducted to remove hDEFB2 after 48 h of culture. Gross morphology of cultured WDs was evaluated. **Results:** Between e17.5 and e20.5, the period when fetal plasma testosterone raises and WD morphological differentiation occurs in the rat, an increase in *Defb2* and *Tlr4* and a decrease in *Ccr6* mRNA levels were detected, indicating that these innate immunity components are expressed and differently regulated during WD development. The influence of androgens on *Defb2* mRNA expression was confirmed, since testosterone/androgen receptor signaling sustained the expression levels of *Defb2* mRNA in 72 h cultured WDs, an effect prevented by testosterone/OH-flutamide co-incubation. hDEFB2-treated WDs were shorter in length after 72 h and 96 h of culture when compared to the control ducts. These effects were rescued following washout of hDEFB2 and change to normal culture medium, showing the specificity of the recombinant protein-induced effects. **Conclusion:** The present data shed light in the role of innate immunity components during epididymal morphogenesis and imply a role for DEFB2 in the androgen-induced morphological differentiation of WD.

Funding support: FAPESP, CNPq and CAPES (Brazil).
Ethics approval: CEUA-Unifesp-EPM#1776201213.

Lipopolysaccharide-induced acute epididymitis alters the functional alpha-1 adrenoceptor subtype in the rat distal cauda epididymis

Andre Mueller[1,2], Alan Andrew S Silva[1], André S Pupo[1], Erick J R Silva[1]*

[1] São Paulo State University (Unesp), Institute of Biosciences, Department of Pharmacology, Botucatu, São Paulo, Brazil
[2] Federal University of Mato Grosso, Institute of Health Sciences, Sinop, Mato Grosso, Brazil

Introduction: The cauda epididymis (CE) is densely innervated by sympathetic postganglionic fibers, which release noradrenaline (NA) as a neurotransmitter to contract the epididymal smooth muscle layer through activation of alpha-1A adrenoceptors (alpha1A-ARs). This sympathetic innervation is important for sperm transport along the cauda epididymis and sperm emission during the seminal emission phase of ejaculation. By using an experimental model of epididymitis in rats, we have previously reported that this inflammatory condition induced an acceleration of sperm transit time in the epididymis. We hypothesize that epididymitis affects the contractility of the CE smooth muscle via changes in alpha1A-ARs responses, thus contributing to epididymal sperm transit time increase. Therefore, our aim was to investigate the alpha1-ARs in the contractility of CE smooth muscle in an experimental model of acute epididymitis induced by the injection of lipopolysaccharide (LPS) from *E. coli* into the lumen of the vas deferens. **Methods:** adult male Wistar rats (90-110 days-old) were anesthetized with ketamine:xilazine. Epididymitis was induced by the retrograde injection of ultrapure LPS from *E. coli* O55:B55 (25 µg) into the lumen of the vas deferens. Sham-operated rats underwent a similar procedure, but were treated with sterile saline only (control). Rats were killed 6 h after treatment; their distal CE ducts were isolated, uncoiled and cleaned of adherent tissues and intraluminal content. CE ducts were mounted in organ baths (30°C, 95%O_2/5%CO_2). Cumulative concentration-response curves (CRCs) to NA, buspirone or oxymetazoline were obtained in the absence and presence of prazosin and BMY7378 (non-selective alpha1- and selective alpha1D-ARs antagonists, respectively). The potencies of agonists (pD_2) and antagonists (pA_2) were calculated. In addition, segments of distal CE duct from untreated rats were incubated in vitro in sterile nutrient solution containing or not 300 ng/ml LPS for 4h, followed by contraction studies. **Results:** LPS-induced epididymitis increased the potency of CE duct contraction to NA in comparison to control group (6-fold increase). The NA-induced contractions of the CE duct were competitively antagonized by prazosin with similar affinities in LPS-treated and control groups ($pA_2 \cong 8.92$). However, the affinity of BMY7378 in the CE duct from LPS-treated rats was >50-fold higher than in control, with a Schild slope less than unity thus suggesting that the contractile responses of the CE duct are mediated by more than one alpha1-AR subtype under inflammatory conditions. The selective alpha1D agonist buspirone was ~3-fold more potent in the LPS-treated group and its CRCs were right-shifted by BMY7378, displaying an affinity consistent with alpha1D receptor ($p \cong_2$ 8.67). Supporting this data, after in vitro LPS exposure the response of the CE duct to NA was counteracted by BMY7378 ($pA_2 \cong 8.50$ LPS); and oxymetazoline (alpha1A partial agonist) was ~6-fold less potent in comparison to control. **Conclusion:** LPS-induced epididymitis changes the functional alpha1-AR subtype in the CE smooth muscle, resulting in a heterogeneous receptor population (alpha1A- and alpha1D-ARs) involved in the CE contractile response during acute inflammation. Our results showed that inflammation induced changes in sympathetic post-ganglionic neurotransmission in the epididymis that may affect sperm transit in this organ and contribute to the impairment of male fertility.

Financial Support: Fapesp (08/50423-7 and 2015/08227-0).
Ethics Committee approval: 749-CEUA.

Mechanistic insights into epididymosome-sperm interactions

Wei Zhou[1*S], Simone J. Stanger[1], Amanda L. Anderson[1], Geoffry N. De Iuliis[1], Adam McCluskey[2], Eileen A. McLaughlin[1,3], Matthew D. Dun[4,5], Brett Nixon[1]

[1] Priority Research Centre for Reproductive Science, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia
[2] Priority Research Centre for Chemical Biology, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia
[3] School of Biological Sciences, University of Auckland, Auckland 1142, New Zealand
[4] School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, The University of Newcastle, Callaghan, NSW 2308, Australia
[5] Hunter Medical Research Institute, Cancer Research Program, New Lambton Heights, NSW 2305, Australia

The mammalian epididymis is responsible for the provision of a highly specialized environment in which spermatozoa acquire functional maturity and are subsequently stored in preparation for ejaculation. Making important contributions to both processes are epididymosomes, small extracellular vesicles released from the epididymal soma via an apocrine secretory pathway. While considerable effort has been focused on defining the cargo transferred between epididymosomes and spermatozoa, comparatively less is known about the mechanistic basis of these interactions. To investigate this phenomenon, we have utilized an *in vitro* co-culture system to track the transfer of biotinylated protein cargo between mouse epididymosomes and recipient spermatozoa. Our data indicate that epididymosome-sperm interactions are initiated via tethering of the epididymosome to receptors restricted to the post-acrosomal domain of the sperm head. Thereafter, epididymosomes mediate the transfer of protein cargo to spermatozoa via a process that is dependent on dynamin, a family of mechanoenzymes that direct intercellular vesicle trafficking. Notably, upon co-culture of sperm with epididymosomes, dynamin 1 undergoes a pronounced relocation between the peri- and post-acrosomal domains of the sperm head. This repositioning of dynamin 1 is potentially mediated via its association with membrane rafts and ideally positions the enzyme to facilitate the uptake of epididymosome-borne proteins. Accordingly, disruption of membrane raft integrity or pharmacological inhibition of dynamin both potently suppress the transfer of biotinylated epididymosome proteins to spermatozoa. Together, these data provide new mechanistic insight into epididymosome-sperm interactions with potential implications extending to the manipulation of sperm maturation for the purpose of fertility regulation.

Presence of aberrant epididymal tubules revealing undifferentiated epithelial cells and absence of sperm in a combined Neuraminidase 3 and 4 deficient mouse model

Louis Hermo[1]*, Regiana Oliveira[1], Alexey V. Pshezhetsky[2], Carlos R. Morales[1]

[1] Department of Anatomy and Cell Biology, McGill University, Montréal, Quebec, Canada
[2] Division of Medical Genetics, Centre Hospitalier Universitaire Sainte-Justine, University of Montréal, Montréal, Quebec, Canada

Mammalian neuraminidases consist of a family of four enzymes (Neu1-4) responsible for the removal of sialic acids from glycoproteins and glycolipids. Plasma membrane- and intracellular-associated neuraminidases (Neu3 and Neu4) participate in many biological processes such as control of cell differentiation and growth. This study details a morphological analysis of the structure of the epididymis of 3-month-old *neu3*$^{-/-}$ *neu4*$^{-/-}$ mice as visualized with the light and electron microscope. While lysosomal abnormalities were evident in epithelial cells of the epididymis, of particular note was the finding of aberrant epididymal tubules (ATs) juxtaposed against or alongside the tubules of the larger main epididymal duct. Such ATs were prominent especially in the caput and cauda regions of the epididymis. ATs were of different sizes and shapes but much smaller than the tubules of the main duct. At times ATs impinged on tubules of the main duct affecting the shape and configuration of their epithelial cells at that site, reducing them to a low cuboidal appearance. More than one AT could be encountered in a given cross sectional profile of a tubule of the main duct. ATs were encased by myoid cells, which they shared with those of the main duct where the two overlapped with each other. However, a distinct intertubular space did not exist between tubules of the main duct and the ATs.While some cross sectional profiles of ATs appeared as a dense mass of cells, others displayed a distinct lumen. The former were composed of a congealed mass of undifferentiated cells as compared to the epithelial cells forming tubules of the main duct. However, while the cells forming the congealed mass demonstrated ER cisternae and a prominent Golgi apparatus, they did not envelop a central lumen. Basal cells were noted at the base of these congealed masses. In the case of the ATs displaying a lumen, the undifferentiated epithelium bordering the lumen consisted of low cuboidal cells and occasional basal cells. Junctional complexes were noted apically between the adjacent cuboidal epithelial cells. The lumen of these ATs did not contain sperm, but was at times was filled with the microvilli of the lining epithelial cells. In the cauda region, ATs often ran parallel to tubules of the main duct for a considerable distance and were seen as a congealed mass of cells that further along their length gave rise to ATs with a distinct lumen. At times ATs appeared to have fused as evidenced by the coalition of their lumen. Despite the presence of the ATs, tubules of the main duct appeared intact showing differentiated epithelial cells and a lumen containing sperm; *neu3*$^{-/-}$ *neu4*$^{-/-}$ mice were fertile. The finding of ATs in the epididymis of *neu3*$^{-/-}$ *neu4*$^{-/-}$ mice suggests that offshoots of the epithelial cells forming the main duct appear during early embryonic development but which are incapable of remaining connected to it. This is suggested by the fact that the ATs do not reveal sperm in their lumen and by their undifferentiated appearance due to the absence of lumicrine factors derived from a testicular source. Hence the removal of sialic acids appears to play an essential role in the proper development of tubules of the main epididymal duct and which in the absence of Neu3 and Neu4 leads to formation of ATs.

Supported by CIHR and NSERC.

Both aging and SOD1 null mutation cause segment-specific patterns of oxidative stress-induced damage along the mouse epididymis

Alicia Klaassen[1S], Anaïs Noblanc[1*], Bernard Robaire[1,2]

Department of Pharmacology and Therapeutics ([1]) and of Obstetrics and Gynecology ([2]), Centre for Research in Reproduction and Development, McGill University, Montreal, Quebec, Canada

There is growing evidence that the quality of sperm decreases with age and that the children of older fathers have a higher incidence of birth defects and genetic mutations. The free radical theory of aging postulates that the accumulation of damage enacted by excess reactive oxygen species (ROS) is the primary mechanism to account for age-dependent reproductive system damage. This theory links dysregulation of the antioxidant defense system to damage seen during aging. A previous study from our laboratory (Biol Reprod. 95(3):60, 2016) demonstrated the effects of the absence of superoxide dismutase 1 (SOD1), an enzyme involved in removing ROS, on several key endpoints of the male reproductive system, including an abnormal phenotype in the aged SOD1$^{-/-}$ mouse epididymis. In this study, we determine the impact of SOD1 null mutation on the histopathology of epididymides of young (4-months-old) and old (18-months-old) mice and assess whether this null mutation results in tissue morphology modifications and in an altered pattern of oxidative damage to nucleic acids and lipids along the epididymis. In wild-type mice, aging induces an increase in tubule diameter along the entire epididymis with an accumulation of more spermatozoa in the lumen, even in the initial segment where they are normally not or rarely observed in young mice. We also noticed the appearance of round cells in the lumen of older mice, throughout the epididymidis. In the corpus epididymidis of older mice, clusters of small vacuoles are seen in some epithelial cells and, in the cauda epididymidis, the myoid cell layer is thicker than that in young wild-type mice. The absence of SOD1 in the mouse epididymis exacerbates the phenotype of accumulation of spermatozoa and round cells in the lumen, as well as the thickening of the smooth muscle layer in the cauda epididymidis of young SOD1$^{-/-}$ mice when compared to old wild-type mice. However, these features are not worsened by aging in old SOD1$^{-/-}$. Only the epididymal epithelium height is decreased in old SOD1$^{-/-}$ mice compared to young SOD1$^{-/-}$ mice and old wild-type mice. The presence of vacuoles in some epithelial cells of the corpus epididymidis is not visible is young SOD1$^{-/-}$ mice and not exacerbated in old SOD1$^{-/-}$ mice compared to old wild-type mice. Both oxidative nucleic acid damage (*via* 8-hydroxyguanosine staining) and lipid peroxidation (*via* 4-hydroxynonenal staining) increased with age. The epididymides of young SOD1$^{-/-}$ mice showed similar markers of oxidative stress as those seen in wild type, aged epididymides, and the epididymides of aged SOD1$^{-/-}$ demonstrated a more accentuated response, thus indicating an exacerbation of an oxidative damage accumulation-related aging phenotype in SOD1 deficient mice. During aging as well as in the SOD1 null mutant, the nucleic acid oxidation increased progressively along the epididymal tubule, while the 4-HNE staining is absent in the caput epididymidis, is predominant in the epithelium of the corpus epididymidis, and rarely observed in the cauda epididymidis. These results indicate the importance of the SOD1 in preventing oxidative stress-induced damage and points to the need for future studies into the mechanism of ROS-related tissue dysfunction to better understand and treat the effects of aging on male fertility.

Supported by a team grant from the Canadian Institutes for Health Research.

Mouse Quiescin Sulfhydryl Oxidases Exhibit Distinct Epididymal Luminal Distribution With Segment-Specific Sperm Surface Associations

Tse-En Wang[1,2*S], Sheng-Hsiang Li[4], Brett Nixon[5], Pei-Shiue Jason Tsai[1,2,3]

[1] Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan
[2] Graduate Institute of Veterinary Medicine, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan
[3] Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, Taipei, Taiwan
[4] Department of Medical Research, Mackay Memorial Hospital, Tamshui, Taiwan
[5] Priority Research Centre for Reproduction, School of Environmental and Life Sciences, Discipline of Biological Sciences, University of Newcastle, Callaghan, New South Wales, Australia

Sulfhydryl oxidation is part of the sperm maturation process essential for the acquisition of sperm fertilization competency and its structural stabilization; specific sulfhydryl oxidases that fulfil these roles have yet to be identified. Here we investigate the involvement of one atypical thiol oxidase family called quiescin Q6/sulfhydryl oxidase (QSOX). We show that QSOX isoform 1 and 2 exhibit complementary distribution throughout the epididymal duct, but that each variant possesses distinct sub-cellular localization within the epididymal principal cells. While QSOX2 was exclusively present in the Golgi apparatus of the proximal epididymis, QSOX1c, the most profusely express QSOX1 variant was abundantly present in the cauda luminal fluids. Immunohistochemistry studies together with proteomic identification in isolated epididymosomes supported the release of QSOX2, but not QSOX1c, via an apocrine secretory pathway. Furthermore, we demonstrate for the first time, distinct association of QSOX1c and QSOX2 with the sperm acrosome and implantation fossa, during different stages of their epididymal maturation. In conclusion, our study provides the first comprehensive comparisons between QSOX1 and QSOX2 in the mouse epididymis, revealing their distinct epididymal distribution, cellular localization, mechanisms of secretion and sperm membrane association. Together, these data suggest that QSOX1 and QSOX2 have discrete biological functions in male germ cell development.

Role of Snail 1 (SNAI1) in the Regulation of Claudin1 in the Rat Epididymis

Julie Dufresne[1]*, Mary Gregory[1], Evemie Dubé[1], Daniel G. Cyr[1]

[1] Laboratory for Reproductive Toxicology. INRS-Institut Armand-Frappier, University of Quebec, Laval, QC, Canada

We have previously shown that epididymal tight junctions are altered in azoospermic men and that the decreased expression of a single claudin (CLDN) is sufficient to compromise these tight junctions, thus highlighting the need to understand the regulation of epididymal CLDNs. Furthermore, studies have reported that the expression of *Cldn1* occurs at higher levels in the basal cells of the epididymis. SNAIL1 (SNAI1), a transcription factor, binds to E-box motif of the *Cadherin1* (*CDH1*; E-Cadherin) and CLDN1 promoter to down-regulate their expression in certain cell types. There are two E-box response elements in mouse and human *CLDN1* promoters, but only one in rat. The present objectives were to determine the presence of SNAI1 in rat epididymis and whether or not SNAI1 regulates epididymal CLDN1. *SNAI1* was expressed in all regions of adult rat epididymis and immunofluorescence indicates that SNAI1 is primarily in the nucleus, as well as in the cytoplasm, of principal cells but was not detected in basal cells. *Snai1* mRNA levels were high in young animals until postnatal day 21, and decreased thereafter. Cells of the rat principal cell line, RCE1, which express CLDN1 were transiently transfected with a murine SNAI1 expression vector. Transfected cells expressed significantly lower *Cldn1* mRNA levels than untransfected cells. Co-transfection of cells with the SNAI1 expression vector and a luciferase construction containing the CLDN1 promoter resulted in decreased transactivation of the reporter gene. Tight junctions are formed in cells from human cell lines developed from fertile patients. In contrast, cells from cell lines derived from infertile obstructive azoospermic patients, do not form tight junctions. Comparison of these cell lines indicated that *Snai1* is expressed at higher levels in cells from infertile patients. To determine the role of the second E-Box response elements on the human CLDN1 promoter, a second E-box response element was inserted into the rat CLDN1 promoter. The modified *CLDN1* promoter construct resulted in lower basal transactivation levels, and addition of exogenous SNAI1 increased the repression of *CLDN1* transactivation. Thus SNAI1 can act as a down-regulator of CLDN1 in both the rat and human epididymal cells and this effect is mediated primarily via the distal E-Box response element. The expression of SNAI1 in principal cells may explain the lower expression levels of CLDN1 in principal cells.

Supported by CIHR.

The protein cargo of mouse epididymosomes

Geoffry N. De Iuliis[1,3]*, Hanah Hart[1], Matthew D. Dun[2,3], Brett Nixon[1,3]

[1] Priority Research Centre for Reproductive Science, School of Environmental and Life Sciences, Faculty of Science, The University of Newcastle, Callaghan, NSW, Australia
[2] School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, The University of Newcastle, Callaghan, NSW, Australia
[3] Hunter Medical Research Institute, New Lambton Heights, NSW, Australia

The full functional potential for the spermatozoon is acquired during epididymal transit, and remarkably this feat is achieved in the complete absence of nuclear gene transcription or *de novo* protein translation. Instead, we now understand that epididymal maturation is driven by the complex external milieu in which sperm are bathed within lumen of the epididymal tubule. A key element of this dynamic microenvironment are epididymosomes, small membrane encapsulated vesicles that are secreted from the epididymal soma. These vessels have been implicated in the direct transfer of biomolecular cargo from the regulatory epithelial cells lining the epididymal lumen, to the maturing gamete. Here, we report comparative proteomic profiling of epididymosomes isolated from different segments of the mouse epididymis using isobaric-tagged based quantification, coupled with high resolution LC-MS/MS. A total of 1700 epididymosome proteins were identified and quantified via this proteomic method, including 350 proteins encapsulated within the vesicle, 550 proteins uniquely identified in membrane-enriched fractions, and almost 800 proteins that were represented in both fractions. Notably, the soluble epididymosome cargo presented more pronounced segment to segment comparative differences than that of the membrane-enriched fractions. Exploring the gene ontology of the epididymosome proteomic cargo revealed a substantial portion of proteins mapping to the cellular component of 'extracellular exosome' and to the biological processes of 'transport', 'oxidation-reduction', 'translation', and 'metabolism'. Such findings take on added significance in view of our demonstration that epididymosomes are able to convey at least a portion of this protein cargo to both the head and mid-piece of maturing spermatozoa.

Maternal low-protein diet exerts effects on serum aldosterone levels and VEGFR-2, AQP1 and AQP9 expression in epididymis of rat offspring

Marilia Martins Cavariani[1*S], Talita de Mello Santos[1], Dhrielly Natália Pereira[1], Patrícia Fernanda Felipe Pinheiro[1], Luiz Gustavo de Almeida Chuffa[1], Raquel Fantin Domeniconi[1]

[1] Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

During pregnancy, adequate protein intake is recommended to ensure the additional nitrogen demand required by both mother and fetus. At this stage there is an increase in protein turnover to meet the requirements for rapid embryo growth, making the protein restriction model one of the most characterized early growth restriction models studied until today. It has already been observed that maternal protein restriction caused sperm alterations on male offspring associated with epididymal functions, such as sperm motility, viability and concentration. However, the causes of these alterations have not yet been elucidated and there is no information in the literature that explains how the protein restriction at an early stage of development affects epididymal function. Several studies have demonstrated the influence of aldosterone on the fluid absorption from epididymal lumen in addition to its modulation over vascular endothelial growth factor (VEGF), thus being able to influence the epididymis vascular pattern. Aquaporins (AQP) are transmembrane proteins involved in the fluid reabsorption/secretion dynamics in the epididymal intraluminal environment, playing an important role in the process of sperm maturation and concentration. Thus, the aim of this study was to verify the effects of maternal protein restriction on offspring aldosterone serum levels, VEGF and VEGF receptor (VEGFR-2) expression as well as on AQP1 and AQP9 expression in different stages of postnatal epididymal development. For this purpose pregnant females *Wistar* rats were distributed in normoproteic (NP) and low-proteic (LP) groups and fed *ad libitum* during gestation and lactation with normoproteic and low-proteic diets, respectively. After weaning, the male rats received standard diet until the ages of 21, 44 and 120 days when they were euthanized and had their blood and epididymis collected to serum aldosterone assay and VEGF, VEGFR-2, AQP1 and AQP9 expression evaluation, respectively. It was observed that although maternal protein restriction during pregnancy and lactation has increased aldosterone serum levels only in the 21-day LP animals, the expression of epididymal VEGF was not altered in any of analyzed ages. However, it was observed a decrease in the expression of this growth factor receptor (VEGFR-2) in the epididymis caput of LP animals on postnatal day (PND) 21. Maternal protein restriction caused a decrease in AQP1 and AQP9 expression on epididymal caput and increased the expression of these proteins in corpus and cauda epididymis of male offspring at all analyzed ages, despite these alterations have been significant only for LP animals in the PND 44. It was concluded that maternal protein restriction permanently changed the structure or functioning of offspring epididymis, since altered aldosterone serum levels of male pups and the expression of VEGFR-2, AQP1 and AQP9 in important periods of epididymis postnatal development.

Financial Support: FAPESP

Maternal protein restriction during gestation and lactation causes male estrogenization altering the epididymis development in rat offspring

Talita de Mello Santos[1*S], Marilia Martins Cavariani[1], Patrícia Fernanda Felipe Pinheiro[1], Luiz Gustavo de Almeida Chuffa[1], Raquel Fantin Domeniconi[1]

[1] Institute of Biosciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

Gestation and lactation are important periods of organism development. During these periods some conditions, as nutrition deficiency, could affect maternal homeostasis and change the development of some systems leading to short/long-term effects on the offspring. Previous studies have shown that low protein in maternal diet, during intrauterine period, alters both spermatozoa morphology and motility in male rat offspring, impairing these animals fertility at adult age. During spermatic transit through epididymis, a complex process called sperm maturation allows spermatozoa conversion into motile and fertilization-competent cells. The impairment of this important epididymal function could be a consequence of an abnormal epididymal development. Sex hormones and growth factors act as regulators of this development process. Androgens and estrogens are fundamental for the maintenance of epididymal structure and morphology of its cells. The androgen gets to epididymis by testicular fluid and blood flow. Estrogen biosynthesis occurs through testosterone conversion by P450 aromatase enzyme on epididymal luminal space and epithelium. Wnt9b is one of the proteins of Wnt pathway that is wide expressed on the epithelium of developing epididymis. A failure in Wnt signaling may be directly related to inadequate differentiation of epididymal tissue and spermatozoa maturation. Little is known about epididymis developmental processes and how congenital defects could be involved with male infertility, so it is important to understand the mechanisms that regulate this organ development. The aim of this study was to investigate the effects of maternal protein restriction on male offspring epididymal development of *Wistar* rats. Pregnant female *Wistar* rats were divided into two groups that received either normoprotein (17%; NP) or low-protein (6%; LP) diet *ad libitum* during perinatal phase. At postnatal days (PND) 7 and 14 male pups were euthanized and the blood was collected for testosterone and estradiol serum levels analyzes. The epididymis was collected to verify androgen receptor (AR), estrogen receptor-α (ER-α), estrogen receptor-β (ER-β) and Wnt9b protein levels by Western Blotting method. Testosterone and estradiol serum levels as well as ER-α expression increased in the animals whose mothers received low-protein diet at both ages. At PND 7 and 14 the semi-quantitative analyzes showed a decrease in the AR and Wnt9b protein levels on LP animals. The only contrasting result between the two ages was for ER-β in the epididymis of restrict animals which was higher at PND 7 and lower at PND 14. The conclusion is that maternal protein restriction during gestational and lactacional periods causes an estrogenization in the epididymis development altering the patterns of sex hormones receptors and Wnt9b signalization.

Financial Support: Fapesp/Capes

Male Contraceptive Initiative (MCI) Strategy, Research, and Grant Activities, 2017-18

Logan Nickels[1]*, Mitch Eddy[1], David Sokal[1]

[1] Male Contraceptive Initiative, Raleigh, NC, USA

Introduction: Globally, nearly half of all pregnancies are unplanned. This persistent occurrence of unplanned pregnancies indicates that contraceptive needs are still not being met. One of the largest gaps in contraceptive need is in novel options for men. Men have only two modern contraceptive options available, and no reversible method that is as safe and reliable as The Pill. Further, they have no long-acting reversible contraceptive options.

Objective: MCI's objective is to facilitate research and development of novel, reversible male contraceptives for people around the world and to build awareness of the status of novel male contraceptive methods. In 2014, we founded a nonprofit organization to pursue this objective: the Male Contraceptive Initiative (MCI), a 501(c)3 nonprofit, at MaleContraceptive.org.

Methods: MCI is using multiple approaches to meet our objectives:

1. Networking with and funding researchers, inside and outside the US, to help advance research and providing connections and access to field experts and resources.
2. Promoting awareness, educating the public through media, advocacy and education.
3. Conducting and disseminating research to answer questions regarding the feasibility and acceptability of novel safe and reversible male contraceptives.
4. Informing policy by networking and advocating with governmental and non-governmental agencies.

Results:

1. We are developing funding programs for institutions and startups, as well as support for young researchers such as travel grants, and fellowships.
2. We have published research that models the estimated potential impact of a male Pill and long-acting reversible contraceptive for men (e.g. reversible vasectomy) on unintended pregnancies in three countries.
3. In 2017, we issued a request for proposals and subsequently awarded a three-year, $500,000 grant to Vibliome Therapeutics to extend their work on a novel kinase inhibitor.
4. In 2018, we awarded 4 two-year, $150,000 "Seed Grants" designed to promote early research studies to identify novel male contraceptives.
5. In 2018, we piloted a student fellowship program and are offering travel grants to young researchers to attend relevant professional conferences around the world.
6. We have been featured in prominent media outlets such as NPR, Bloomberg, Scientific American, and The Wall Street Journawl.

Conclusion: We are making significant progress in the facilitation of research and development of novel, reversible male contraceptives for people around the world and the building of awareness of the status of novel male contraceptive methods.

Suppression of Mouse Sperm Motility by Contraceptive anti-EPPIN S21C Antibody

Alan Andrew S. Silva[1], Noemia A.P. Mariani[1], Tamiris R.F. Raimundo[1], Helio Kushima[1], Erick J.R. Silva[1]*

[1] Department of Pharmacology, Institute of Biosciences, São Paulo State University, Botucatu, SP, Brazil

Introduction: New male contraceptive methods will expand contraceptive options for men, thus facilitating family planning and health. The protein EPPIN (Epididymal Protease Inhibitor) is a promising target for male contraception due to its selective expression in the testis and epididymis, critical role in sperm function and druggable properties. In men, EPPIN is present on the surface of spermatozoa, where it inhibits sperm motility acquisition upon ejaculation by binding to the seminal vesicle-secreted protein semenogelin-1 (SEMG1). Studies demonstrated that anti-EPPIN S21C antibody, raised against C-terminal epitope Ser_{103}-Cys_{123} of human EPPIN, can mimic SEMG1-EPPIN binding on the sperm surface, thus inhibiting the motility of human spermatozoa. The development of a mouse model to study EPPIN's functions will facilitate its development as a contraceptive target, as well as the safety and efficacy evaluation of potential EPPIN-binding drugs. Although EPPIN is immunolocalized on the surface of mouse epididymal spermatozoa, its role on mouse sperm function is elusive. To gain insights into the physiological roles of EPPIN in mice, we evaluated the effects of the contraceptive anti-EPPIN S21C antibody on mouse sperm motility in vitro. **Methods:** Adult (90-110 days old) male C57BL/6 mice (n=3) were euthanized and their cauda epididymides were dissected, placed in HTF medium supplemented with 0.75% BSA, and cut with scissors to release sperm into the medium. After 10 min of incubation (37°C, 5%/95% CO_2/air), the sperm suspension (2.5 x 10^5 spermatozoa/ml final concentration) was incubated in HTF medium containing 0.4 mg/ml pre-immune serum (PIS, control) or anti-EPPIN S21C antibody, which cross-reacts with mouse EPPIN. We assessed sperm motility after 30, 60, 90 and 120 min of incubation by computer-assisted sperm analysis (CASA) using a CEROS II system at 37°C with a frame rate acquisition of 60 Hz for 1.5 s. Sperm tracks were classified as motile, progressive, and static. We evaluated the following sperm kinematic parameters: average path velocity (VAP; µm/s), straight line velocity (VSL; µm/s), curvilinear velocity (VCL; µm/s), lateral head displacement (AHL; µm), straightness (STR; %), and linearity (LIN; %). Data were analyzed by Student's t-test; $p<0.05$ was considered significant. **Results:** S21C antibody reduced sperm motility and progressive motility in a time-dependent manner in comparison to control, reaching significance at 60 min. S21C antibody significantly decreased sperm kinematic parameters that described progressive (VAP) and vigour (VCL and ALH) movements at all time points analyzed in comparison to PIS control. In addition, S21C antibody reduced progression parameter VSL at 30 and 60 min, and increased STR at 120 min. **Conclusion:** Our results support the hypothesis that EPPIN's function as a modulator of sperm motility is conserved between mice and humans, highlighting its crucial role in male fertility. The mouse is a suitable experimental model for translational studies on EPPIN's functions and mechanism of action. Overall, our study provides novel insights into the evolution of EPPIN and its physiological role in the male reproductive tract.

Support: Fapesp (2015/08227-0).
Local Ethics committee approval: 703-CEUA.

Optimization of seminal analysis by flow cytometry and SP22 immunostaining, a putative human fertility biomarker

Josiane de Lima Rosa[1]*, Camila de Paula Freitas Dell'Aqua[2], Carolina Mendonça Gorgulho[3], Thamiris Moreira Figueiredo[1], Jorge Willian Franco de Barros[1], Ramão Souza de Deus Júnior[1], Gabriela Missassi[1], Ramon Kaneno[3], Gary Klinefelter[4], Wilma De Grava Kempinas[1]

[1] Laboratório de Biologia e Toxicologia da Reprodução e do Desenvolvimento, Instituto de Biociências, Departamento de Morfologia; [2] Faculdade de Medicina Veterinária e Zootecnia, Departamento de Reprodução Animal e Radiologia Veterinária; [3] Instituto de Biociências, Departamento de Microbiologia e Imunologia; Universidade Estadual Paulista - UNESP, Botucatu, São Paulo, Brazil.
[4] U.S. Environmental Protection Agency, Office of Research and Development, Reproductive Toxicology Division, Research Triangle Park, North Carolina, USA.

Introduction: Several sperm and seminal plasma proteins have been associated with the fertile potential. The sperm membrane protein SP22 (22kDa) was identified in a study with epididymal toxicants and its amino acid sequence shows 90% homology with the human Park protein. Despite its testicular origin, during sperm transit through the epididymis it is redistributed and when spermatozoa reach the epididymal cauda SP22 acquires its characteristic location in the equatorial segment of the spermatozoid head. This location shows uniformity among several mammalian species, including humans, and the inhibition of *in vivo* and *in vitro* fertilization, obtained by the incubation of spermatozoa with anti-rSP22 antibodies, suggests the participation of the protein in the spermatozoa-oocyte interaction during the fertilization process. Since spermatogenesis in men is less efficient than in other mammals, recovering enough spermatozoa for all analysis is a difficult or impossible task with only one ejaculate. Flow cytometry, in addition to allowing functional analysis to be performed concomitantly, requires a reduced amount of cells. **Objectives:** The present study aims to standardize functional analysis by flow cytometry and the SP22 immunolocalization in human sperm. **Methods:** Seminal samples of 6 men were collected for validation of the probes in the cytometer and SP22 immunolocalization standardization. For the evaluation of plasma and acrosomal membrane integrity (PAM) and mitochondrial potential and superoxide production (MPS) the following associations were used: propidium iodide (PI) and FITC-PSA (*Pisum sativum* agglutinin conjugated to fluorescein isothiocyanate); YO-PRO (YP: damaged plasma membrane cells), MitoStatus (MST: mitochondrial potential) and MitoSOX (MS: generation of superoxide anion in the mitochondrial matrix), respectively. Semen aliquots were divided into positive control (100% alive, T100) and negative (100% dead; T0). T0 was subjected to 10 consecutive cycles of freezing in liquid nitrogen and thawing in a water bath at 46°C (flash-frozen) for induction of plasma and acrosomal membrane injury, halting of mitochondrial activity and generation of superoxide anion. At the end of these 10 cycles, an aliquot of T0 was mixed with an aliquot of T100 in the ratio of 1:1, which gave a sample with 50% live and dead spermatozoa (T50). After preparation of T0 and T50, an aliquot of each control and T50 diluted in PBS 0.5% BSA at the concentration of 1×10^6 spermatozoa/ml and incubated at 37°C for 15 min and 20 min in the dark, for PAM and MPS, respectively. At the end of this period, at least 10,000 cells per sample were analyzed. This study was approved by Ethics Committee of Botucatu Medical School (UNESP) and the individuals provided written consent. **Results and Conclusion:** By histograms analysis, it was possible to observe that for the PI, FITC and MS probes at T100 the majority of cells were negatively labeled, indicating absence of plasma membrane and acrosomal membrane and low production of superoxide anion in the mitochondrial matrix. As to the MST probe most of the cells in the T100 were positively labeled, indicating the high mitochondrial potential. The immunolocalization of SP22 confirmed labeling in the equatorial region of the spermatozoa head. In the study continuation, we intend to quantify sperm protein SP22, in an attempt to incorporate this potential fertility biomarker in epidemiological and clinical studies, as a predictive parameter of human fertility/infertility. **Financial Support:** FAPESP (2015/13941-3)

Functions of cystic fibrosis transmembrane conductance regulator (CFTR) in the human epididymal epithelial organoids

Shih–Hsing Leir[1]*, James Browne[1], Scott E. Eggener[2] and Ann Harris[1]

[1] Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH, USA
[2] Section of Urology, University of Chicago Medical Center, Chicago, IL, USA

The cystic fibrosis transmembrane conductance regulator (CFTR) is an ATP- and cAMP-dependent chloride channel located in the apical membrane of many epithelial cells. The epididymis plays a key role in sperm maturation, and animal studies using mouse and swine models have shown that CFTR is important for maintaining normal epididymal function. Additionally, more than 97% of men with cystic fibrosis (CF) are infertile due to obstruction or degeneration of the genital ducts. However, the structural and cellular dissimilarities in the epididymis between animals and humans indicate that a human cell model is required to study the role of CFTR in maintaining human epididymis function. We established cultures of human epididymis epithelial (HEE) cells and used these to address CFTR function. Non-cancerous human epididymis tissue was obtained from patients undergoing orchiectomy following a clinical diagnosis of testicular cancer. The tissues were dissected into three different anatomical regions: caput (head), corpus (body) and cauda (tail), digested with collagenase, and grown in CMRL-1066 medium. The caput cells had the highest CFTR mRNA expression, followed by the corpus and cauda. When cultured in Matrigel, the caput HEE cells formed organoids after 5-10 days and can be passaged for long-term culture. The contribution of CFTR to HEE organoid function was assessed using activation of CFTR by forskolin (FSK) and a pharmacological CFTR potentiator (VX-770), followed by blocking with CFTR inhibitor. By using this method, we found that FSK and HEE treatment induced HEE organoid swelling, and that the swelling is CFTR dependent. Our results indicate that cultured HEE organoids are a good model to assay differentiated functions of the human epididymis, which will help determine CFTR-dependent cellular processes. This culture model will also reveal how epididymis function is impaired in CF, which may help to treat infertility in male CF patients.

Metabolomic profiling by 1H-NMR of human seminal plasma and database-driven analysis reveal new features for glycerophosphocholine

Marie Walschaerts[1], Sandra Arnault[1], Emmanuelle Obeuf[1], Cécile Canlet[2], Laurent Debrauwer[2], François Isus[1], Myriam Daudin[1], Sébastien Dejean[3], Nicolas Savy[3], Safouane Hamdi[1]*

[1] Groupe de Recherche en Fertilité Humaine, EA3694, Université Paul-Sabatier, CHU de Toulouse, Toulouse, France
[2] UMR 1331 TOXALIM, Plateau Metatoul AXIOM, Université Paul-Sabatier, INRA, INP, Toulouse, France
[3] Equipe Statistique et Probabilités, Institut de Mathématiques de Toulouse, Université Paul-Sabatier, Toulouse, France

Background: Seminal plasma (SP) is a complex mixture that dilutes and transports spermatozoa and is recognized as an anatomical and functional reflect of the male genital tract. Therefore, SP exploration is informative for the etiological diagnosis of male infertility. Recently, ^{1}H NMR studies of human biofluids have attracted much attention as a powerful tool to understand pathophysiological processes at metabolites level.
Aim: To perform and to compare untargeted metabolomic profiling by ^{1}H-NMR of seminal plasma from normozoospermic (NZ) and azoospermic (AZ) patients.
Patients and methods: SP samples from 47 consecutive NZ (according to WHO 2010 criteria) and 38 consecutive AZ were stored after routine investigations and analyzed by batches on a Bruker DRX 600 MHz NMR Spectrometer. A manual signal assignment of metabolites was performed and spectrum-shaped metabolomics data from NZ and AZ patients were transformed by the wavelet method before a Sparse-PLS-DA-based comparison. For further statistical analysis, data of 993 NZ patients and 461 AZ patients referred to our Andrological Center between 2005 and 2015 were extracted from the database.
Results: In preliminary experiments, ^{1}H- and ^{13}C–RMN spectra (1D, 2D COSY, 2D HSQC) were obtained and manual signal assignment retrieved 30 unambiguous metabolites. Sparse PLS-DA on wavelets-transformed spectra identified several intervals of chemical shift with significant differences between NZ and AZ. In the 2,9–3,9 ppm interval, 3 metabolites could be identified: choline, fructose and glycerophosphocholine (GPC). Enzymatic assays confirmed a difference for total seminal GPC between NZ and AZ (respectively 6.89 ± 4.06 and 3.11 ± 3.32 µmol, $p<0.0001$) but not for total choline or fructose. Comparing NZ and AZ from the center database confirmed this difference in total GPC (respectively 7.9 ± 7.6 and 3.1 ± 3.0 µmol, $p<0.001$). A detailed analysis of the NZ dataset revealed that total GPC is negatively correlated to patients' age ($p=0.0003$) and BMI ($p=0.003$), positively correlated to spermatozoa concentration ($p<0.0001$) but not to sperm motility or viability.
Conclusion: Metabolomic profiling by 1H-NMR of SP and statistical analysis of 1454 patients shed light on GPC and revealed its link with spermatozoa concentration, opening new research perspectives.

Impairment on sperm quantity and quality in adult rats after arsenic exposure during prepuberty

Felipe Couto Santos[1], Ana Cláudia Ferreira Souza[1], Luiz Otávio Guimarães Ervilha[1], Mariana Machado-Neves[1]*

[1] Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

The epididymis plays an important role in the sperm maturation, which consists in the acquisition of forward motility and fertilizing ability. Epithelial cells lining the caput, corpus, and cauda regions are strongly involved in the different steps of this process due to their distinct functions and gene expression profiles. It is known that the differentiation of epididymal epithelial cells occurs during prepuberty, from the postnatal day (PND) 21 to 49, being this period critical for the establishment of epididymis functions. There is a paucity of information concerning the impact of arsenic exposure during the period of differentiation of this organ and the sperm quality in adult rats. Therefore, we aimed to evaluate the effect of arsenic exposure during the prepubertal period on reproductive competence in adulthood, focusing on sperm parameters. For that, after weaning (PND 21), male pups were divided into control group (n=5) and arsenic group (n=5). While control animals received filtered water *ad libitum*, other animals received 10 mg/L arsenic as sodium arsenite ($AsNaO_2$; SIGMA) in drinking water *ad libitum*, from PND 21 to 49. Thereafter, the animals were maintained until reach sexual maturity on PND 70. They were then euthanized (CEUA process number 96/2017), and epididymides were obtained for the assessment of sperm number and sperm transit time in epididymis. In addition, sperm from epididymis cauda were used for evaluating their motility, morphology, and structural integrity of plasma and acrosomal membranes. The results were analyzed by Student's T-test, and differences were considered significant when $p<0.05$. Our results showed that arsenite exposure reduced the number of spermatozoa in the caput/corpus and cauda epididymis compared to control group, even when this counting was analyzed per g epididymis ($p<0.05$; Table 1). These findings suggest damage in the seminiferous epithelium after arsenite exposure with consequences to sperm production. Moreover, animals exposed to arsenite presented lower percentage of motile sperm and sperm with intact membranes than control animals ($p<0.05$; Table 1). It is known that spermatozoa with damaged acrosomal and plasma membranes are not able to fertilize eggs and preserve cellular homeostasis, respectively. These damages may be related to disruption in spermiogenesis or sperm maturation, as both membranes pass through biochemical and morphological alterations during sperm transit in the epididymis. We concluded that exposure to sodium arsenite during prepuberty compromises reproductive competence at adulthood, reducing sperm quantity and quality in the epididymis.

Table 1. Sperm count in epididymis and sperm quality of adult Wistar rats exposed to sodium arsenite during prepuberty

Parameters	Control	10 mg/L arsenite
Caput/corpus epididymis sperm number (x10^6/organ)	94.8 ± 3.1	60.4 ± 2.4*
Caput/corpus epididymis sperm number (x10^6/g organ)	290.0 ± 6.6	193.5 ± 3.5*
Sperm transit time in the *caput/corpus* epididymis (days)	3.1 ± 0.2	3.2 ± 0.4
Cauda epididymis sperm number (x10^6/organ)	126.4 ± 9.0	69.4 ± 4.7*
Cauda epididymis sperm number (x10^6/g organ)	619.0 ± 23.7	389.0 ± 9.7*
Sperm transit time in the *cauda* epididymis (days)	4.4 ± 0.2	3.7 ± 0.3
Sperm parameters		
Sperm motility (%)	76.5 ± 2.3	62.0 ± 4.8*
Sperm with intact membranes (%)	32.3 ± 1.3	8.1 ± 1.2*
Normal sperm morphology (%)	93.8 ± 2.4	92.6 ± 2.5

Mean ± standard error mean. * Means significant difference ($p<0.05$) by Student's t-test.

Combined treatment with antibiotic and dexamethasone prevents duct obstruction and reduces adaptive immune responses in a long-term model of mouse epididymitis

Britta Klein[1]*, Rukmali Wijayarathna[2], Ralf Middendorff[1], Kate A. Loveland[2,3,4], Mark P. Hedger[2,3,4], Andreas Meinhardt[1,2]

[1] Dept. of Anatomy and Cell Biology, JLU, Giessen, Hesse, Germany
[2] Hudson Institute of Medical Research, Clayton, Victoria, Australia
[3] Dept. of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia
[4] Dept. of Molecular and Translational Sciences, Monash University, Clayton, Victoria, Australia

Antibiotic treatment is the standard therapy for human epididymitis caused by uropathogenic *E. coli* (UPEC). Despite effective elimination of the bacteria, around 50% of patients subsequently present with fertility problems, likely due to duct obstructions.
Using a mouse model of UPEC-induced epididymitis, this study explored the disease pathogenesis over time (day 10, i.e. acute vs. day 31, i.e. chronic phase), and the effects of an antibiotic, anti-inflammatory (corticoid) or combined treatment.
At day 10, macroscopical signs of acute epididymal inflammation can be detected at different strengths in all groups, with mildly better epididymal tissue preservation apparent following combined treatment. Moreover, the number of epididymal live bacteria and local inflammatory cytokine expression were significantly reduced in antibiotic and combined treated animals.
By day 31, the macroscopical signs of an acute inflammation were resolved in all groups, irrespective of the treatment. Similarly, local cytokine expression levels had decreased to sham levels in all groups. Live epididymal bacteria are undetectable following antibiotic or combined treatment. Of note, following combined treatment, the epididymal integrity appears to be better preserved, with no visible duct obstructions and no persisting inflammatory sites.
Interestingly, at day 31, the tissue-inherent IgG as well as serum IgM and IgG levels in the combined-treated group were lower compared to all other infection groups. This suggests that in long-term, the combined treatment is able to resolve both innate and adaptive immune responses in UPEC epididymitis, with active adaptive immunity possibly being responsible for the more severe tissue damage following antibiotic-only treatment.

Positive correlation between sperm DNA integrity and sperm number in pubertal rats exposed to binge drinking

Priscila Marianno[1], Joana N. Simas[1], Vanessa Vendramini[1]*

[1] Laboratory of Developmental and Reproductive Biology (LabRed), Unifesp, São Paulo, São Paulo, Brazil

Introduction: The heavy episodic alcohol consumption called binge drinking has been the subject of studies within the scientific community. The pattern of use is characterized by the consumption of large amounts of alcohol in a short period of time, which brings blood alcohol concentration to 0.08 g/dL or above, levels considered to be toxic. Heavy drinking behavior is prevalent among adolescents and young adults and tends to decrease with age. It is known that alcohol is associated with changes on male reproductive system, however, yet there are no studies showing whether the episodic use of excessive quantities of alcohol during puberty interferes in sperm production and if a possible toxic effect persists throughout the reproductive age. **Objective:** The aim of this study was to evaluate whether the exposure of pubertal Wistar rats to alcohol in binge pattern impacts some male reproductive parameters. **Methods:** The animals were subjected to treatment with multiple binges (2 consecutive sessions over 4 weeks) at a dose of 5 g/kg, starting at 51 days of age and evaluated at two different ages: 75 and 130 days. At the euthanasia day, the testes, epididymis, ventral prostate and seminal vesicles were collected and weighed. Qualitative and quantitative analysis of sperm collected from the right epididymis tail was carried out. **Results:** The episodic exposure to alcohol altered the body weight gain during treatment. Sperm concentration/ml and morphology of treated rats were reduced at 75 days of age in a significant manner when compared to control rats. In this same age sperm chromatin integrity was not statistically significant. No alterations were observed after the cessation of the use, suggesting that the type of damage is temporary and can be recovered. **Conclusion:** the negative correlation between the susceptibility of sperm DNA to fragmentation and sperm concentration obtained from epididymis cauda assessed immediately after treatment points out to a concerning effect of this pattern of alcohol consumption on the fertility and sperm genome quality of young users.

Ethics Authorization Number: 1425130716

Resveratrol improves spermatic parameters and gestation index in rats submitted to left-sided varicocele from peripuberty

Talita B. Mendes[1], Joana N. Simas[1], Camila C. Paccola[1], Samara U. de Oliva[1], Vanessa Vendramini[1*], Sandra M. Miraglia[1]

[1] Laboratory of Developmental and Reproductive Biology (LabReD), Unifesp, São Paulo, São Paulo, Brazil

Introduction: Varicocele is the most common cause of male infertility and is related to increased oxidative stress. As a consequence, damage occurs in the plasma membrane, in the mitochondria and in the sperm DNA, affecting the viability, capacity and acrosome reaction. Antioxidants, including resveratrol, are substances with properties to protect against damage caused by oxidative stress. **Objective:** To investigate the protective action of resveratrol against reproductive damage caused by experimental varicocele. **Methods:** One hundred and twenty-two male peripubertal rats (41 days postpartum, dpp) were distributed in 4 groups: Control Sham (S); Varicocele (V); Resveratrol (R) and Varicocele treated with resveratrol (VR). Left unilateral varicocele was induced in the animals of the V and VR groups (41 dpp) by partial ligation of the left renal vein (2-0 cotton thread and having as a diameter pattern an epidural catheter). Animals of the S group were submitted to the same surgical procedure, but they did not have the ligation performed. The R and VR groups received 300mg / kg / day of resveratrol (per gavage), up to 99 dpp. In the animals submitted to euthanasia (100 dpp), we evaluated: daily sperm production and sperm transit in the epididymis; numerical density of apoptotic cells (TUNEL method) in the epididymis; sperm mitochondrial function (Mitotracker Green FM); gestation index. Statistical analysis was undertaken using ANOVA-SNK ($p \leq 0,05$). **Results:** The varicocele (V) group presented increased frequency of TUNEL-positive cells in the epididymis and reduced mitochondrial function in relation to the other experimental groups (S, R and VR). The parameters of absolute number of spermatozoa and transit time were also reduced in the V group when compared to groups S and R. The gestation index of the V group was reduced in relation to the S, R and VR groups. **Conclusion:** These results suggest that daily administration of resveratrol in varicocelized rats from peripuberty improves sperm and reproductive quality in adulthood.

Ethics Authorization Number: 3264050315

Altered histomorphometry and steroid receptors immunostaining pattern on rat prepubertal testes and epididymis, after prenatal exposure to betamethasone

Jorge Barros[1,2*S], Cibele Borges[2], Tainá Pacheco[1,2], Ana Flávia Lozano[1,2], Wilma Kempinas[2]

[1] Graduate Program in Cell and Structural Biology, Institute of Biology, State University of Campinas (UNICAMP), Campinas, Brazil
[2] Department of Morphology, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, Brazil

Introduction: During pregnancy endogenous glucocorticoids are required to promote fetal tissues maturation. However, in case of preterm birth risk, therapy with betamethasone, a synthetic glucocorticoid, is applied in order to promote fetal lung maturation, which decreases neonatal mortality and morbidity. This hormonal subclass is highly related to the fetal reprogramming concept and, in previous studies, our Laboratory reported that prenatal exposure to betamethasone leads to late reproductive disorders in rats. **Objective:** Thus, the present study aimed to evaluate the impact of betamethasone on rat prepubertal testicular and epididymal development, after intrauterine exposure to this synthetic glucocorticoid. **Methods:** Pregnant Wistar rats were allocated into two groups: BM (n=4-5), treated with 0.1mg/Kg/day of betamethasone; and Control (n=5-6), treated with saline; BM and Control groups were treated with intramuscular injection on gestational days 12, 13, 18 and 19, critical days of prenatal reproductive development in rats. We performed testicular and epididymal histomorphometric analysis at postnatal days (PND) 7, 14 and 28, and immunohistochemistry for androgen (AR) and α-type estrogen (ERα) receptors in both organs at same ages. **Results:** Histomorphometric analysis revealed a decrease in Leydig cells nuclear volume at PND 7, 14 and 28. Seminiferous tubules at PND 14 and 28 presented lower tubular diameter and number of Sertoli cells in BM group. Immunohistochemistry assay for AR showed lower immunostaining pattern in testicles at PND 7 and 28, and in epididymis at PND 28 of BM group. Furthermore, immunostaining pattern for ERα were also reduced only in the testicles of BM group, at PND 14 and 28. **Conclusion:** Our results, in rats, suggest that prenatal exposure to betamethasone potentially causes reproductive programming and impairs male rat postnatal reproductive development, which raises concerns about the use of betamethasone on human antenatal therapy.

Financial support: FAPESP grant #2017/02764-9

Effects of Prostaglandin E2 on Gap Junction Protein Alpha 1 (Connexin 43) in the Rat Epididymis

Mary Gregory[1]*, Daniel G. Cyr[1]

[1] Laboratory for Reproductive Toxicology, INRS-Institut Armand-Frappier, University of Quebec, 531 boul. des Prairies, Laval, QC, H7V 1B7, Canada

Gap junctions are responsible for intercellular communication. In the adult mammalian epididymis, gap junction protein alpha 1 (GJA1) is localized between basal and either principal or clear cells. Mutations to GJA1 gene, resulting in decreased phosphorylation of GJA1, resulted in decreased sperm motility and velocity parameters, indicating that GJA1 plays a significant role in epididymal function. *Gja1* mRNA levels during postnatal development decreased with age, and this decrease coincides with basal cell differentiation. The present objective was to determine the influence of basal cells and prostaglandin E2 (PGE2) on GJA1 in the rat epididymis. Prior to the differentiation of basal cells at days 7 and 14, GJA1 is present at the apical lateral margins between adjacent epithelial cells and throughout the cytoplasm. While some GJA1 co-localized with TJP1 (tight junction protein 1, also known as ZO1), not all GJA1 was co-localized with TJP1. When basal cells begin to differentiate and migrate at the base of the epithelium at day 21, GJA1 is present both at the basal region of the epithelium and throughout the cytoplasm. By day 28, GJA1 was localized primarily at the base of the epithelium, and was associated with basal cells, as identified by TP63 staining, and between basal and principal cells. This pattern of localization remained consistent through to adulthood. As basal cells differentiated, they expressed prostaglandin-endoperoxide synthase 1 (PTGS1) and this was concomitant with changes in GJA1 localization. To assess the effects of PGE2 on GJA1, RCE cells were exposed to PGE2 (50 μM) for 3 hrs. PGE2 increased levels of *Gja1* mRNA in RCE cells while levels of *Gjb1* (Cx32), *Gjb2* (Cx26), *Gjb4* (Cx30.3) and *Gjb5* (Cx31.1) were unaltered. PGE2 increased protein levels of both GJA1 and phospho-GJA1. Furthermore, phospho-AKT, CTNNB1, and phospho-CTNNB1 were all increased by PGE2 treatment, which is consistent with studies on regulatory pathways implicated in the regulation of GJA1 in mouse embryonic stem cells. Total AKT levels were not altered. Levels of the tight junction protein, claudin1, which is also expressed in basal cells, were also not altered by PGE2. Data suggest that the development of the epididymal epithelium and the differentiation of epididymal basal cells regulate the targeting of GJA1 intercellular communication, and that this effect appears to be mediated, in part, by PGE2.

Supported by NSERC and CRC.

Index

Index

Index

List of Participants

Badaruddin Abbasi ..... babukhsh@iau.edu.sa
Raheleh Aram ..... raheleh.aram@iaf.inrs.ca
Maria Christina W. Avellar ..... avellar@unifesp.br
Jorge Willian Franco de Barros ..... jorge.willian1@hotmail.com
Maria Agustina Battistone ..... mbattistone@mgh.harvard.edu
Clemence Belleannee ..... clemence.belleannee@crchudequebec.ulaval.ca
Elise Boivin-Ford ..... elise.boivin-ford@mcgill.ca
Cibele dos Santos Borges ..... cibelesantosborges@gmail.com
Sylvie Breton ..... breton.sylvie@mgh.harvard.edu
James A Browne ..... jab410@case.edu
Mingju Cao ..... mingju.cao@mcgill.ca
Guillermo Carvajal ..... guillec6@hotmail.com
Flavia Lorena Carvelli ..... lorecarvelli@gmail.com
Marilia Martins Cavariani ..... marycavariani@hotmail.com
Hong Chen ..... ch4973@gmail.com
Chen Chu ..... chen_chu@dfci.harvard.edu
Colin Conine ..... colin.conine@umassmed.edu
Gail A. Cornwall ..... gail.cornwall@ttuhsc.edu
Patricia S Cuasnicu ..... pcuasnicu@gmail.com
Daniel Cyr ..... daniel.cyr@iaf.inrs.ca
Mojtaba Dashtizad ..... dashtizad@nigeb.ac.ir
Geoffry Nunzio De Iuliis ..... geoffry.deiuliis@newcastle.edu.au
Talita de Mello Santos ..... talita_mellosantos@yahoo.com.br
Regiana Lucia de Oliveira ..... regiana.oliveira@mail.mcgill.ca
Darius J. Devlin ..... darius.devlin@bcm.edu
Joël R. Drevet ..... joel.drevet@uca.fr
Julie Dufresne ..... julie.dufresne@iaf.inrs.ca
Edward Mitchell Eddy ..... mitch@malecontraceptive.org
Marzieh Eskandari Shahraki ..... marzieh.eskandari78@gmail.com
Samuel Guilerme Fernandes ..... samuelguilerme@gmail.com
Lucas Garcia A Ferreira ..... garciaii_antuerpia@hotmail.com
Heather Elizabeth Fice ..... heather.fice@mail.mcgill.ca
Geanne Arantes Freitas ..... geanne_af@hotmail.com
Thomas Garcia ..... thomas.garcia@bcm.edu
George L Gerton ..... gerton@pennmedicine.upenn.edu
Laura Girardet ..... lauragirardet@hotmail.fr
Mary Gregory ..... mary.gregory@iaf.inrs.ca
Yihua Gu ..... gu_yihua@hotmail.com
Rachel Guiton ..... rachel.guiton@uca.fr
Barbara Hales ..... barbara.hales@mcgill.ca
Safouane Hamdi ..... safouane.hamdi@univ-tlse3.fr

List of Participants

Ann Harris	ann.harris@case.edu
Thorben Hau	thorben.hau@anatomie.med.uni-giessen.de
Mark Peter Hedger	mark.hedger@hudson.org.au
Louis Hermo	louis.hermo@mcgill.ca
Rex A. Hess	rexhess@illinois.edu
Barry Thomas Hinton	bth7c@virginia.edu
Masahito Ikawa	ikawa@biken.osaka-u.ac.jp
Daniel Stephen Johnston	daniel.johnston@nih.gov
Aimee Lee Katen	aimee.katen@mcgill.ca
Wilma De Grava Kempinas	wilma.kempinas@unesp.br
Britta Klein	britta.klein@anatomie.med.uni-giessen.de
Pierre Leclerc	pierre.leclerc@crchul.ulaval.ca
Christine Légaré	christine.legare@crchul.ulaval.ca
Shih-Hsing Leir	sxl1180@case.edu
Ana Flávia Quiarato Lozano	anaaquiarato@gmail.com
Puttaswamy Manjunath	manjunathp555@gmail.com
Andreas Meinhardt	andreas.meinhardt@anatomie.med.uni-giessen.de
Ralf Middendorff	ralf.middendorff@anatomie.med.uni-giessen.de
Camilla Moreira Ribeiro Leite	ribeiro.cmr@gmail.com
Mariana Neves	mmachadoneves@yahoo.com.br
Logan Michael Nickels	logan@malecontraceptive.org
Brett Nixon	brett.nixon@newcastle.edu.au
Anaïs Noblanc	anaisnoblanc@hotmail.fr
Cristian O'Flaherty	cristian.oflaherty@mcgill.ca
Marie-Claire Orgebin-Crist	m-c.orgebin-crist@Vanderbilt.Edu
Christopher Pearl	pearlch@gvsu.edu
Laurie Pinel	laurie.pinel@iaf.inrs.ca
Erick José Ramo da Silva	ejrsilva81@gmail.com
Bernard Robaire	bernard.robaire@mcgill.ca
Josiane de Lima Rosa	josi.lr@outlook.com
Tricia Marie Rowlison	rowlisonT@si.edu
Fabrice Saez	fabrice.saez@uca.fr
Ana Maria Salicioni	asalicioni@vasci.umass.edu
Huijuan Shi	shihuijuan2011@163.com
Wai Chi Shum	shumw@shanghaitech.edu.cn
Petra Sipilä	petra.sipila@utu.fi
Robert Sullivan	robert.sullivan@crchul.ulaval.ca
Pradeep S. Tanwar	pradeep.tanwar@newcastle.edu.au
Darya Tourzani	dtourzan@umass.edu
Roland R. Tremblay	roland.r.tremblay@crchudequebec.ulaval.ca
Pei--Shiue Jason Tsai	psjasontsai@ntu.edu.tw

List of Participants

Terry Turner ..... ttt@virginia.edu
Vanessa Vendramini Vilela ..... vane.vanila@gmail.com
Pablo E. Visconti ..... pvisconti@vasci.umass.edu
Allison Voisin ..... allison.voisin@uca.fr
Tse-En Joan Wang ..... f05629003@ntu.edu.tw
Rukmali Wijayarathna ..... rukmali.wijayarathna@hudson.org.au
Ru Yanfei ..... ruyanfei@sibcb.ac.cn
Baoli Zhang ..... zhangbl@shanghaitech.edu.cn
Yonglian Zhang ..... ylzhang@sibcb.ac.cn
Wei Zhou ..... wzhou3@uon.edu.au

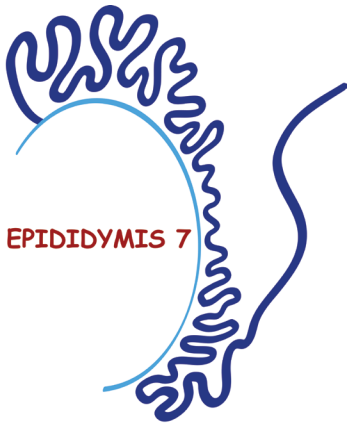
List of Participants - Last update: August 23, 2018

Notes

Notes

Notes

Notes



MONTREAL 2018