

Estrogen metabolism to investigate carcinogenicity of *Schistosoma haematobium*

Mónica Botelho
UPSDNT
07/02/2014

50 μ m



Instituto **Nacional de Saúde**
Doutor Ricardo Jorge

OUTLINE

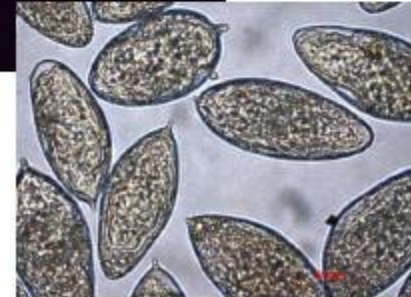
1. *Schistosoma haematobium*: the neglected schistosome

2. Development of functional tools for schistosomes

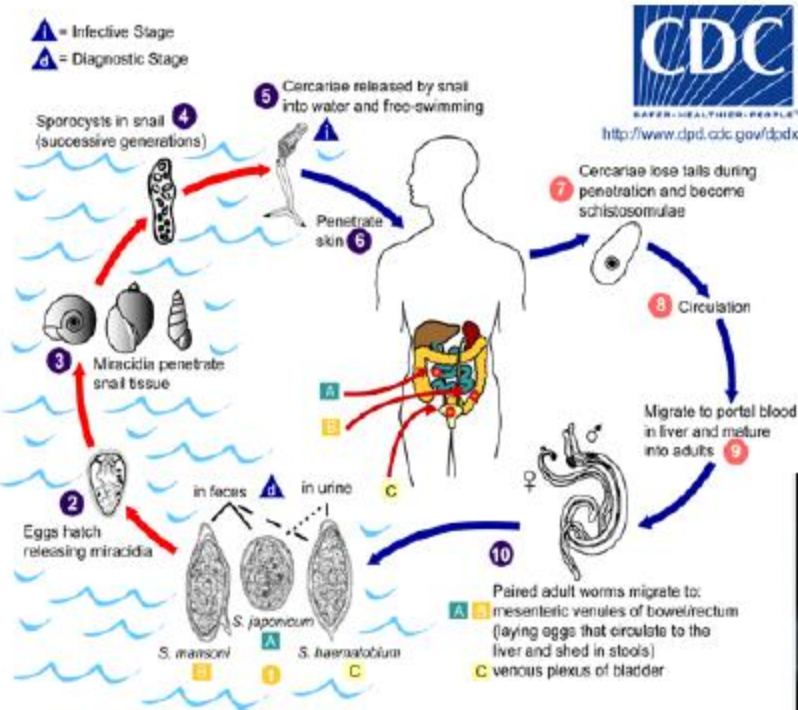
- . Culture of developmental stages of *Schistosoma haematobium*
- . Animal models of *S. haematobium* induced bladder cancer

3. Carcinogenic potential of *S. haematobium* eggs

- . Effect of egg extract on urothelial cells
- . Catechol-estrogens isolated from *Schistosoma haematobium*



Schistosomes: Life cycle

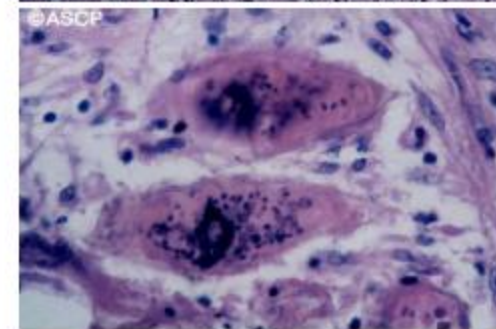
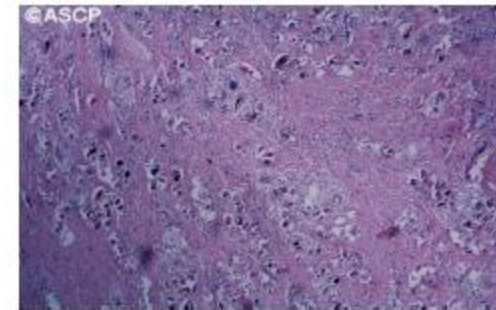


Urogenital Schistosomiasis

- Eggs provoke granulomatous inflammation that leads to small fibrotic nodules known as "sandy patches", ulceration, and pseudopolyposis of the vesical and ureteral walls. Urinary granulomas.
- Dysuria, pollakisuria, proteinuria and HEMATURIA
- Bacterial superinfection
- Obstructive uropathy. Hydronephrosis



PJ Hotez et al., Lancet 2010



Gryseels et al 2006

Orihel and Ash

The most neglected schistosome among schistosomes

Table 1. Number of citations in PubMed over the last five years, 2008–2012.

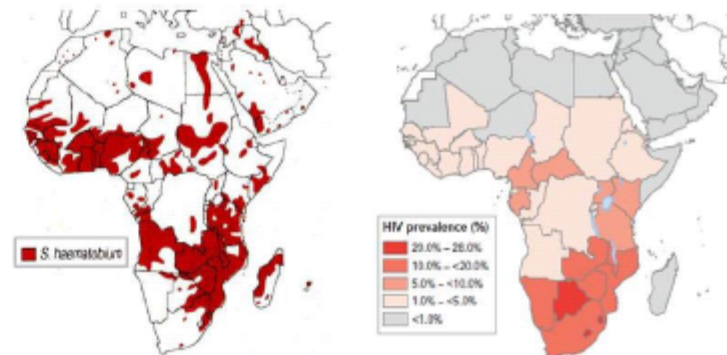
Parasite Species	Approximate Number of Human Cases	Number of PubMed Citations over the Last Five Years ^b	PubMed Citations per Millions of Human Cases	References
<i>Schistosoma japonicum</i>	1 million	644	644	Steinmann et al. 2006 [1]
<i>Schistosoma mansoni</i>	54 million ^a	1,371	25	Van der Werf et al. 2003 [3]
<i>Schistosoma haematobium</i>	112 million ^a	342	3	Van der Werf et al. 2003 [3]

a. Sub-Saharan Africa only

b. Search conducted on July 14, 2012

PJ Brindley and PJ Hotez, *PLoS NTDs* 2013

1. Group 1 carcinogen responsible for a unique squamous cell carcinoma of the bladder
2. Female Genital Schistosomiasis (FGS) – Infertility ?
3. FGS: 3 – 4 times increased risk in acquiring HIV infection



PJ Hotez et al., *PLoS NTDs* 2013

The neglected schistosome

- Absence of available animal models of urogenital schistosomiasis
- Absence of (1) *in vitro* culture methodologies for developmental stages and (2) Functional Genomic toolkit to address basic biological questions
- In 2012 *Schistosoma haematobium* got into the postgenomic era with *S. mansoni* and *S. japonicum* (in 2009)



The *Schistosoma japonicum* genome reveals features of host-parasite interplay

The Schistosoma japonicum Genome Sequencing and Functional Analysis Consortium*

The genome of the blood fluke *Schistosoma mansoni*

Matthew Berriman¹, Brian J. Haas^{2,3}, Philip T. LoVerde⁴, R. Alan Wilson⁵, Susan T. Mashiyama^{6,10}, Bissan Al-Lazikani¹¹, Luiza F. Andrade¹², Pete Daniella C. Bartholomeu³, Gaëlle Blandin³, Conor R. Caffrey³, Avril C. Art Delcher⁷, Ricardo DeMarco^{3,13,10}, Appolinaire Djikeng⁸, Tina Eyre¹, J. Christiane Hertz-Fowler⁹, Hirohisa Hirai¹⁷, Yuriko Hirai¹⁷, Robin Houst¹, Daniela Lacerda³, Camila D. Macedo^{6,8}, Paul McVeigh¹⁴, Zamin Ning¹, Julian Parkhill¹, Mihaela Perteza⁷, Raymond J. Pierce¹⁵, Anna V. Protasi¹, Marie-Adele Rajandream¹, Jane Rogers¹, Mohammed Sajid¹⁶, Steven Adrian R. Tivey¹, Owen White¹, David L. Williams^{2,18}, Jennifer Wortis¹, Adhemar Zerlotini¹¹, Claire M. Fraser-Liggett³, Barclay G. Barrell¹ & I.

nature
genetics

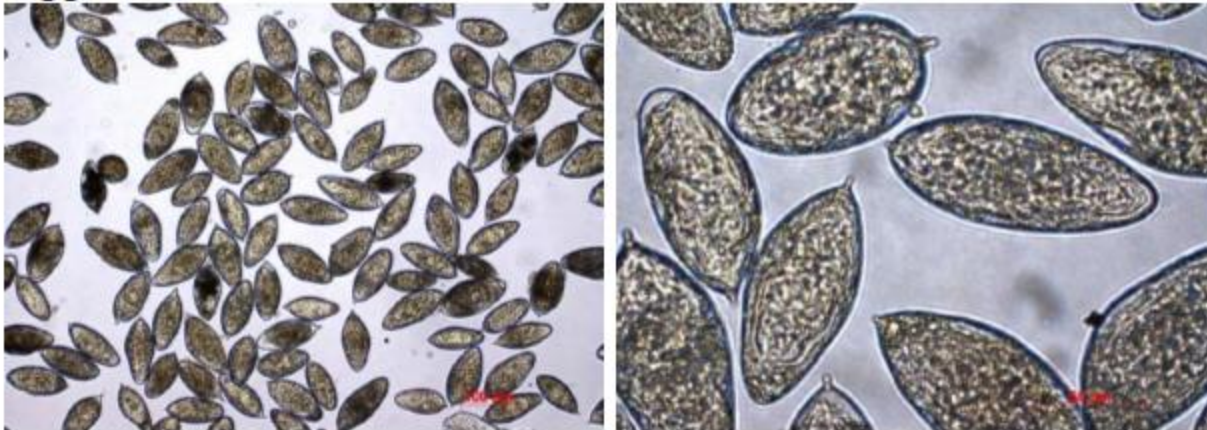
NATURE GENETICS VOLUME 44 | NUMBER 2 | FEBRUARY 2012

Whole-genome sequence of *Schistosoma haematobium*

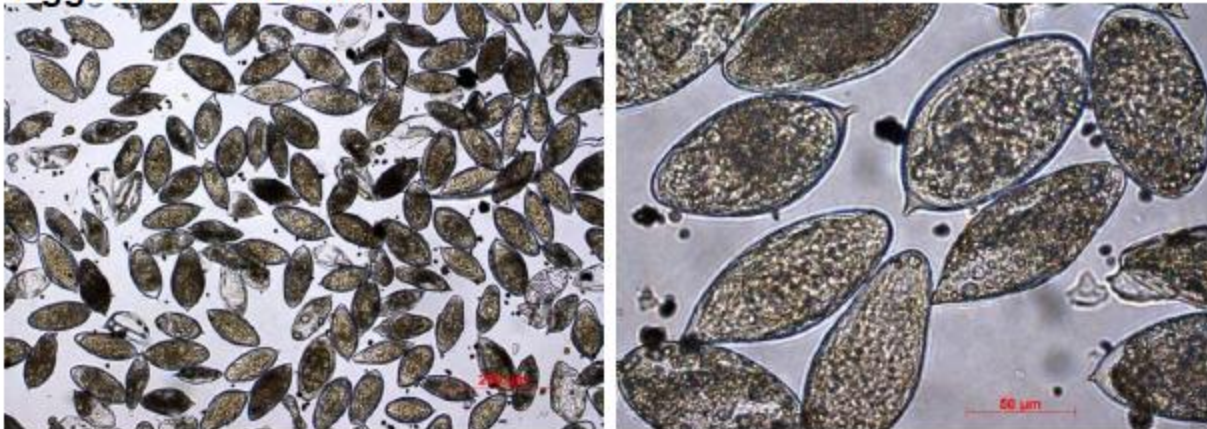
Neil D Young^{1,11}, Aaron R Jex^{1,11}, Bo Li^{2,11}, Shiping Liu², Linfeng Yang², Zijun Xiong², Yingrui Li², Cinzia Cantacessi¹, Ross S Hall¹, Xun Xu², Fangyuan Chen², Xuan Wu², Adhemar Zerlotini³, Guilherme Oliveira³, Andreas Hofmann^{1,4}, Guojie Zhang², Xiaodong Fang², Yi Kang², Bronwyn E Campbell¹, Alex Loukas⁵, Shoba Ranganathan^{6,7}, David Rollinson⁸, Gabriel Rinaldi^{9,10}, Paul J Brindley¹⁰, Huanming Yang², Jun Wang², Jian Wang² & Robin B Gasser¹

***In vitro* culture of *Schistosoma haematobium* developmental stages**

Eggs isolated from liver of infected hamsters



Eggs isolated from intestine of infected hamsters

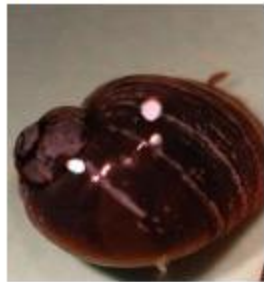


***In vitro* culture of *Schistosoma haematobium* developmental stages**

Adults obtained by portal perfusion from infected hamsters



Cercariae obtained by shedding infected *Bulinus truncatus* snails



Schistosomules obtained by mechanical transformation of cercariae



Animal models of urogenital Schistosomiasis

Urothelial dysplasia and inflammation induced by *Schistosoma haematobium* total antigen instillation in mice normal urothelium

Mónica C. Botelho, M.Sc.^{a,b,*}, Paula A. Oliveira, Ph.D.^c, Carlos Lopes, Ph.D.^{d,e},
José M. Correia da Costa, Ph.D.^a, José C. Machado, Ph.D.^{b,f}

^a CIBP—Centre for Parasite Immunology and Biology, National Institute of Health, Porto, Portugal

^b IPATIMUP—Institute of Pathology and Molecular Immunology of Porto University, Porto, Portugal

^c CECAV—Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

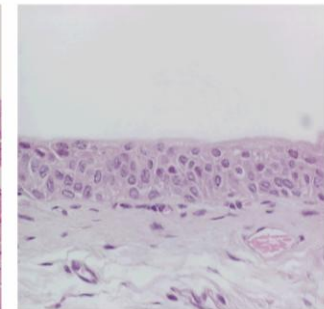
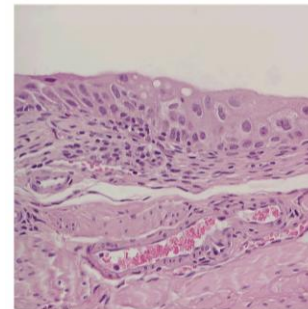
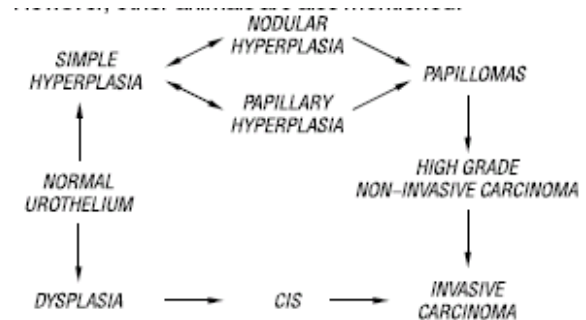
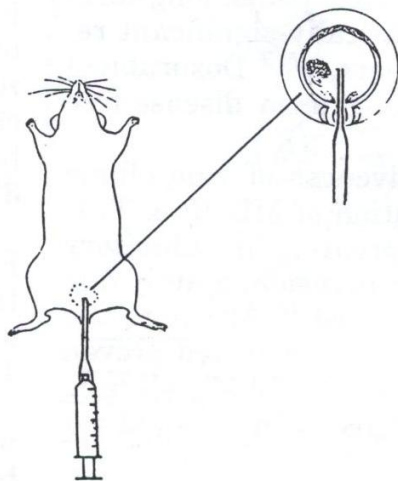
^d ICBAS—Institute of Biomedical Sciences Abel Salazar, Department of Cellular Biology and Immunology, Porto University, Porto, Portugal

^e IPO—Portuguese Institute of Oncology, Department of Pathology, Porto, Portugal

^f FMUP—Faculty of Medicine of Porto University, Porto, Portugal

Received 19 August 2009; received in revised form 25 September 2009; accepted 29 September 2009

Urologic Oncology 29 (2011) 809 – 814



Carcinogenic potential of *S. haematobium* eggs

Tumour-like phenotypes in urothelial cells after exposure to antigens from eggs of *Schistosoma haematobium*: An oestrogen–DNA adducts mediated pathway?

Mónica C. Botelho^{a,b,*}, Nuno Vale^c, Maria João Gouveia^c, Gabriel Rinaldi^{d,e}, Julio Santos^f, Lucio L. Santos^g, Paula Gomes^c, Paul J. Brindley^d, José Manuel Correia da Costa^{a,b}

^aCenter for the Study of Animal Science, ICETA, University of Porto, Portugal

^bINSA, National Institute of Health, Rua Alexandre Herculano, 321, 4000-055 Porto, Portugal

^cCIQUP, Chemistry and Biochemistry Department, Faculty of Sciences, University of Porto, Porto, Portugal

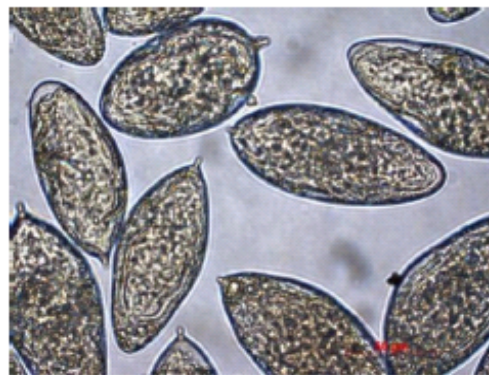
^dDepartment of Microbiology, Immunology and Tropical Medicine, Research Center for Neglected Diseases of Poverty, School of Medicine & Health Sciences, George Washington University Washington, DC 20037, USA

^eDepartamento de Genética, Facultad de Medicina, Universidad de la República, (UDELAR), Montevideo 11800, Uruguay

^fClínica da Sagrada Esperança, Avenida Mortala Mohamed-Ilha de Luanda, Angola

^gExperimental Therapeutics and Pathology Research Group, Portuguese Institute of Oncology, Porto, Portugal

International Journal for Parasitology 43 (2013) 17–26



Normal urothelial cells (HCV 29)

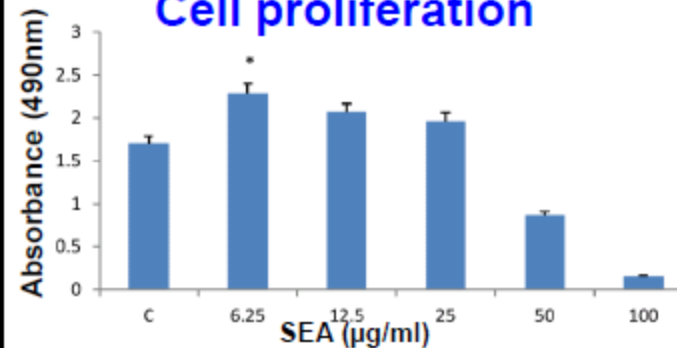
**Cell proliferation
Apoptosis
Oxidative stress
Genotoxicity**



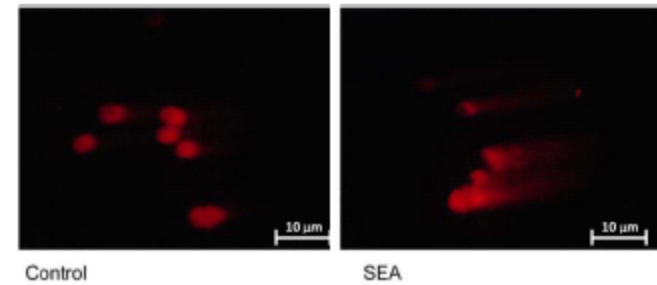
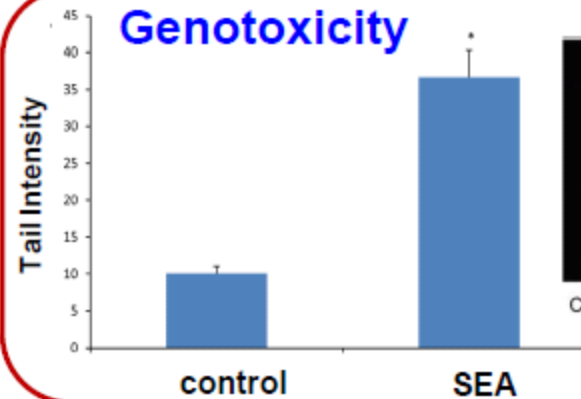
**Liquid Chromatography Diode Array Detection
Electron Spray Ionisation Mass Spectrometry
(LC/UV-DAD/ESI-MS) – investigation of oxysterols (oxidized derivatives of cholesterol)**

Soluble eggs antigens induced tumor-like phenotype in urothelial cells

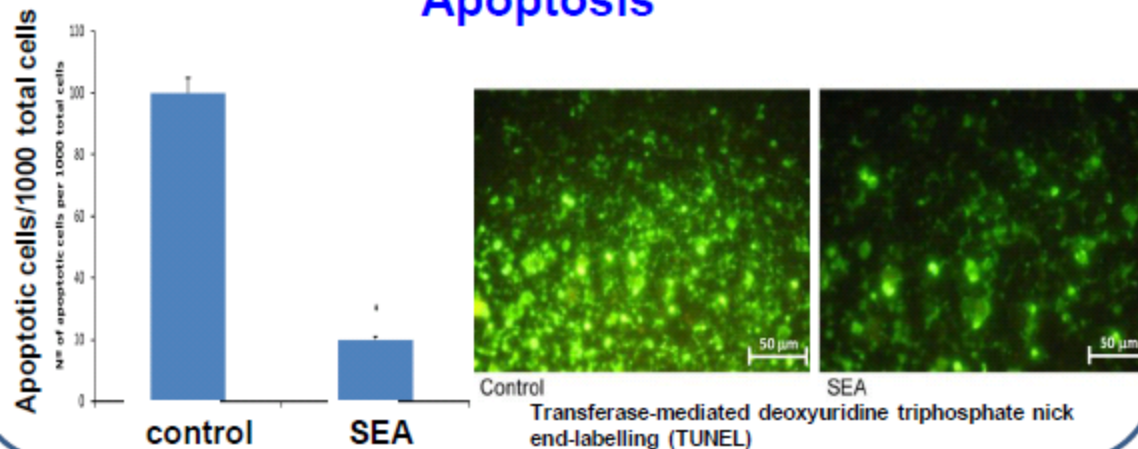
Cell proliferation



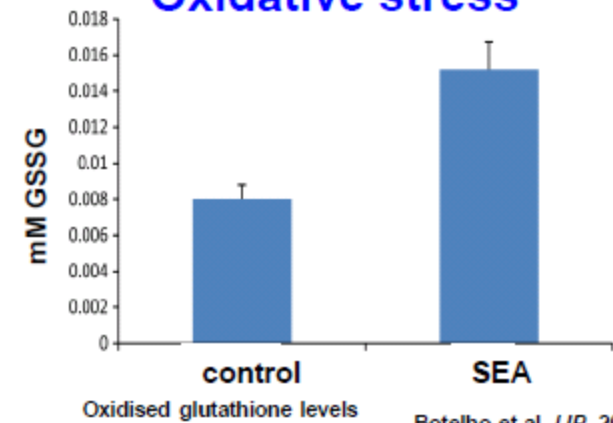
Genotoxicity



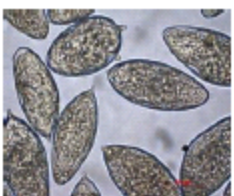
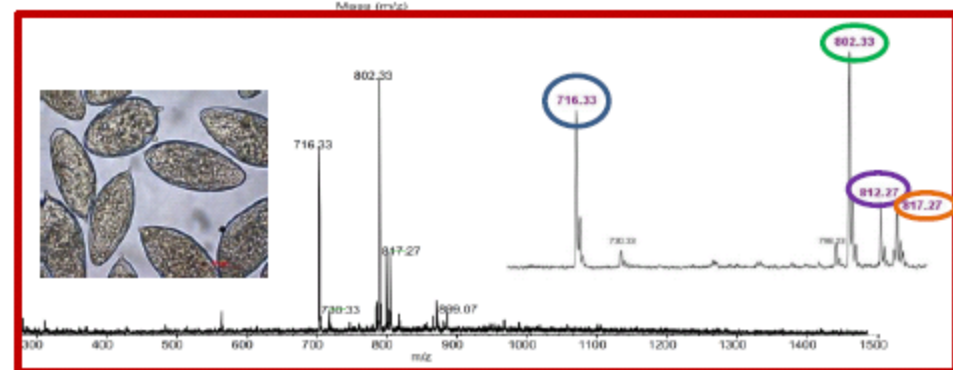
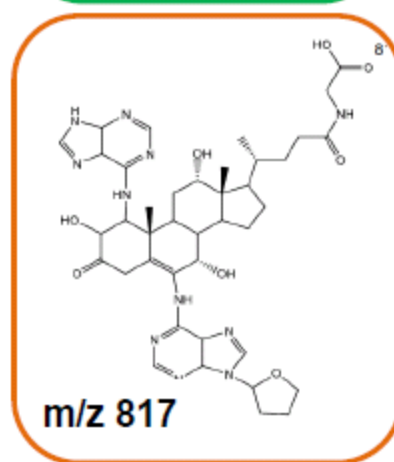
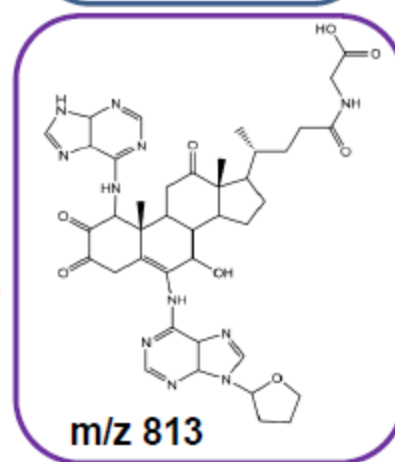
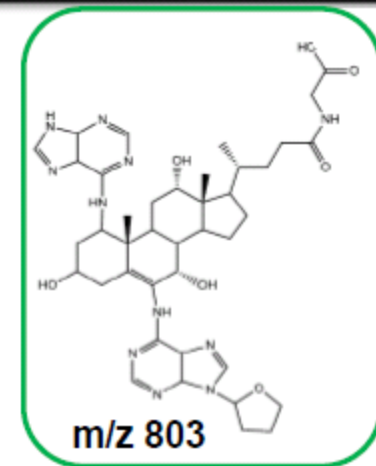
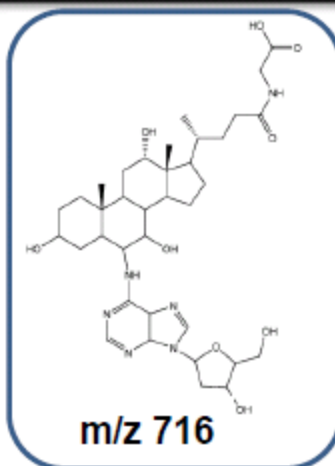
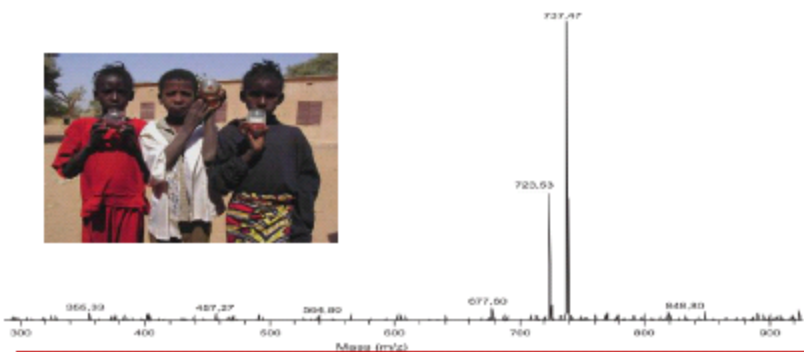
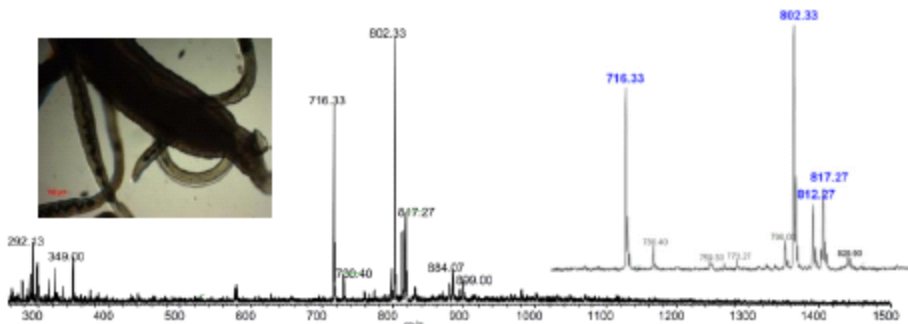
Apoptosis



Oxidative stress



**MS identified molecules extracted from *S. haematobium*
CATECHOL-OESTROGENS (oxidative metabolites derived
from estrogens)**



Pathways for Estrogen Carcinogenesis

Two different complementary pathways together probably contribute to the carcinogenicity of estrogen and to

- **Initiation**
- **Promotion**
- **Progression**

Yager JD, Davidson NE (2006) Estrogen carcinogenesis in breast cancer. *N Engl J Med* 354 (3):270-282. doi:10.1056/NEJMra05077

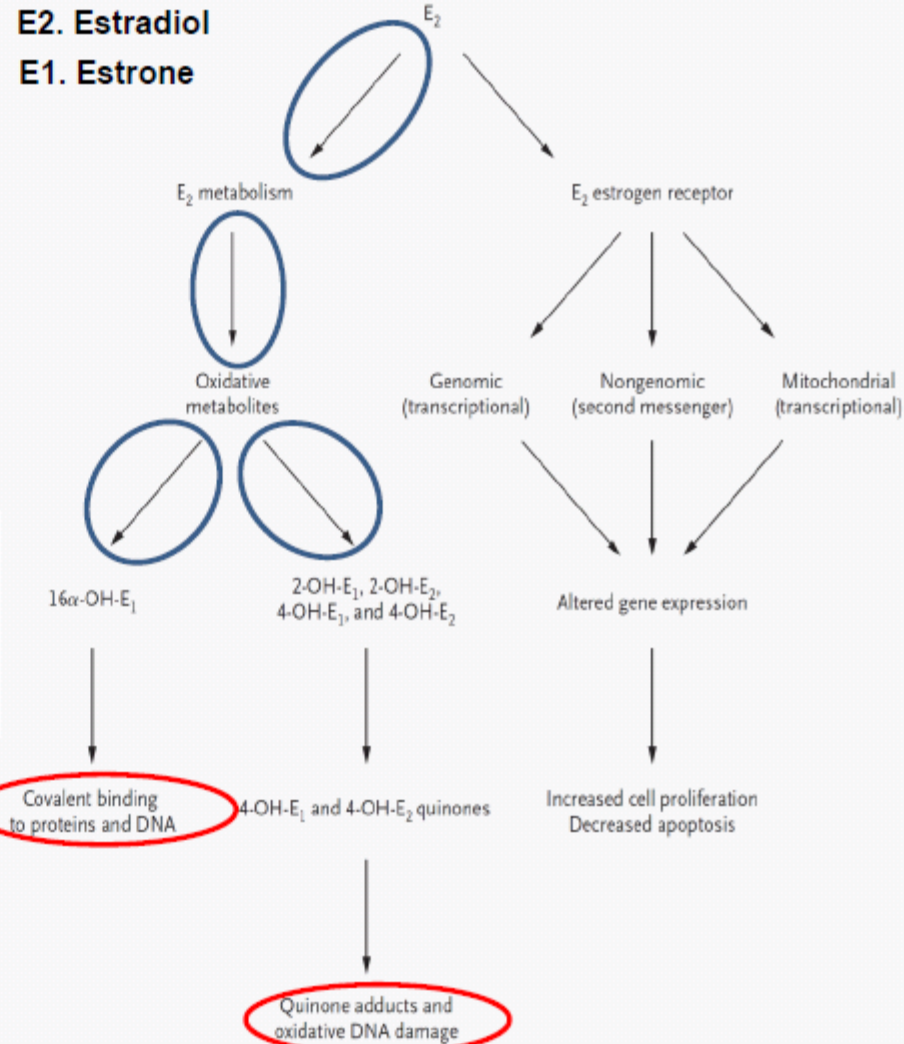
Review *Journal of Steroid Biochemistry & Molecular Biology* 125 (2011) 169-180

Unbalanced metabolism of endogenous estrogens in the etiology and prevention of human cancer

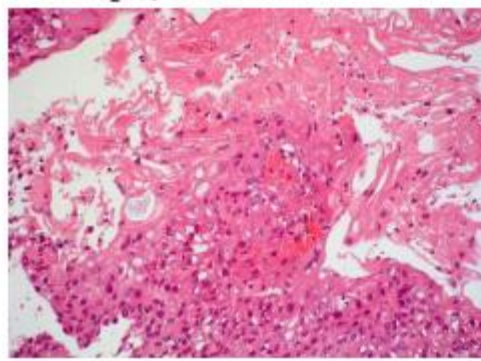
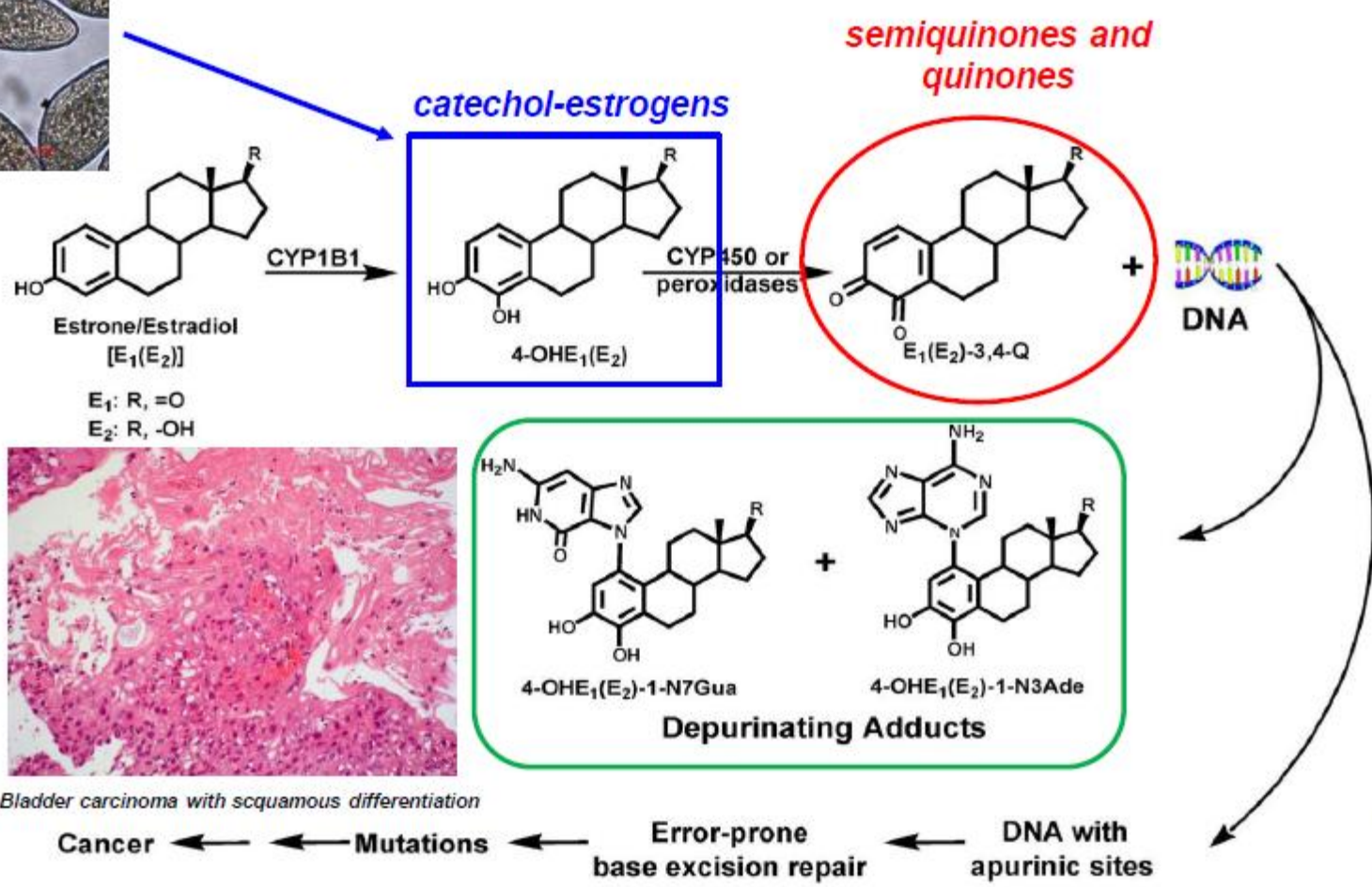
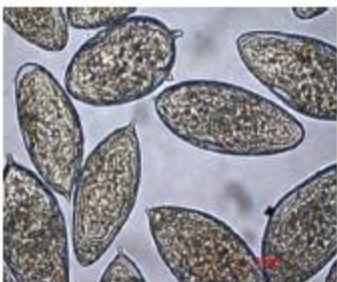
Ercole L. Cavalieri^{a,b,*}, Eleanor G. Rogan^{a,b}

^a Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, United States
^b Department of Environmental, Agricultural and Occupational Health, College of Public Health, University of Nebraska Medical Center, 986805 Nebraska Medical Center, Omaha, NE 68198-6805, United States

In relation to cancer initiation, estrogens should be considered as other chemicals, namely, their physico-chemical and biochemical properties lead them to follow the principles of chemical carcinogenesis elucidated by the Millers [6,7], rather than considering them as hormones. Substantial evidence supports a genotoxicity paradigm for the initiation of cancer by endogenous estrogens. Specific oxidative metabolites of estrogens can react



Major metabolic pathways in cancer initiation by estrogens



Bladder carcinoma with squamous differentiation

Adapted from Cavalieri, 2011
Zahid, 2013

Conclusions

- 1. It is feasible to culture *in vitro* developmental stages of *S. haematobium***
- 2. Soluble extracts from *S. haematobium* eggs induced carcinogenesis of the bladder in animal models**
- 3. Soluble extracts from *S. haematobium* eggs induced tumor-like phenotype in urothelial cells**
- 4. Novel catechol-oestrogen molecules derived from the eggs could be involved in the carcinogenesis process of the bladder**

Future Perspectives

- 1. Functional genomics, such as RNAi to address biological relevant questions related to *S. haematobium* and its carcinogenic potential (e.g. The draft genome of *S. haematobium* encodes a homolog of estradiol 17 β dehydrogenase, also known as 17 β hydroxysteroid dehydrogenase or 17 β HSD, which has a known role in the synthesis of estradiol and testosterone.)**
- 2. Synthesize and/or purify and/or isolate reactive catechol-estrogens.**
- 3. Evaluate impact of catechol estrogens on urothelial cells *in vitro*, at the phenotypic and gene expression levels.**
- 4. Evaluate impact of catechol estrogens in an informative mouse model.**
- 5. Investigate schistosome catechol estrogen–DNA adducts in informative human cases from a schistosomiasis haematobia endemic region of Angola. (Potential for Biomarkers screening)**



Paul J. Brindley
Gabriel Rinaldi



Fátima Gartner
Mário Sousa
Lourdes Bastos



Júlio Santos
Lúcio Lara
Carlos Lopes

