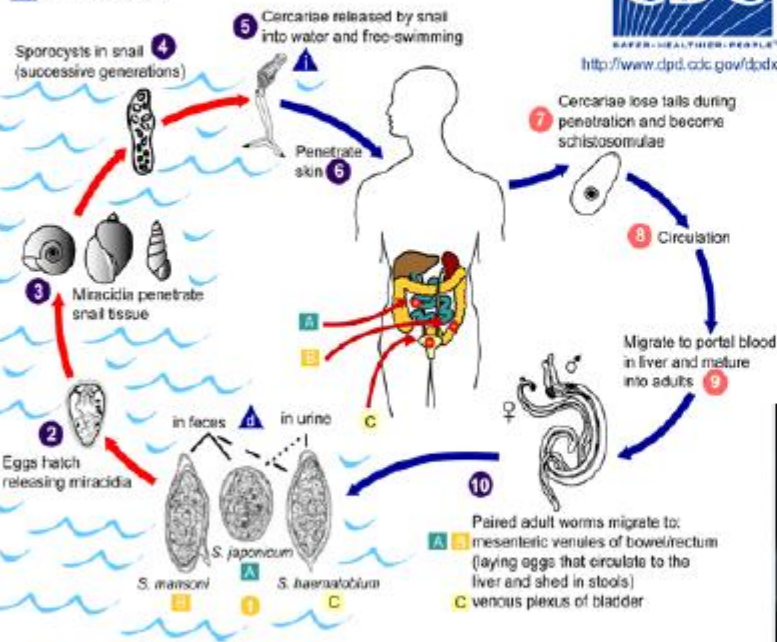


# New insights into the carcinogenesis induced by Schistosomes

Mónica Botelho  
29/08/2015

## Schistosomes: Life cycle

▲ = Infective Stage  
▲ = Diagnostic Stage

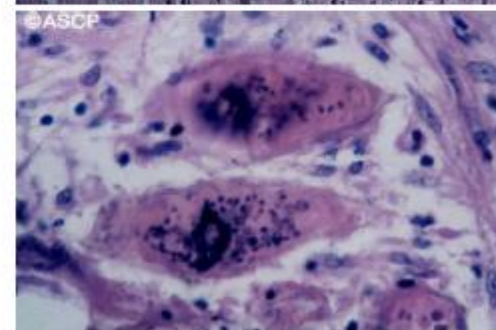
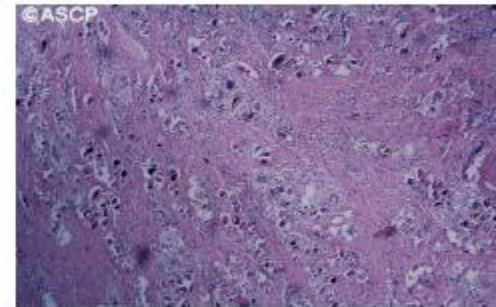


## Urogenital Schistosomiasis

- Eggs provoke granulomatous inflammation that leads to small fibrotic nodules known as “sandy patches”, ulceration, and pseudopolypsis 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

## Top 10 World's deadliest animals

**If you're thinking about sharks, snakes and lions...think again!**



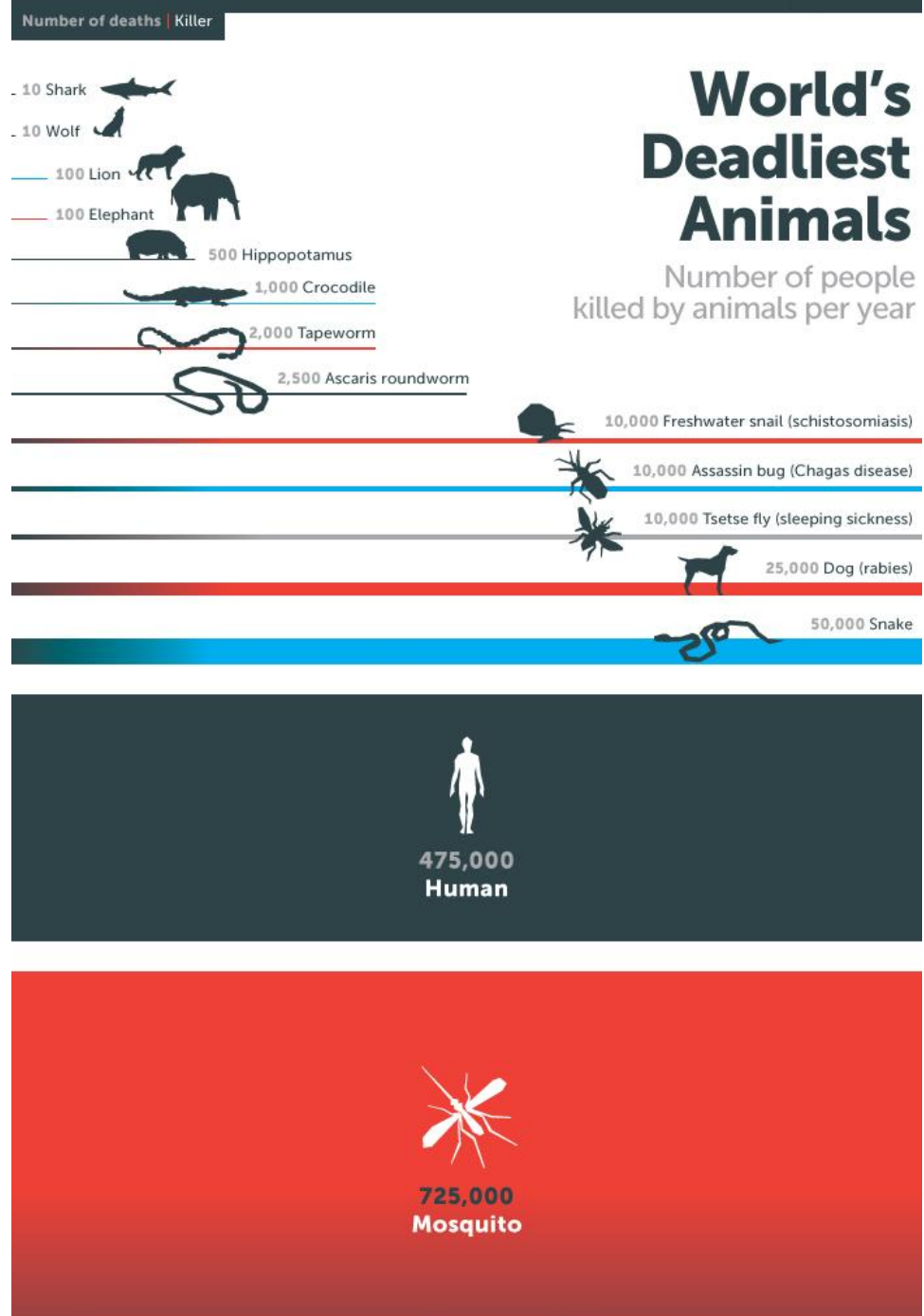
200 000 people killed per year

## Top 10 World's deadliest animals

[gatesnotes](#)  
[The blog of Bill Gates](#)

Mosquito Week

The Deadliest Animal in the World  
 By [Bill Gates](#)  
 | April 25, 2014



**SOURCES:** WHO; crocodile-attack.info; Kasturiratne et al. (doi.org/10.1371/journal.pmed.0050218); FAO (webcitation.org/6OgpS8SV0); Linnell et al. (webcitation.org/6ORL7DBUO); Packer et al. (doi.org/10.1038/2F436927a); Alessandro De Maddalena. All calculations have wide error margins.



# Neglected Tropical Diseases

Bill & Melinda Gates Foundation.  
<http://www.gatesfoundation.org/>

## *What We Do*

### **NEGLECTED INFECTIOUS DISEASES STRATEGY OVERVIEW**

#### **New Diseases**

To improve prospects for curbing six newly targeted diseases—ascaris, trichuris, hookworm, [schistosomiasis](#), Buruli ulcer, and Chagas disease—we are investing in research to better understand their transmission patterns and what tools or interventions are needed to fight them.

# The most neglected schistosome among schistosomes

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

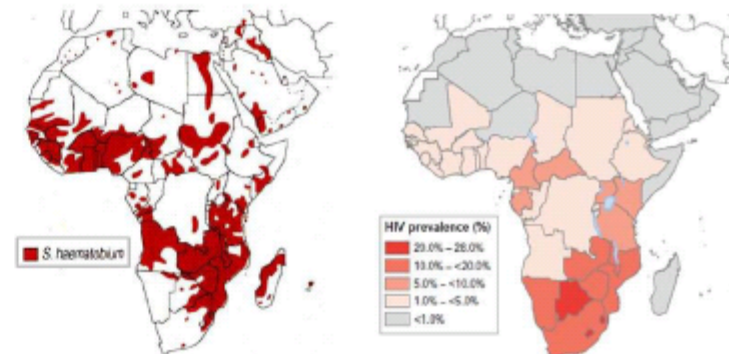
Parasite Species	Approximate Number of Human Cases	Number of PubMed Citations over the Last Five Years <sup>b</sup>	PubMed Citations per Millions of Human Cases	References
<i>Schistosoma japonicum</i>	1 million	644	644	Steinmann et al. 2006 [1]
<i>Schistosoma mansoni</i>	54 million <sup>a</sup>	1,371	25	Van der Werf et al. 2003 [3]
<i>Schistosoma haematobium</i>	112 million <sup>a</sup>	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 Berriman<sup>1</sup>, Brian J. Haas<sup>2,3</sup>, Philip T. LoVerde<sup>4</sup>, R. Alan Wilson<sup>5</sup>, Susan T. Mashiama<sup>6,10</sup>, Bissan Al-Lazikani<sup>11</sup>, Luiz F. Andrade<sup>12</sup>, Pete Daniella C. Bartholomieu<sup>3</sup>, Gaëlle Blandin<sup>3</sup>, Conor R. Caffrey<sup>3</sup>, Avril C. Art Delcher<sup>3</sup>, Ricardo DeMarco<sup>3,13,16</sup>, Appolinaire Djikeng<sup>3</sup>, Tina Eyre<sup>1</sup>, J. Christiane Hertz-Fowler<sup>3</sup>, Hirohisa Hirai<sup>17</sup>, Yuriko Hirai<sup>17</sup>, Robin Houst<sup>3</sup>, Daniela Lacerda<sup>3</sup>, Camila D. Macedo<sup>3,8</sup>, Paul McVeigh<sup>14</sup>, Zamin Ning<sup>1</sup>, Julian Parkhill<sup>1</sup>, Mihaela Pertea<sup>3</sup>, Raymond J. Pierce<sup>15</sup>, Anna V. Protasi<sup>3</sup>, Marie-Adele Rajandream<sup>1</sup>, Jane Rogers<sup>1</sup>, Mohammed Sajid<sup>18</sup>, Steven Adrian R. Tivey<sup>1</sup>, Owen White<sup>19</sup>, David L. Williams<sup>21</sup>, Jennifer Wortis<sup>3</sup>, Adhemar Zerlotini<sup>11</sup>, Claire M. Fraser-Liggett<sup>3</sup>, Barclay G. Barrell<sup>1</sup> &...

nature  
genetics

NATURE GENETICS VOLUME 44 | NUMBER 2 | FEBRUARY 2012

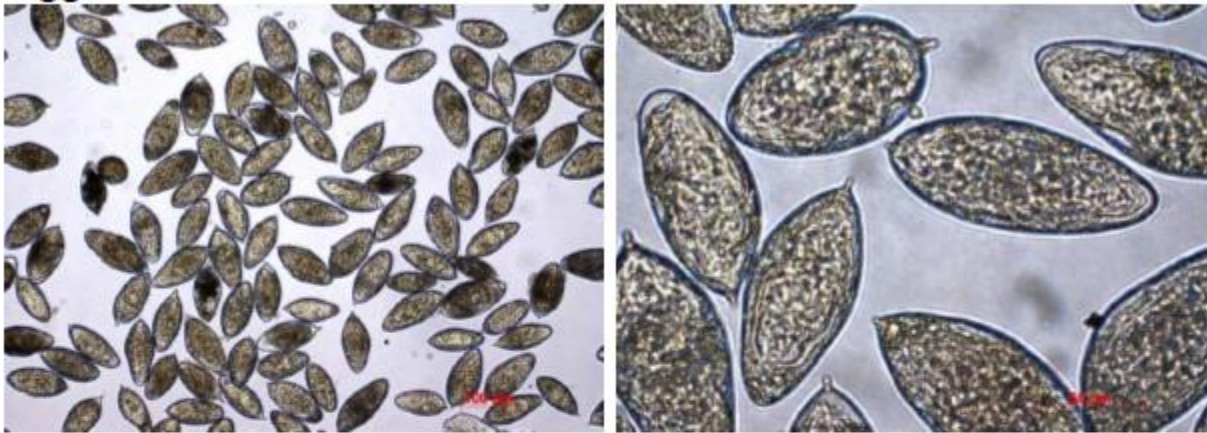
## Whole-genome sequence of *Schistosoma haematobium*

Neil D Young<sup>1,11</sup>, Aaron R Jex<sup>1,11</sup>, Bo Li<sup>2,11</sup>, Shiping Liu<sup>2</sup>, Linfeng Yang<sup>2</sup>, Zijun Xiong<sup>2</sup>, Yingrui Li<sup>2</sup>, Cinzia Cantacessi<sup>1</sup>, Ross S Hall<sup>1</sup>, Xun Xu<sup>2</sup>, Fangyuan Chen<sup>2</sup>, Xuan Wu<sup>2</sup>, Adhemar Zerlotini<sup>3</sup>, Guilherme Oliveira<sup>3</sup>, Andreas Hofmann<sup>1,4</sup>, Guojie Zhang<sup>2</sup>, Xiaodong Fang<sup>2</sup>, Yi Kang<sup>2</sup>, Bronwyn E Campbell<sup>1</sup>, Alex Loukas<sup>5</sup>, Shoba Ranganathan<sup>6,7</sup>, David Rollinson<sup>8</sup>, Gabriel Rinaldi<sup>9,10</sup>, Paul J Brindley<sup>10</sup>, Huanming Yang<sup>2</sup>, Jun Wang<sup>2</sup>, Jian Wang<sup>2</sup> & Robin B Gasser<sup>1</sup>

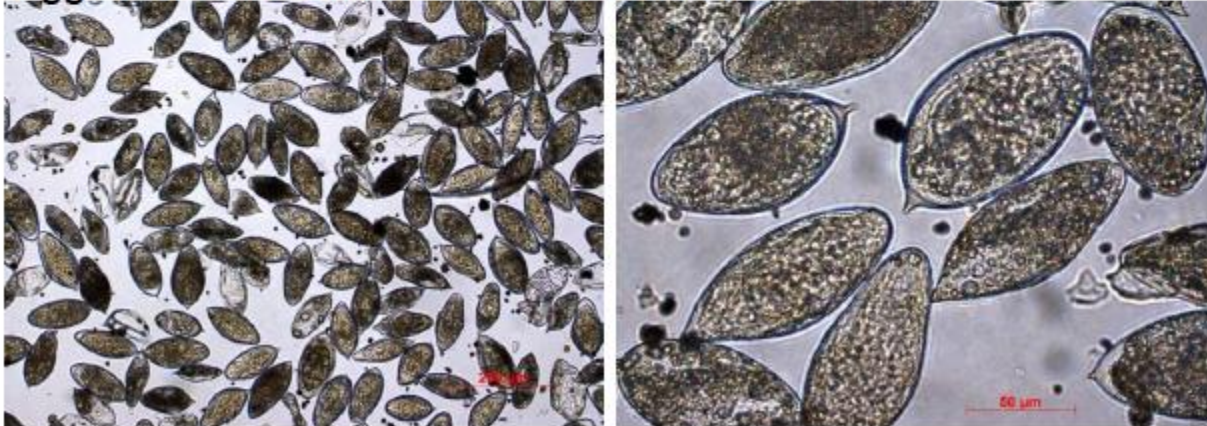


## ***In vitro* culture of *Schistosoma haematobium* developmental stages**

**Eggs isolated from liver of infected hamsters**



**Eggs isolated from intestine of infected hamsters**



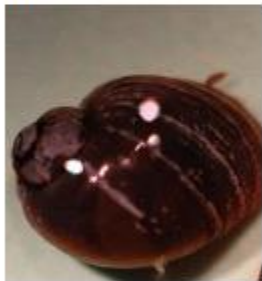


## *In vitro* culture of *Schistosoma haematobium* developmental stages

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 Schistosomiasis associated bladder cancer

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

Mónica C. Botelho, M.Sc.<sup>a,b,\*</sup>, Paula A. Oliveira, Ph.D.<sup>c</sup>, Carlos Lopes, Ph.D.<sup>d,e</sup>,  
José M. Correia da Costa, Ph.D.<sup>a</sup>, José C. Machado, Ph.D.<sup>b,f</sup>

<sup>a</sup> CIBP–Centre for Parasite Immunology and Biology, National Institute of Health, Porto, Portugal

<sup>b</sup> IPATIMUP–Institute of Pathology and Molecular Immunology of Porto University, Porto, Portugal

<sup>c</sup> CECAV–Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

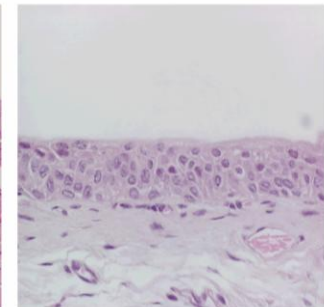
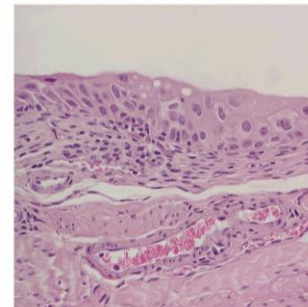
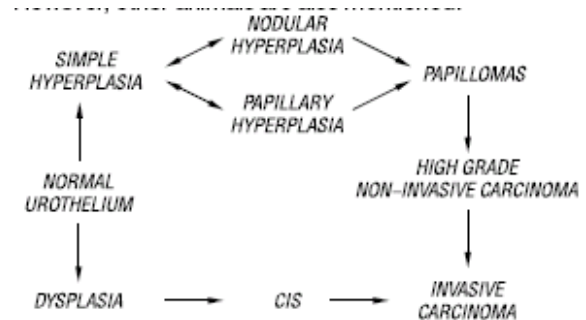
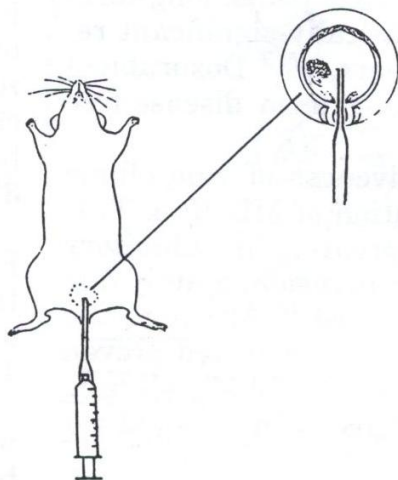
<sup>d</sup> ICBAS–Institute of Biomedical Sciences Abel Salazar, Department of Cellular Biology and Immunology, Porto University, Porto, Portugal

<sup>e</sup> IPO–Portuguese Institute of Oncology, Department of Pathology, Porto, Portugal

<sup>f</sup> 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<sup>a,b,\*</sup>, Nuno Vale<sup>c</sup>, Maria João Gouveia<sup>c</sup>, Gabriel Rinaldi<sup>d,e</sup>, Julio Santos<sup>f</sup>, Lucio L. Santos<sup>g</sup>, Paula Gomes<sup>c</sup>, Paul J. Brindley<sup>d</sup>, José Manuel Correia da Costa<sup>a,b</sup>

<sup>a</sup>Center for the Study of Animal Science, ICETA, University of Porto, Portugal

<sup>b</sup>INSA, National Institute of Health, Rua Alexandre Herculano, 321, 4000-055 Porto, Portugal

<sup>c</sup>CIQUP, Chemistry and Biochemistry Department, Faculty of Sciences, University of Porto, Porto, Portugal

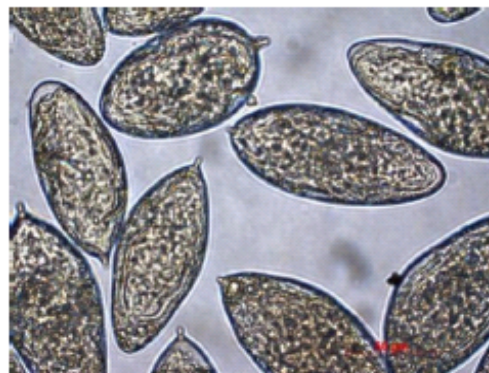
<sup>d</sup>Department of Microbiology, Immunology and Tropical Medicine, Research Center for Neglected Diseases of Poverty, School of Medicine & Health Sciences, George Washington University Washington, DC 20037, USA

<sup>e</sup>Departamento de Genética, Facultad de Medicina, Universidad de la República, (UDELAR), Montevideo 11800, Uruguay

<sup>f</sup>Clínica da Sagrada Esperança, Avenida Mortala Mohamed-ilha de Luanda, Angola

<sup>g</sup>Experimental 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)



# The Hallmarks of Cancer



Leading Edge  
Review

## Hallmarks of Cancer: The Next Generation

Douglas Hanahan<sup>1,2,\*</sup> and Robert A. Weinberg<sup>3,\*</sup>

<sup>1</sup>The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL, Lausanne CH-1015, Switzerland

<sup>2</sup>The Department of Biochemistry & Biophysics, UCSF, San Francisco, CA 94158, USA

<sup>3</sup>Whitehead Institute for Biomedical Research, Ludwig/MIT Center for Molecular Oncology, and MIT Department of Biology, Cambridge, MA 02142, USA

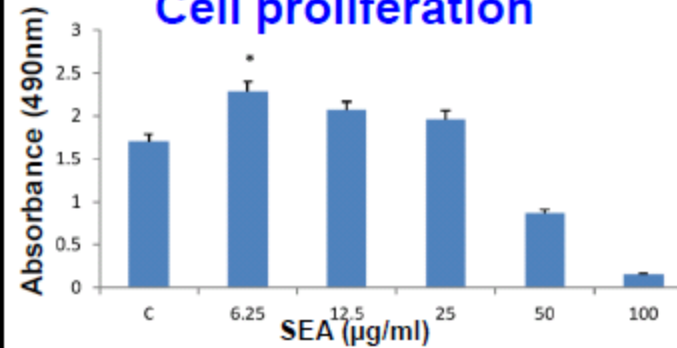
\*Correspondence: [dh@epfl.ch](mailto:dh@epfl.ch) (D.H.), [weinberg@wi.mit.edu](mailto:weinberg@wi.mit.edu) (R.A.W.)

DOI 10.1016/j.cell.2011.02.013

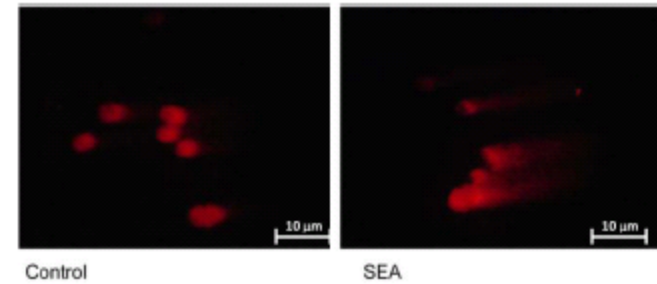
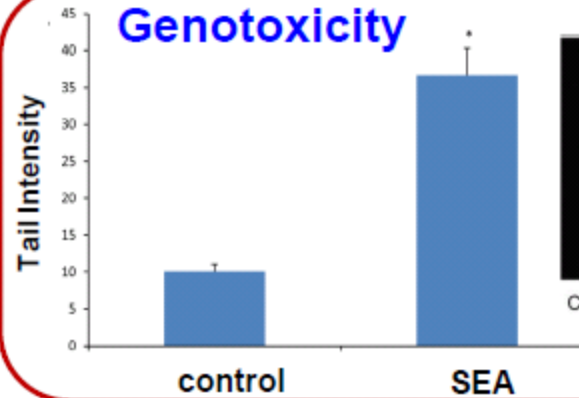
- Proliferation
- Apoptosis
- Migration
- Invasion
- Metastasis
- Angiogenesis
- Metabolism
- Immunity
- Genome instability
- Inflammation

# Soluble eggs antigens induced tumor-like phenotype in urothelial cells

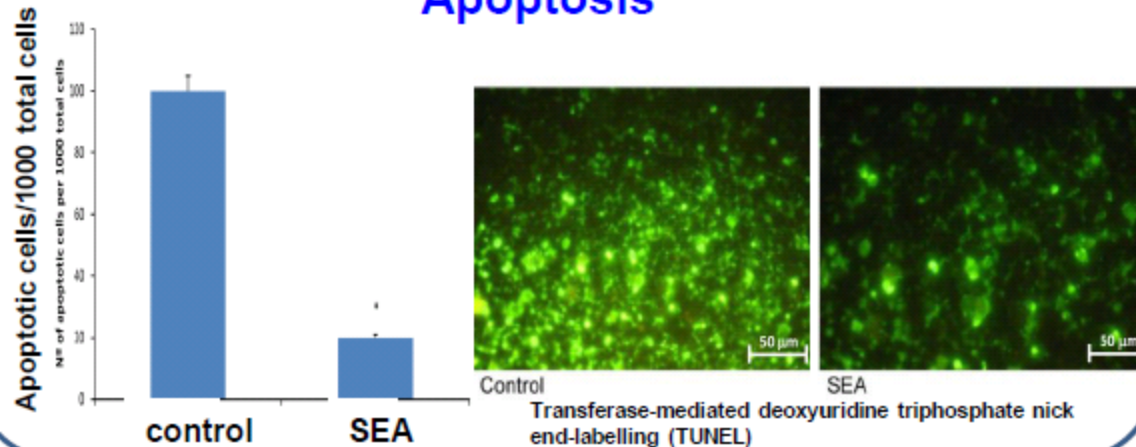
## Cell proliferation



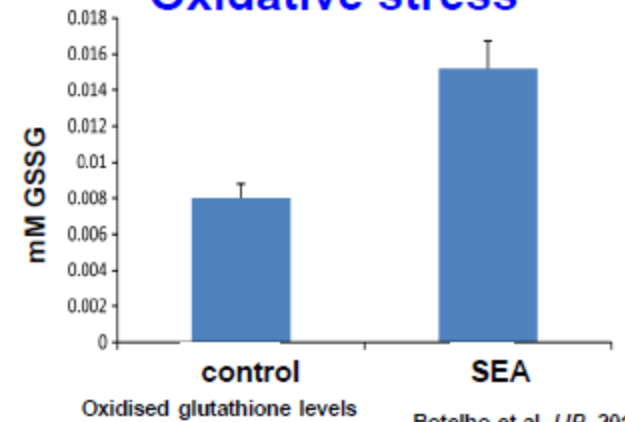
## Genotoxicity

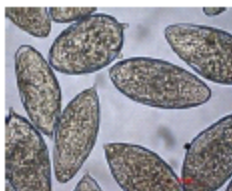


## Apoptosis

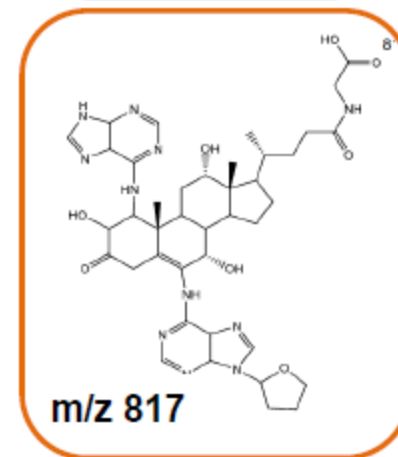
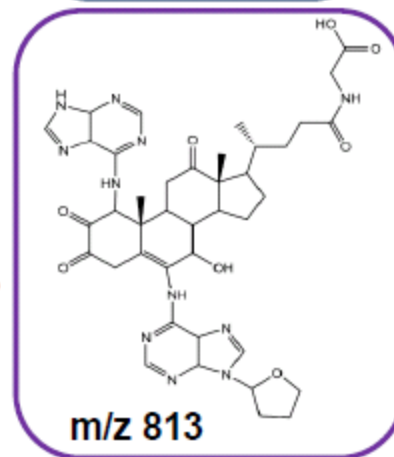
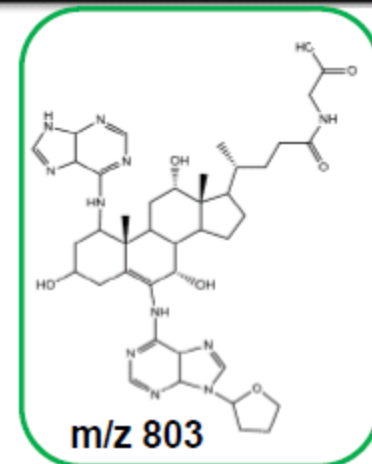
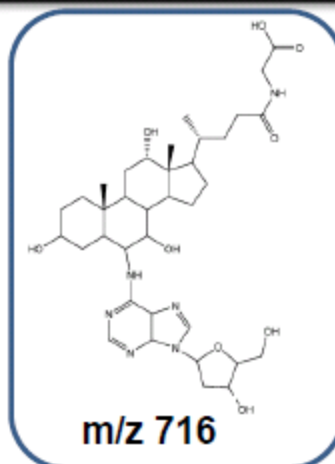


## Oxidative stress



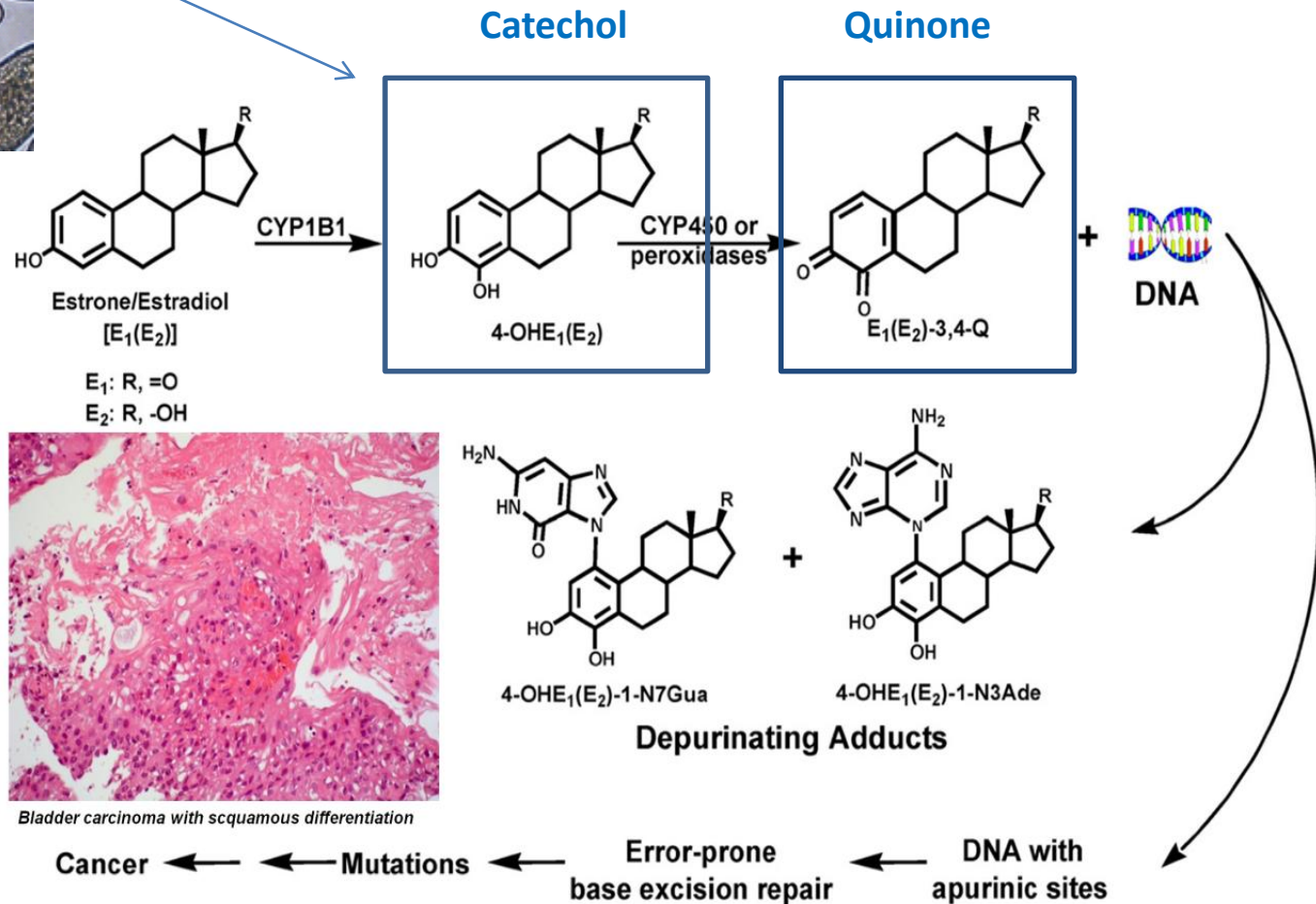
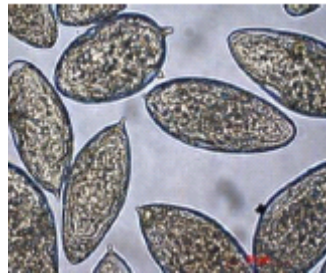


**MS identified molecules extracted from *S. haematobium*  
CATECHOL-OESTROGENS (oxidative metabolites derived  
from estrogens)**





# Pathway for Schistosomiasis Bladder Cancer



# Collaborations



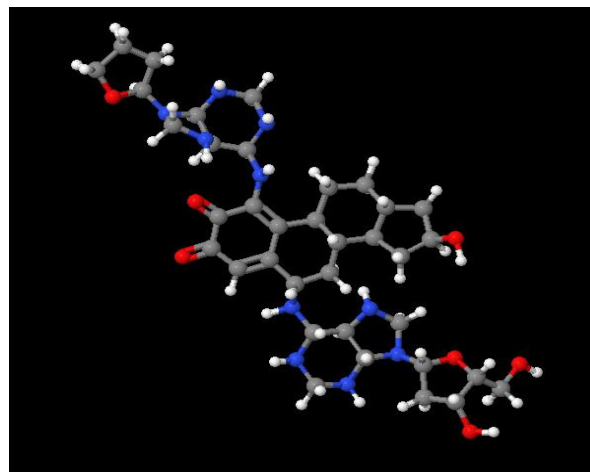
**Paul J. Brindley**  
**Gabriel Rinaldi**



**Júlio Santos**  
**Lúcio Lara**  
**Carlos Lopes**



**Joachim Richter**



**Fátima Gartner**  
**Mário Sousa**



**Alberto Barros**



**Paula Oliveira**

**Thank you for your attention**



# Infertility-associated schistosomiasis

Trends in Parasitology, June 2015, Vol. 31, No. 6

Opinion

CellPress

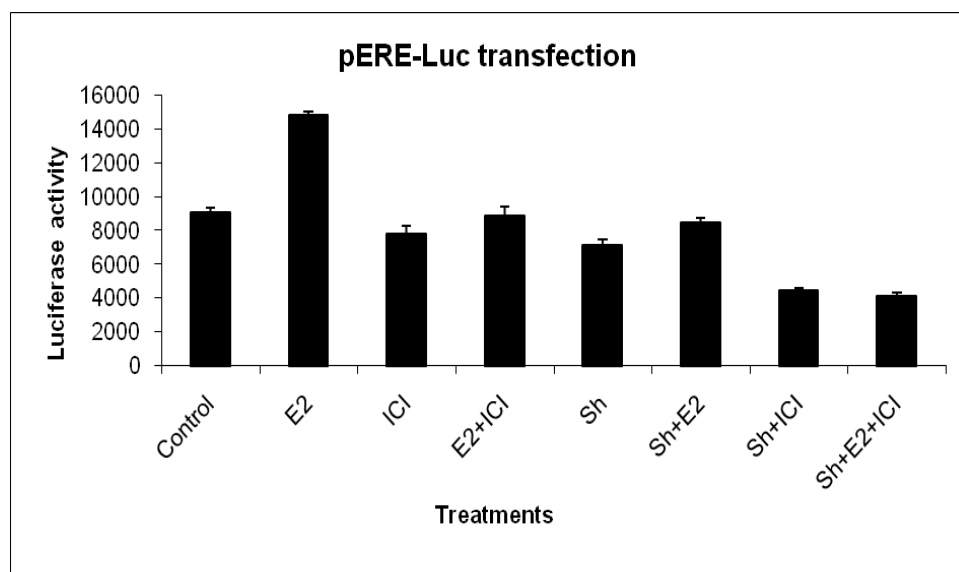
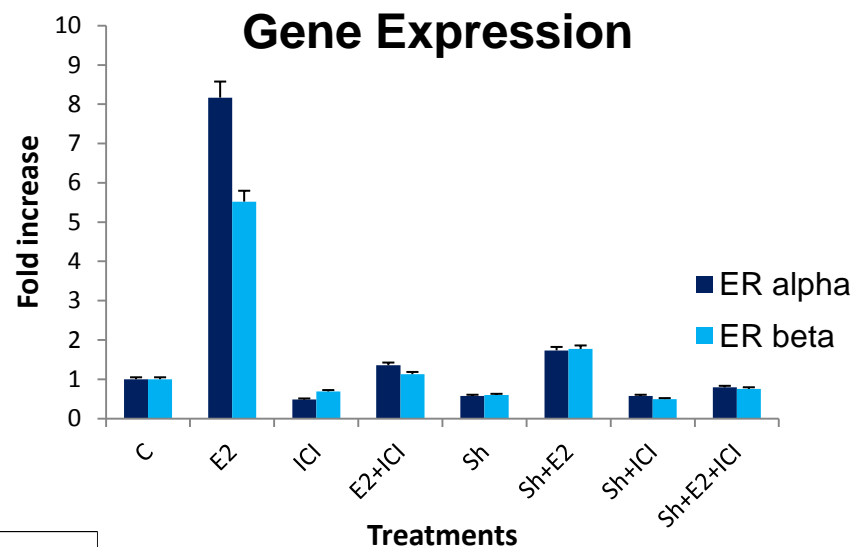
## The role of estrogens and estrogen receptor signaling pathways in cancer and infertility: the case of schistosomes

Mónica C. Botelho<sup>1,2</sup>, Helena Alves<sup>1</sup>, Alberto Barros<sup>3,4</sup>, Gabriel Rinaldi<sup>5</sup>, Paul J. Brindley<sup>5</sup>, and Mário Sousa<sup>6</sup>

- Homonal imbalance caused by estrogen-like molecules produced by schistosomes

# Schistosomes estrogen-like molecules and down-regulation of estrogen receptor

Sex	Age (years)	E2	Range	Testosterone	Range	LH	Range
Female	4	62,8	0-22	<15,0	2-10	0,114	<2,5
Male	12	30,8	0-25	77,5	5-500	1,79	0,2-8,0
Male	14	79,8	0-25	363	5-500	1,89	0,2-8,0
Male	17	45,7	0-25	724	>200	5,89	1,4-7,7
Male	17	31,9	0-25	535	>200	7,65	1,4-7,7
Male	20	68,3	<56,0	982	262-1593	2,87	1,4-7,7



Botelho *et al.* Exp Parasitol 2010

# Infertility-associated Schistosomiasis haematobia in women

## Urinary Estrogen Metabolites and Self-Reported Infertility in Women Infected with *Schistosoma haematobium*

Júlio Santos<sup>1</sup>, Maria João Gouveia<sup>2</sup>, Nuno Vale<sup>2</sup>, Maria de Lurdes Delgado<sup>3</sup>, Ana Gonçalves<sup>4</sup>, José M. Teixeira da Silva<sup>4</sup>, Cristiano Oliveira<sup>4</sup>, Pedro Xavier<sup>4</sup>, Paula Gomes<sup>2</sup>, Lúcio L. Santos<sup>1,5</sup>, Carlos Lopes<sup>1,6</sup>, Alberto Barros<sup>4,7</sup>, Gabriel Rinaldi<sup>8,9</sup>, Paul J. Brindley<sup>8</sup>, José M. Correia da Costa<sup>3,10</sup>, Mário Sousa<sup>11</sup>, Mónica C. Botelho<sup>3,10\*</sup>

Plos One 9 (2014) e96774

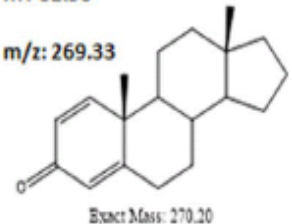
	E + (n= 25)	E - (n= 21)	OR	95% CI	P-value
Fertile women (ages)	2 (29, 63)	6 (28–94)			
Group 2+3 (ages)	15 (19–41)	2 (21–34)	4.33	1.13–16.70	0.03
Group 2 (ages)	9 (18–20)	1 (21)	2.67	0.60–11.80	n.a.
Group 3 (ages)	6 (27–41)	1 (34)	4.75	0.51–44.50	n.a.
Total	17	8			
≤12 years	8	13			
Total	25	21			

Women unable to become pregnant after one year of trial (Self-reported primary infertility - Group 2) and those who had borne fewer children than desired (Self-reported secondary infertility - Group 3).

OR, odds ratio; CI, confidence interval.

RT: 32.96

m/z: 269.33





# Infertility associated *Schistosomiasis mansoni*

## Schistosoma mansoni infection impairs reproduction in mice

Reding C<sup>1</sup>, Reding A<sup>1</sup>, Lopes G<sup>2</sup>, Sousa M<sup>3</sup>, Gartner F<sup>4</sup>, Alves H<sup>5</sup>, Richter J<sup>6</sup>, Oliveira PA<sup>7</sup>, Botelho MC<sup>5</sup>

Unpublished results

- Mating
- Gestational period
- Synchronization
- Number pups

# Infertility associated *Schistosomiasis mansoni*

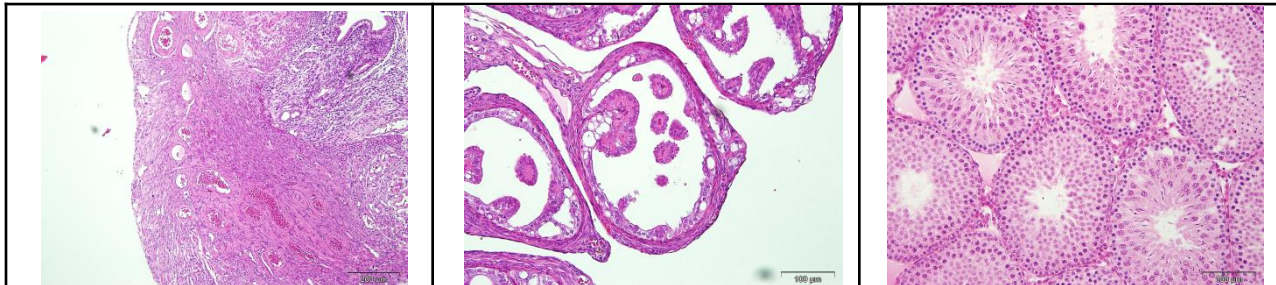
Animals	Gestational length (days)	Synchronicity (days)	Number of pups
2FCx1MC	25	0-1	15.1
2FCX1MI	25.6	0-2	14.5
2FIX1MC	22.8	1-6	13.8
2FIX1MI	21.8	3-8	11.9

Ovary

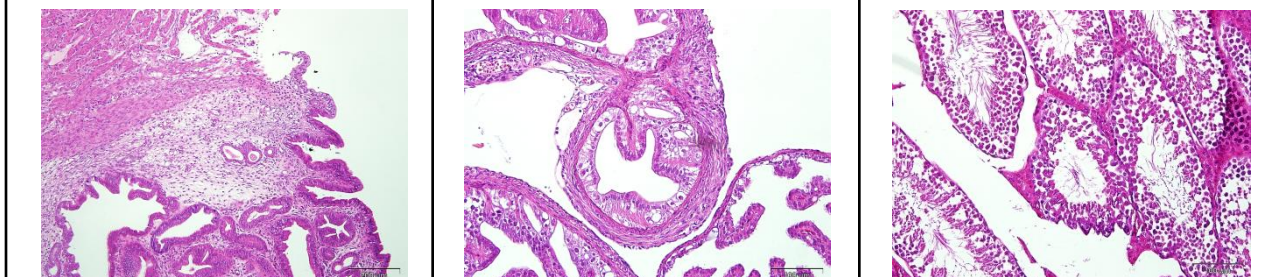
Tube

Testes

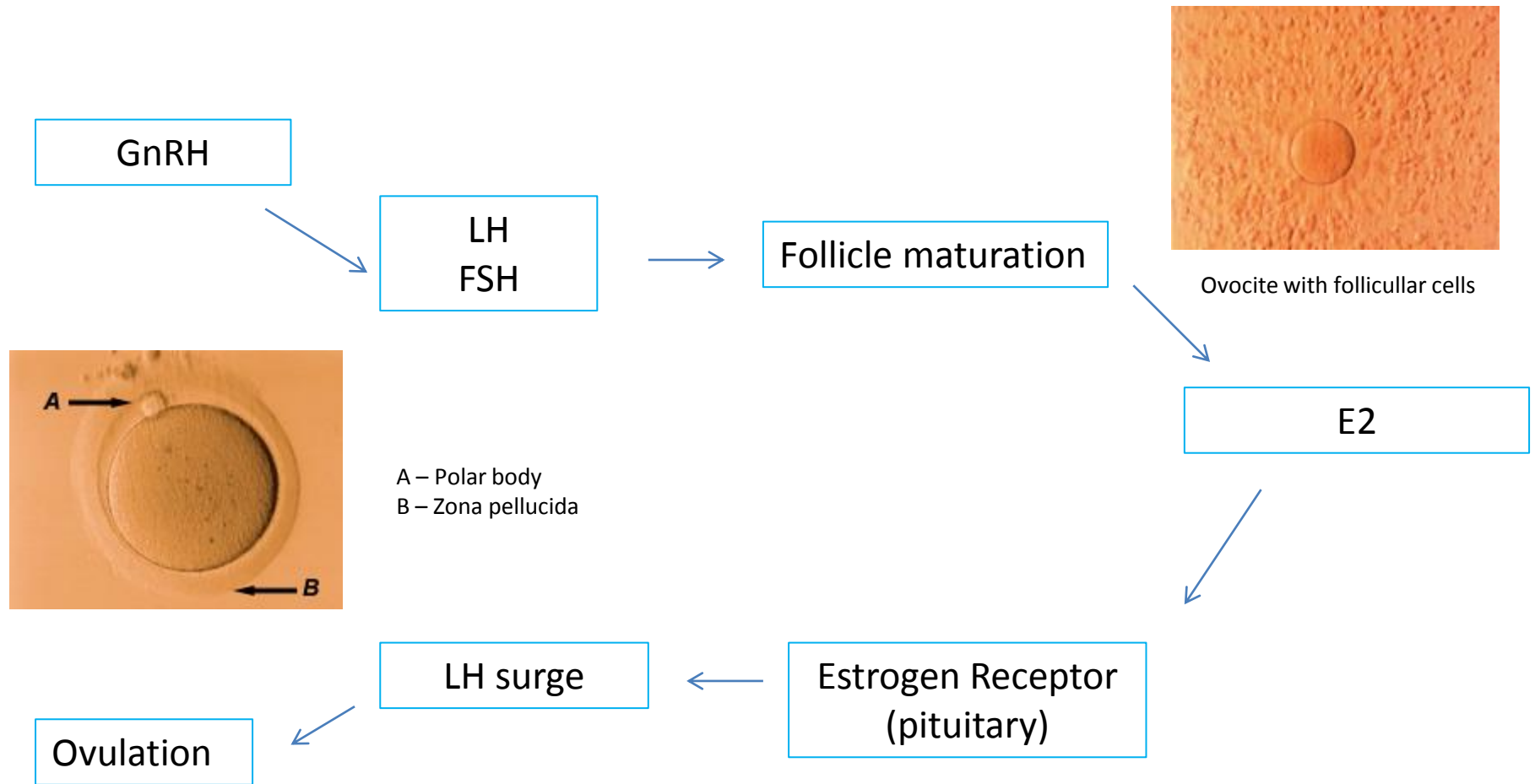
Control



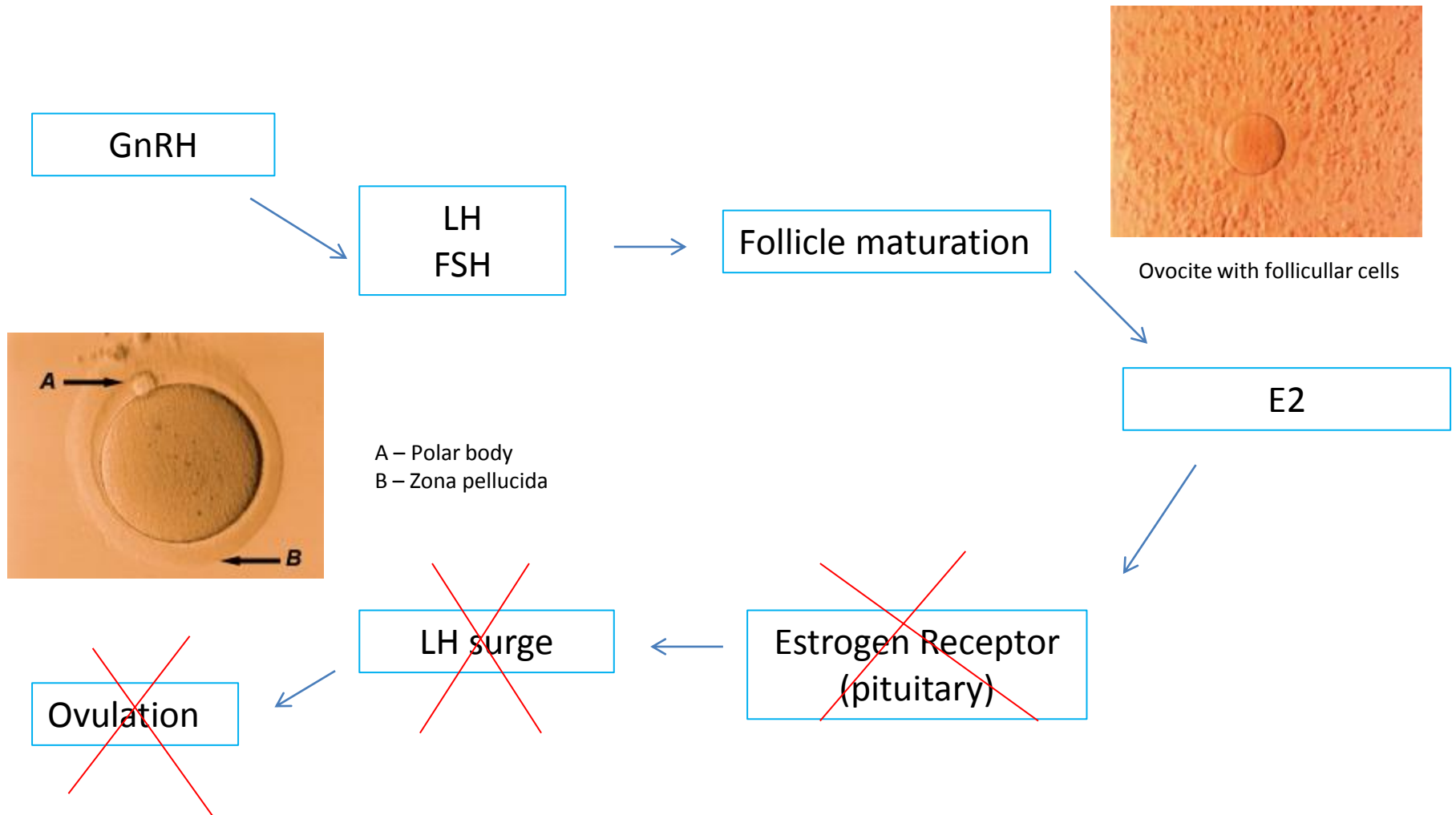
*S. mansoni*



# Pathway for Female Fertility



# Mechanism for Schistosomiasis Female Infertility





# 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 $\beta$  dehydrogenase, also known as 17 $\beta$  hydroxysteroid dehydrogenase or 17 $\beta$  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 regions. (Potential for Biomarkers screening)

# OUTLINE

## **1. *Schistosoma haematobium*: the neglected schistosome**

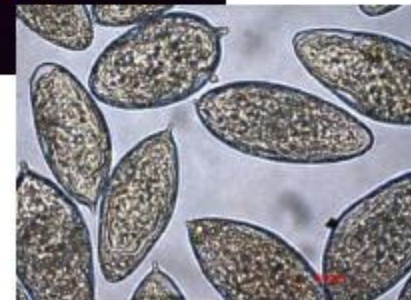
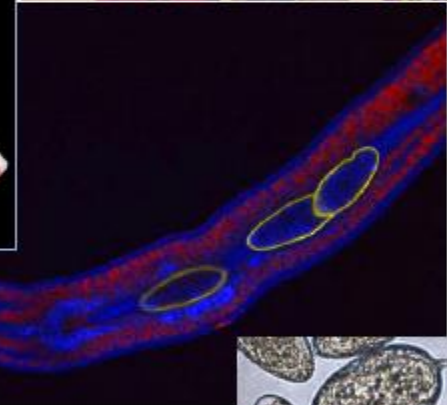
- Facts of figures

## **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*



# The Hallmarks of Cancer

Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

## The Hallmarks of Cancer

## Review

Douglas Hanahan\* and Robert A. Weinberg†

\*Department of Biochemistry and Biophysics and  
Hormone Research Institute  
University of California at San Francisco  
San Francisco, California 94143

†Whitehead Institute for Biomedical Research and  
Department of Biology  
Massachusetts Institute of Technology  
Cambridge, Massachusetts 02142

evolve progressively from normalcy via a series of pre-malignant states into invasive cancers (Foulds, 1954).

These observations have been rendered more concrete by a large body of work indicating that the genomes of tumor cells are invariably altered at multiple sites, having suffered disruption through lesions as subtle as point mutations and as obvious as changes in chromosome complement (e.g., Kinzler and Vogelstein, 1996). Transformation of cultured cells is itself a multistep process: rodent cells require at least two intro-

- Proliferation
- Apoptosis
- Migration
- Invasion
- Metastasis
- Angiogenesis



# Pathways for Estrogen Carcinogenesis and Infertility

Two different complementary pathways probably contribute to estrogen imbalance leading to:

- Initiation and promotion of cancer progression

