

# *Schistosoma haematobium* e cancro da bexiga: a verdade escondida

Mónica Botelho

9/4/2013



- What is schistosomiasis?



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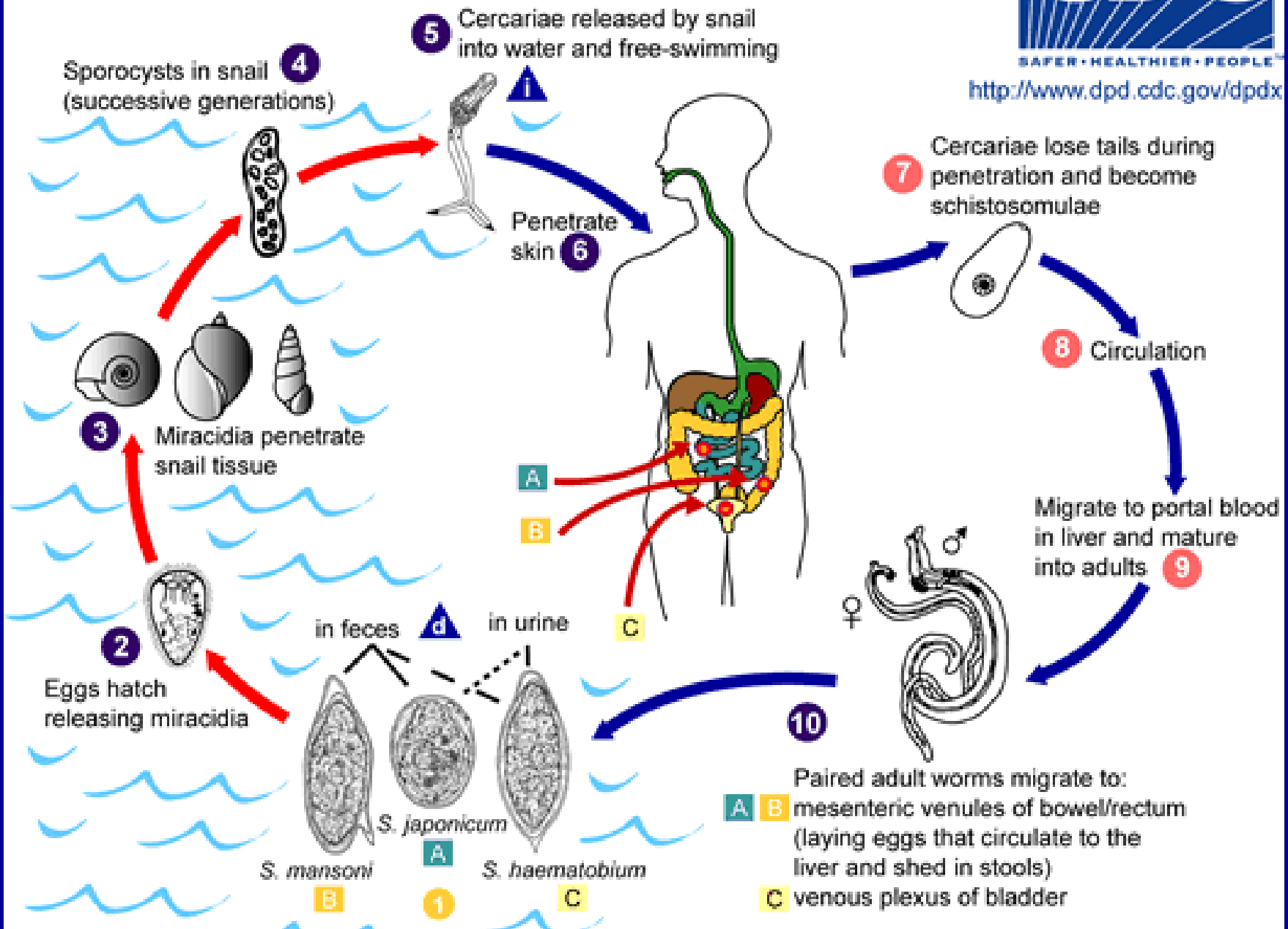
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**i** = Infective Stage  
**d** = Diagnostic Stage





**BEWARE**



**BILHARZIA**

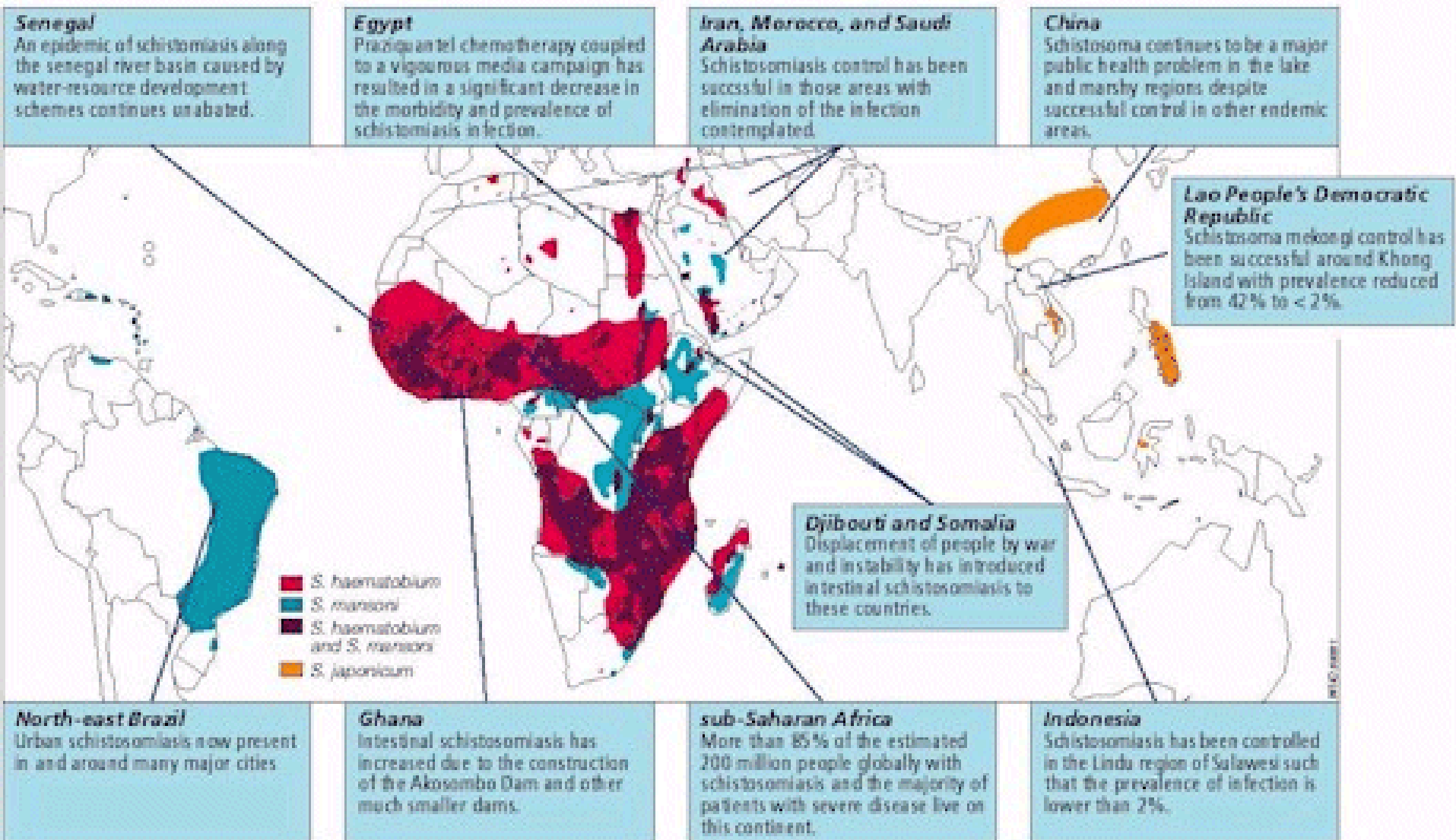
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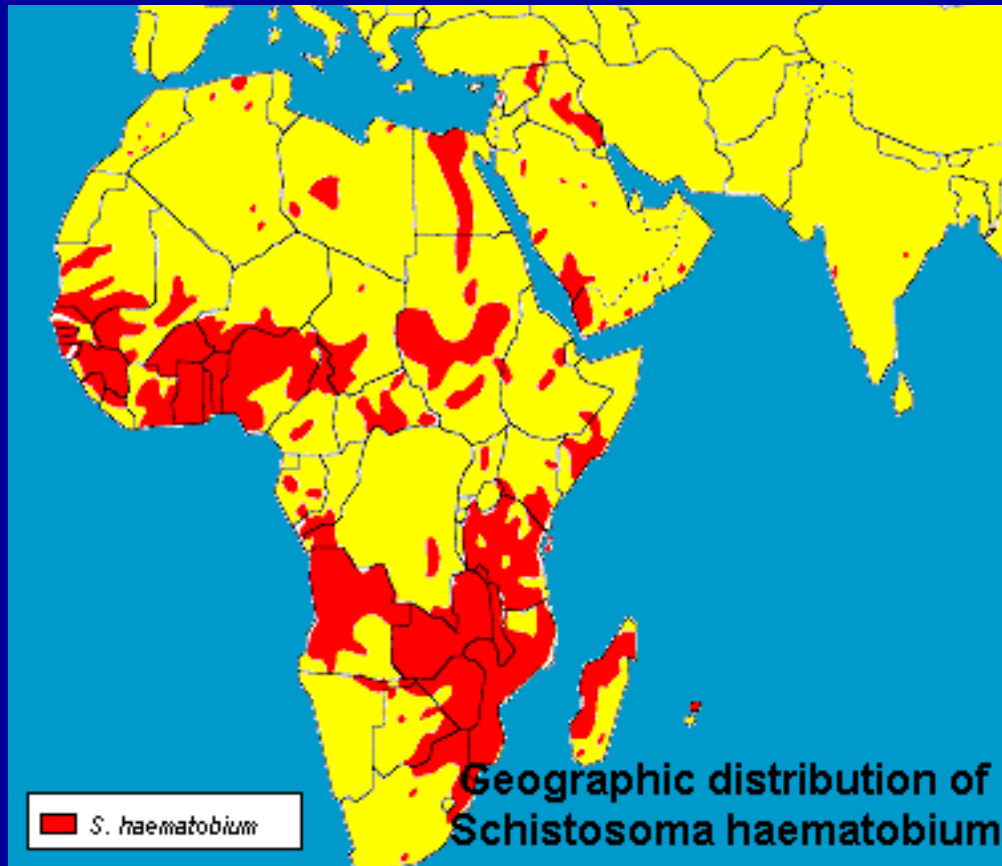
*ERECTED BY ASSOCIATION FOR BILHARZIA  
CONTROL • P.O. BOX 2171 BULAWAYO*

# Schistosomiasis: facts and figures

- Human schistosomes currently infect more than 200 million people in 76 countries worldwide in the endemic areas of Africa, the Caribbean, Central America, South America, East Asia, and the Middle East.

# Global distribution of Schistosomiasis

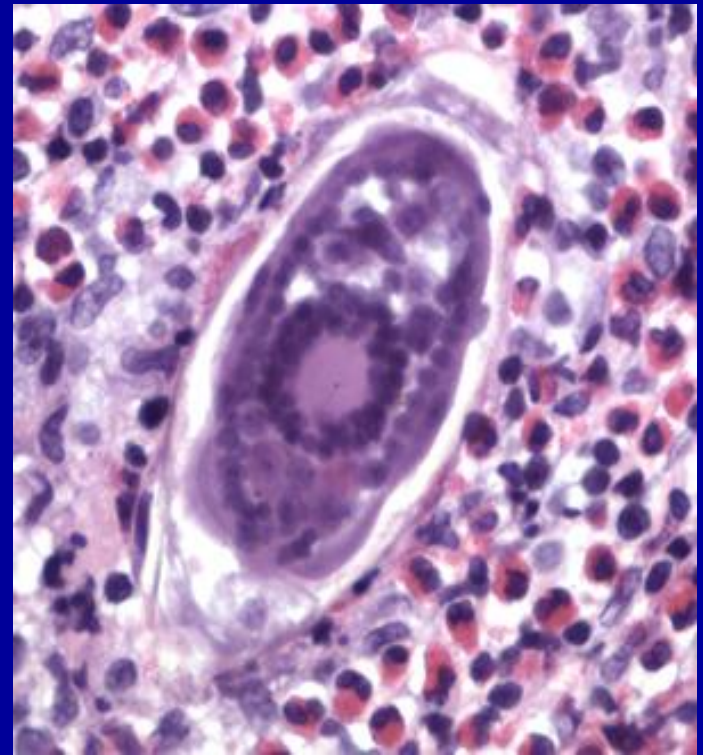




- Is there a role for *S. haematobium* in bladder cancer?

# *S. haematobium* and bladder cancer

- A causal association between the parasite and bladder cancer was postulated in 1911 by Fergusson, but so far proof of this association has remained elusive.



# *S. haematobium* and bladder cancer

Squamous cell carcinoma of the urinary bladder has been associated with *Schistosoma haematobium* infection in many parts of Africa.

A parasite-tumor linkage is further suggested by the predominance of squamous cell (as opposed to transitional cell) morphology of bladder carcinomas seen in *S. haematobium*-endemic areas.

- *What about host endocrine system?*

# Schistosomiasis and host hormones

It has been shown that schistosomes synthesize steroid hormones (Nirde et al, FEBS Letters, 1983).

Schistosomes produce hormone-like signals (Mendonça et al, Parasitology Today, 2000).

Existence of receptors able to bind the molecules of estradiol (Barrabes, Ann Parasitol Hum Comp, 1986; Mendonça et al, Parasitology Today, 2000).

# Schistosomiasis and host hormones

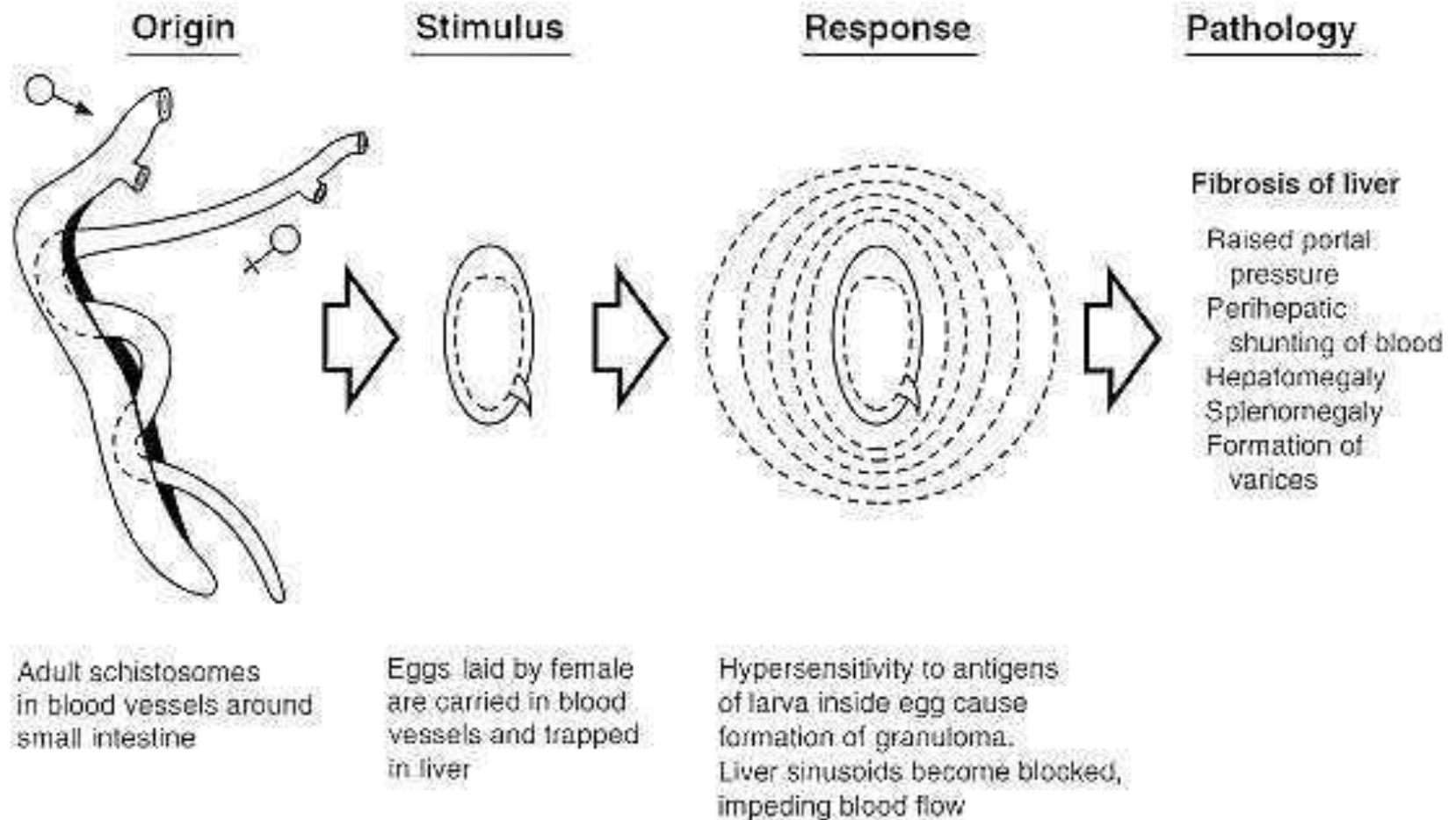
Recent experimental evidence suggests that schistosomes can not only evade immune responses actively but also exploit the hormonal microenvironment within the host to favor their establishment, growth and reproduction (Escobedo et al, Trends in Parasitology, 2005).

# Aims

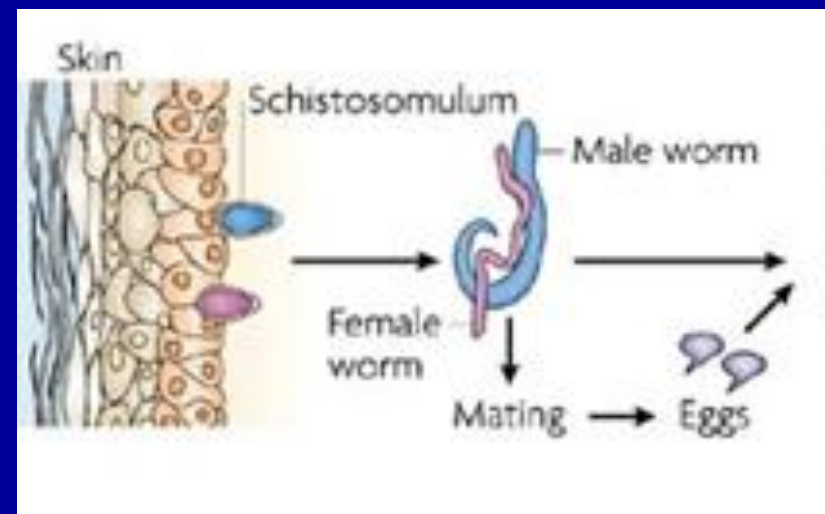
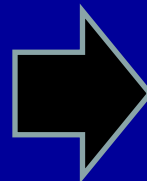
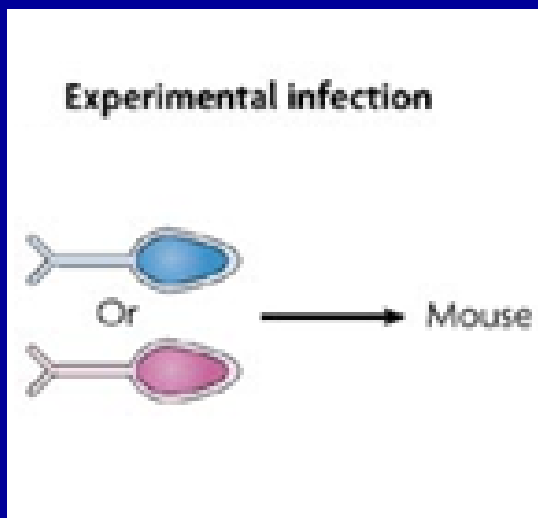
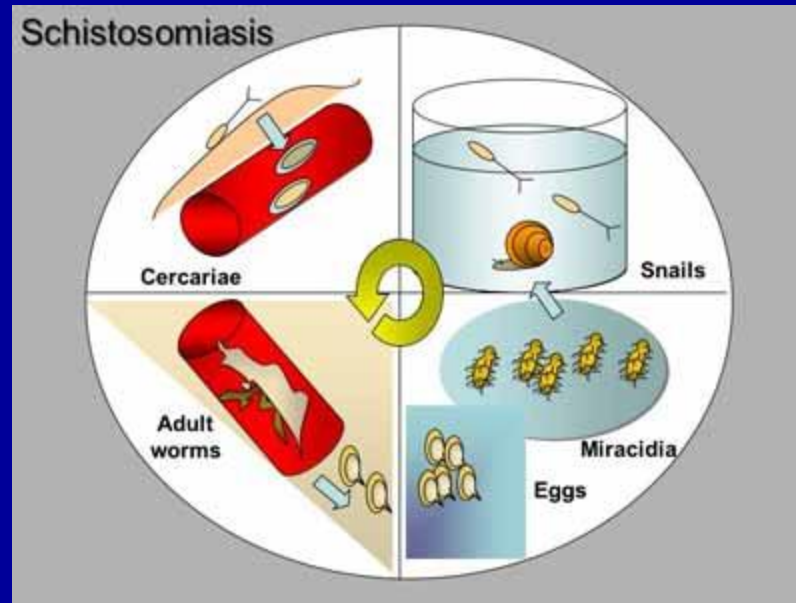
- A) To study the mechanisms underlying the association between *S. haematobium* and SCC of the bladder
- B) To understand how host endocrine system can favor the establishment of schistosomes

*S. haematobium* and bladder  
cancer

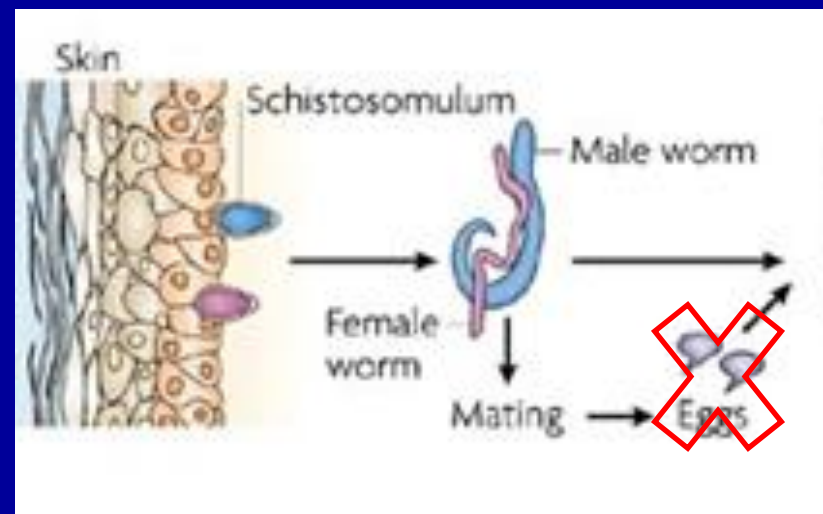
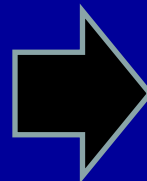
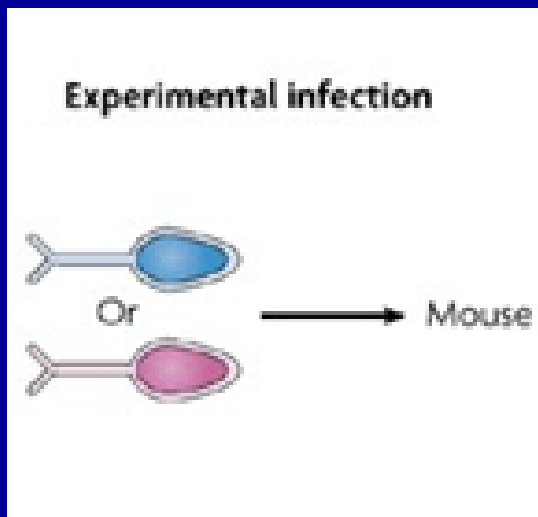
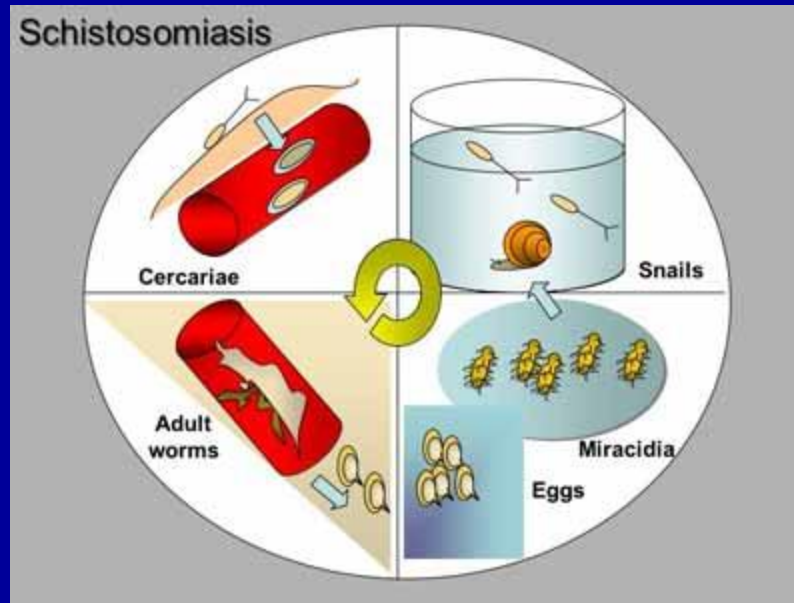
# *Schistosoma haematobium* immature worms induce granulomatous-like immune reaction and hepatic fibrosis similar to parasite eggs



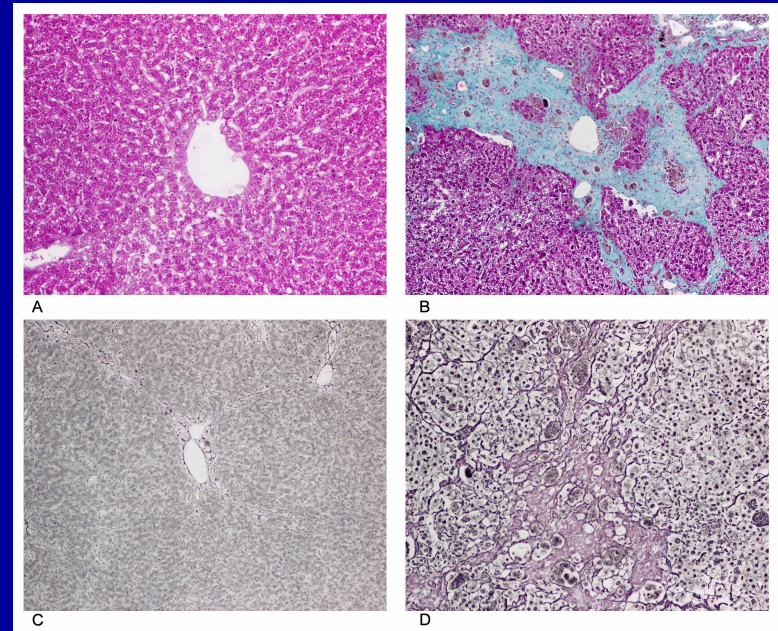
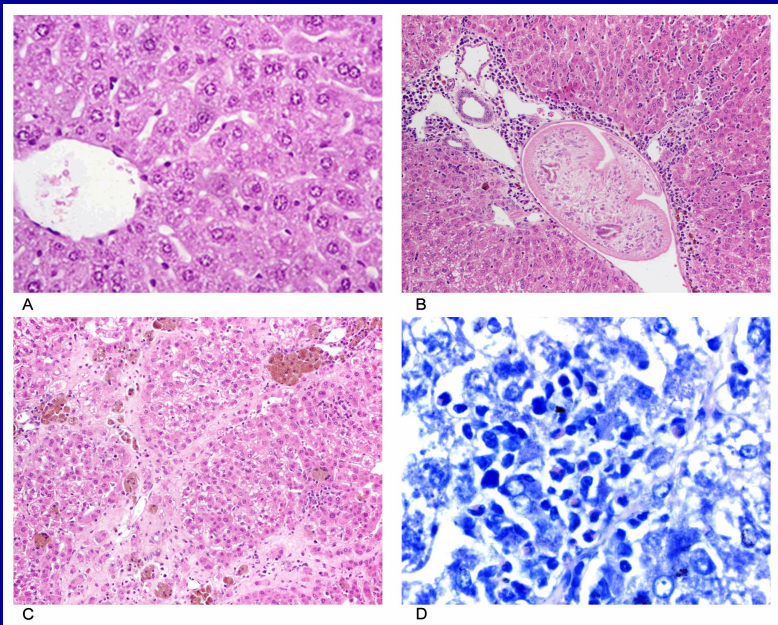
# Methodological strategy



# Methodological strategy



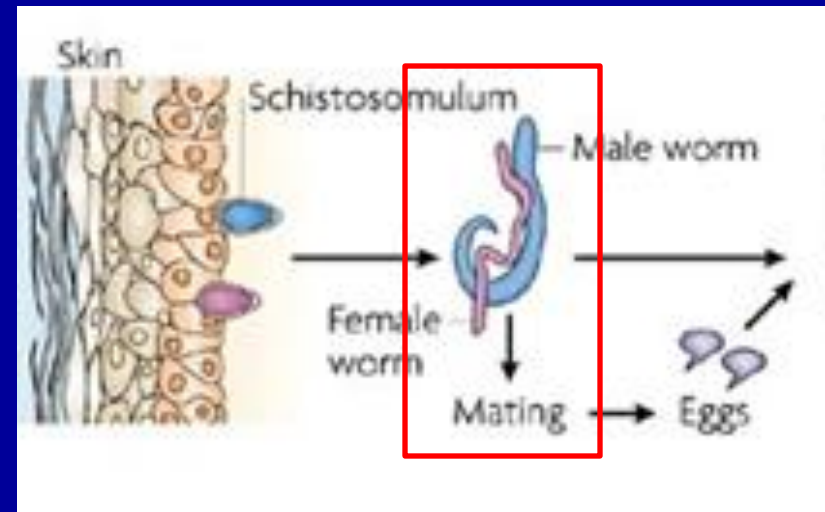
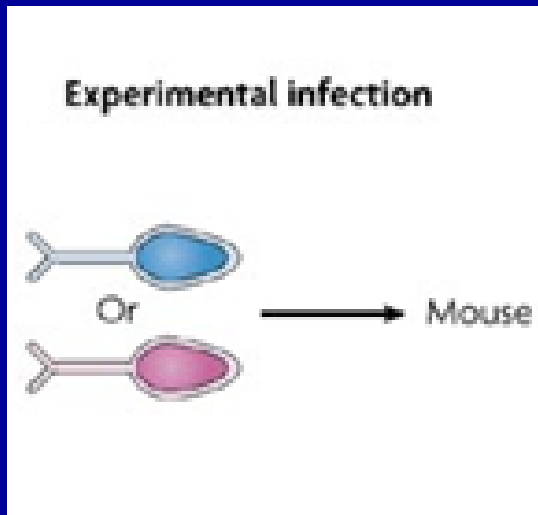
*Schistosoma haematobium* immature worms induce granulomatous-like immune reaction and hepatic fibrosis similar to parasite eggs



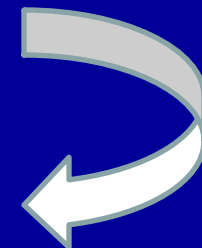
*S. haematobium* total antigen increases the proliferation, decreases the apoptosis and induces invasion of epithelial cells *in vitro*



# Methodological Strategy

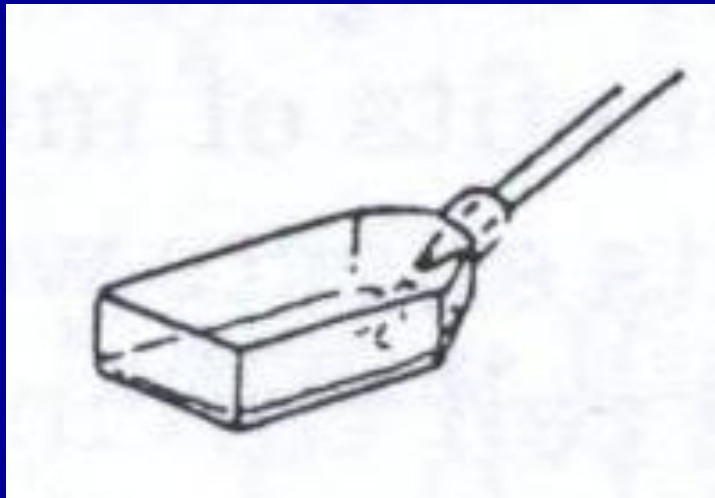


*S. haematobium*  
total antigen (Sh)



# Methodological Strategy

Chinese Hamster  
Ovary (CHO) cells

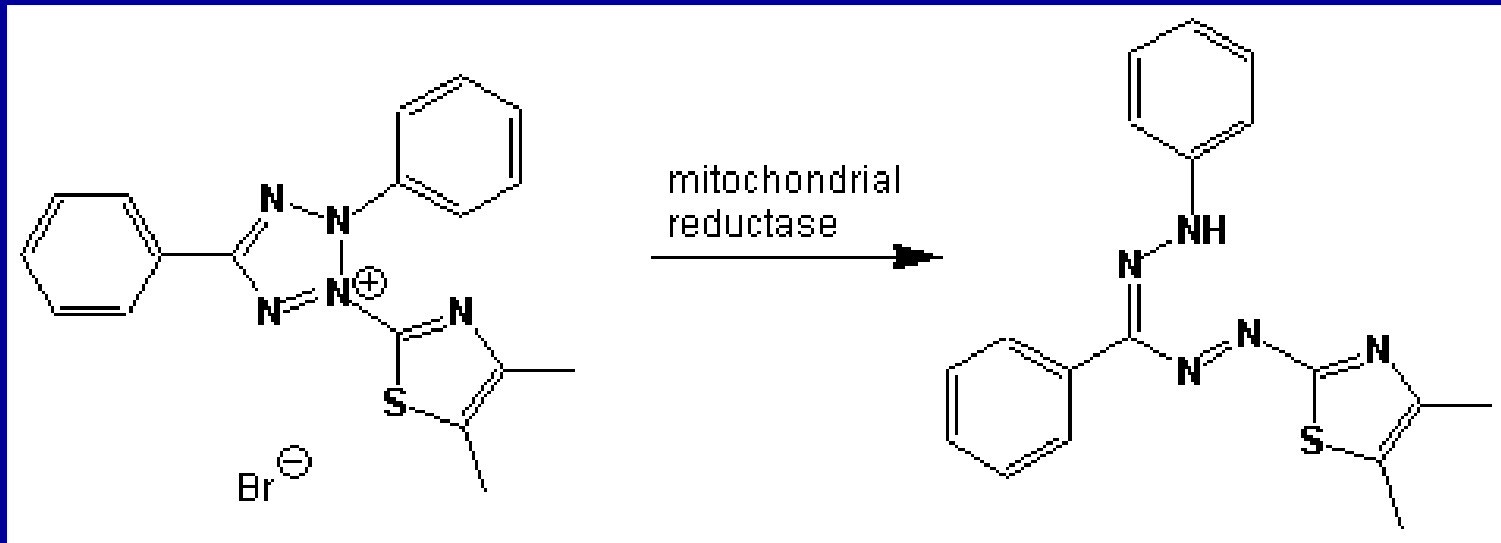


Sh-treatment 48 h



# Methodological Strategy

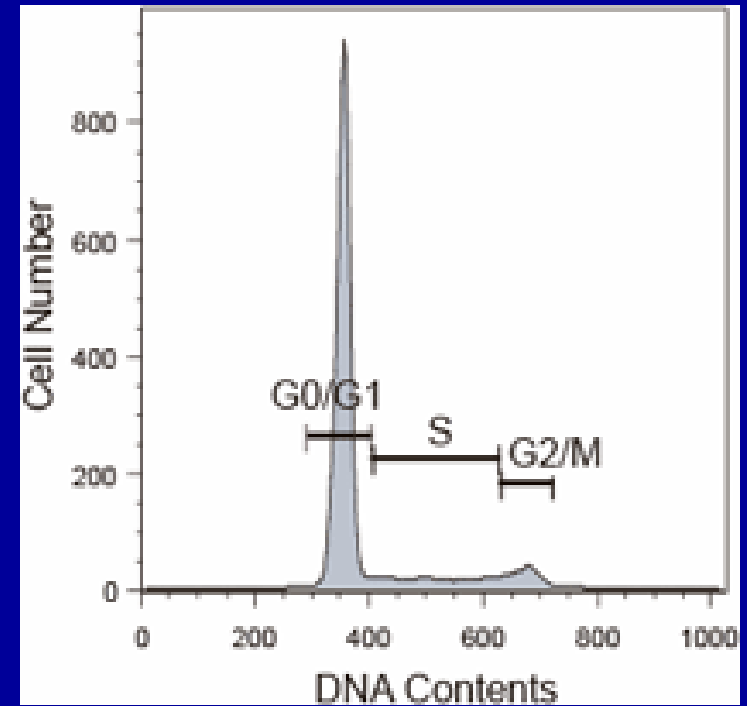
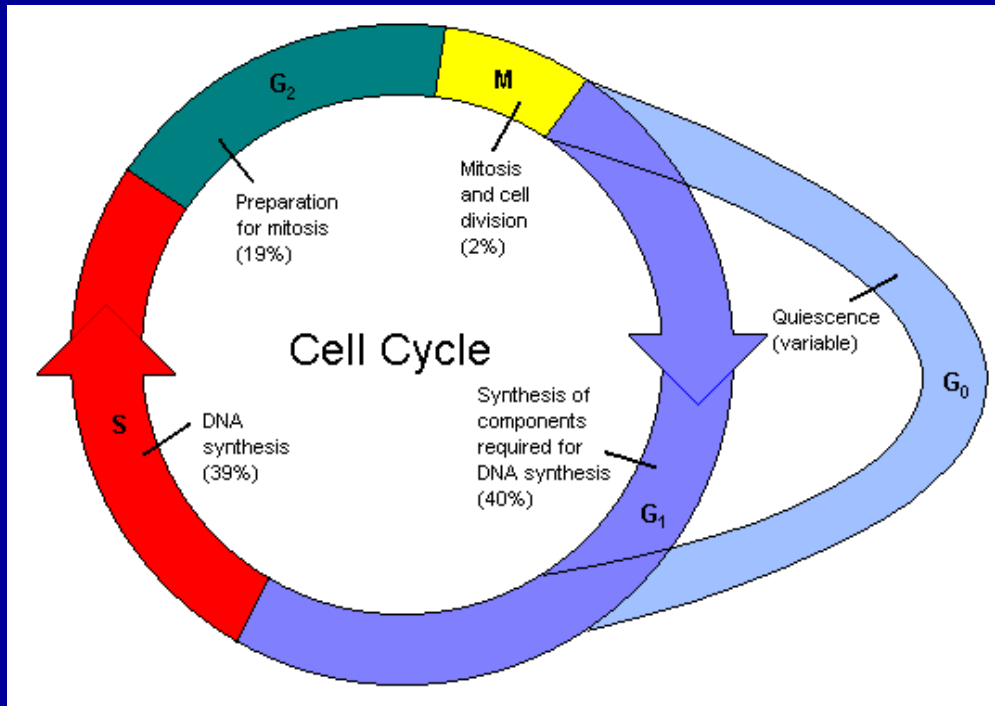
## Proliferation MTT assay



Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells.

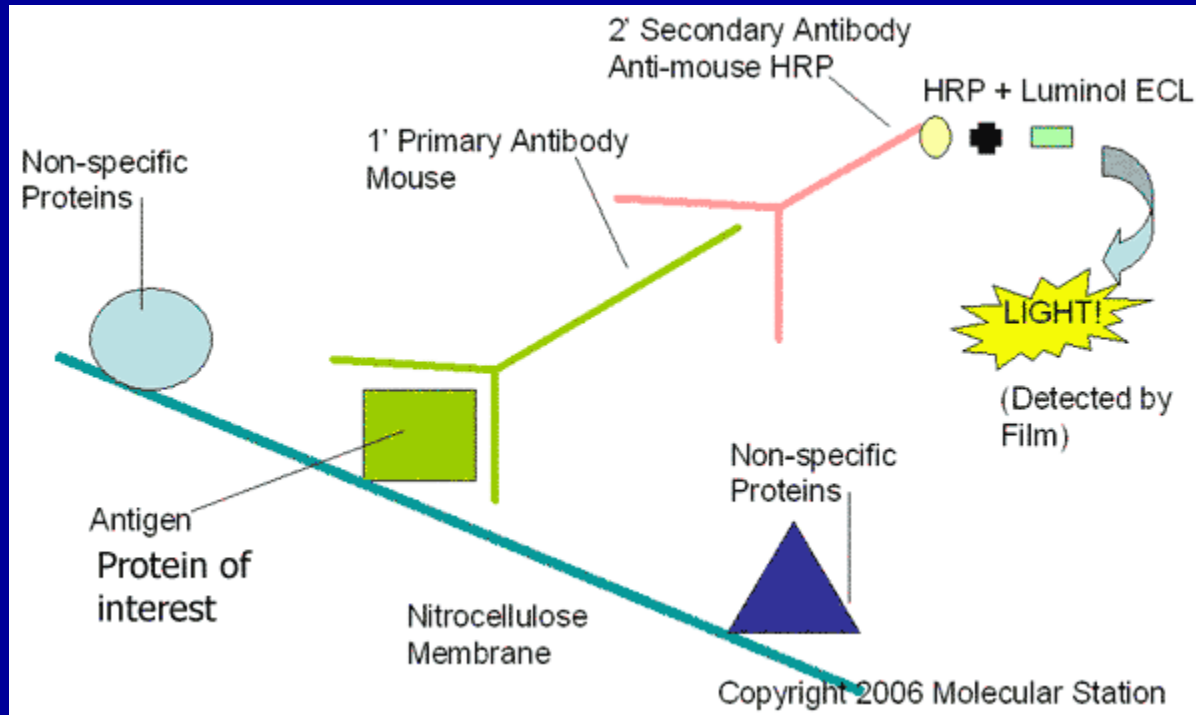
# Methodological Strategy

## DNA content Flow cytometry



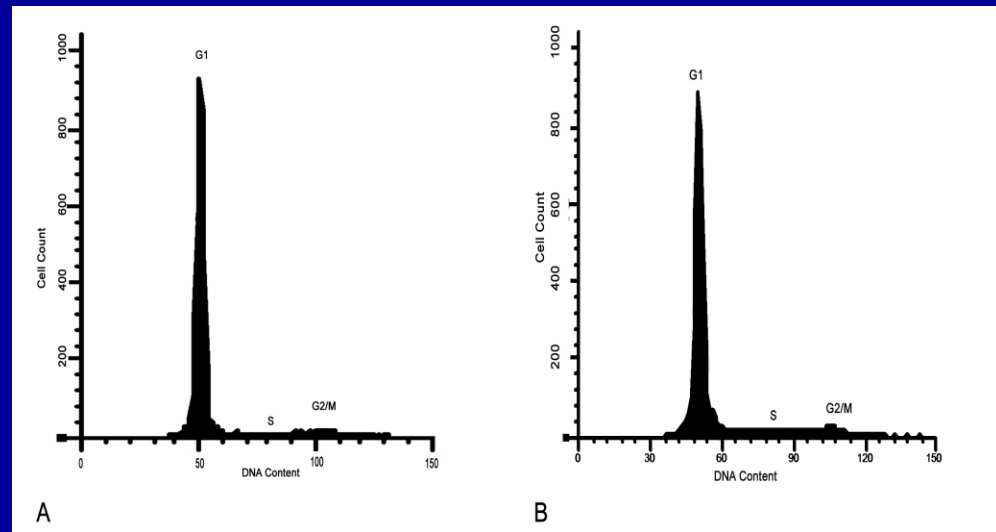
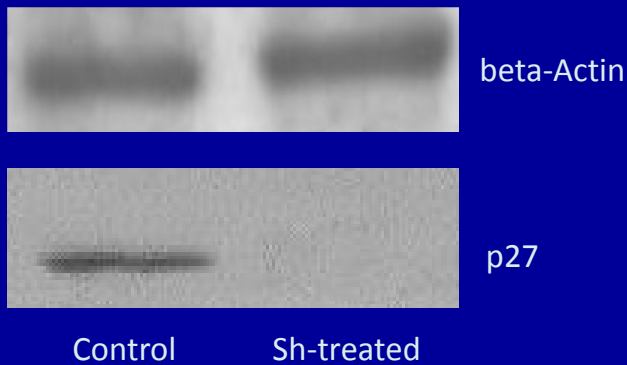
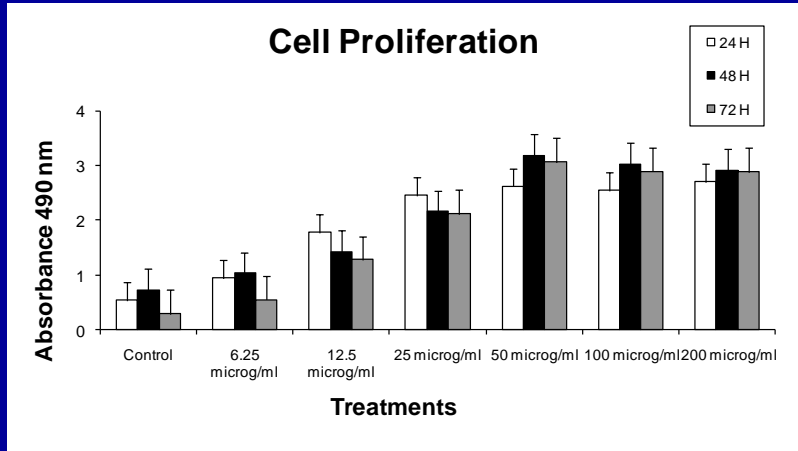
# Methodological Strategy

## Protein expression Western blot



The **western blot** is used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins. The proteins are then transferred to a membrane where they are detected using antibodies specific to the target protein.

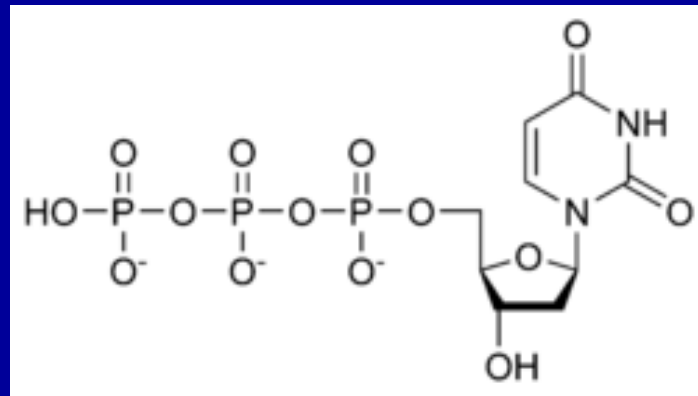
# *S. haematobium* total antigen increases the proliferation, decreases the apoptosis and induces invasion of epithelial cells *in vitro*



# Methodological Strategy

## Apoptosis

TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling)

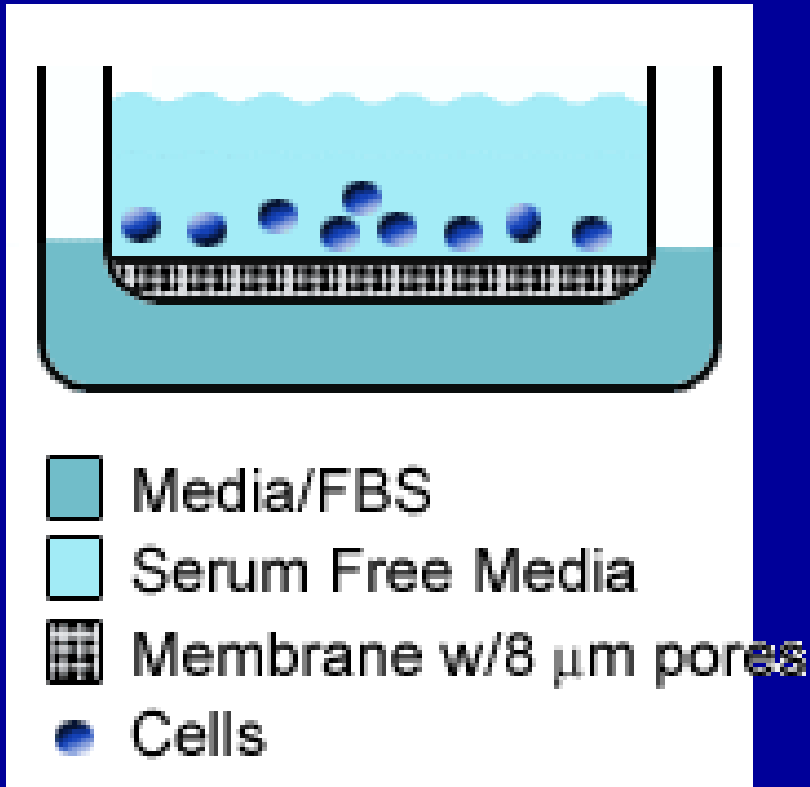


The assay relies on the presence of nicks in the DNA which can be identified by terminal deoxynucleotidyl transferase, an enzyme that will catalyze the addition of dUTPs that are secondarily labeled with a marker. It may also label cells that have suffered severe DNA damage.

# Methodological Strategy

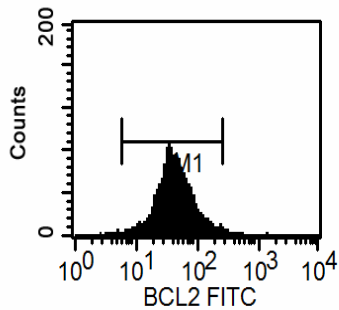
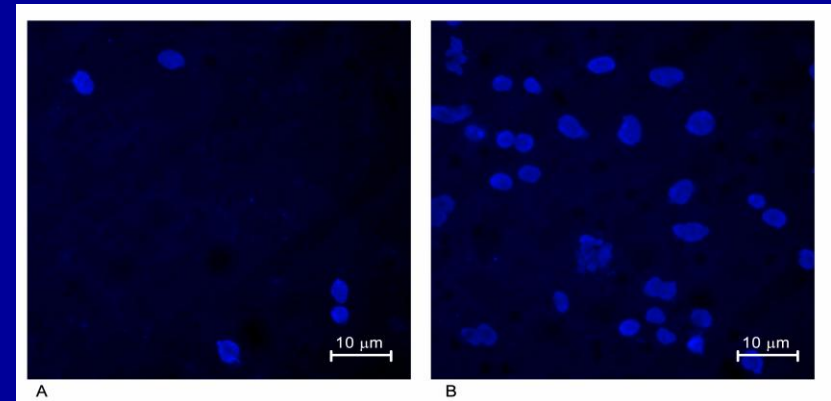
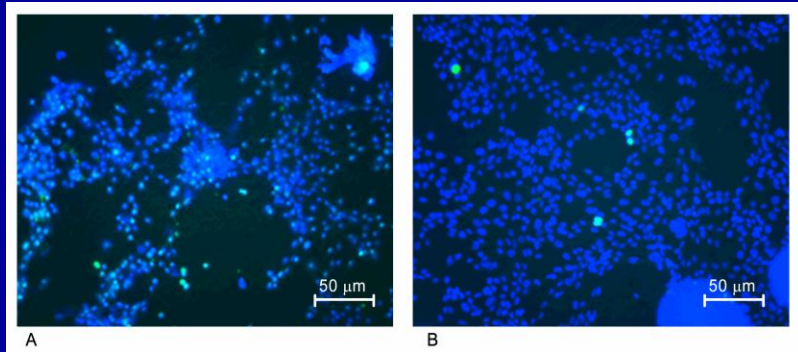
## Invasion

## Boyden chamber

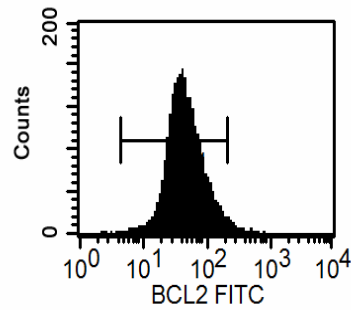


1. Cell suspension is placed in upper chamber and incubated for 24-72 hours at 37 C.
2. Invasive cells pass through the membrane and cling to the bottom of the polycarbonate membrane that is tissue culture-treated to enhance cell attachment.
3. Non-invading cells cannot pass through the membrane and stay in the upper chamber.

# *S. haematobium* total antigen increases the proliferation, decreases the apoptosis and induces invasion of epithelial cells *in vitro*

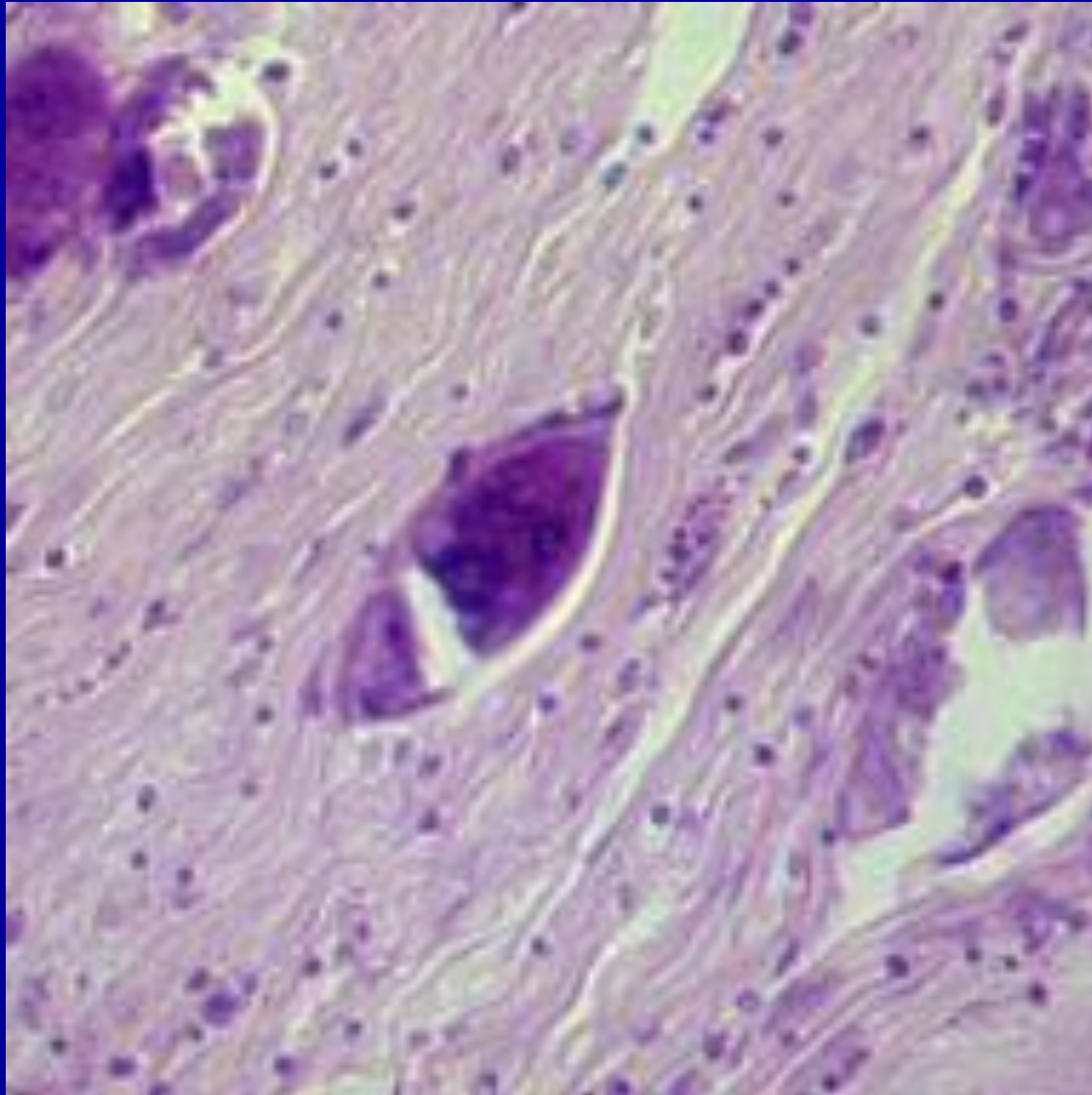


a.



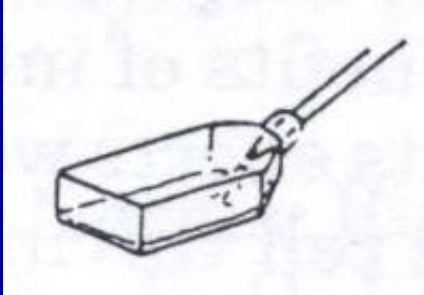
b.

*S. haematobium* total antigen induces tumorigenesis

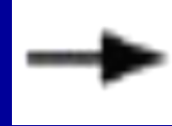
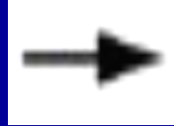


# Methodological Strategy

Chinese Hamster  
Ovary (CHO) cells



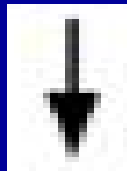
Sh-treatment 48 hours



Nude mice

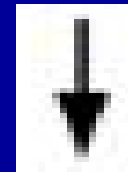


Control CHO cells



No Tumour

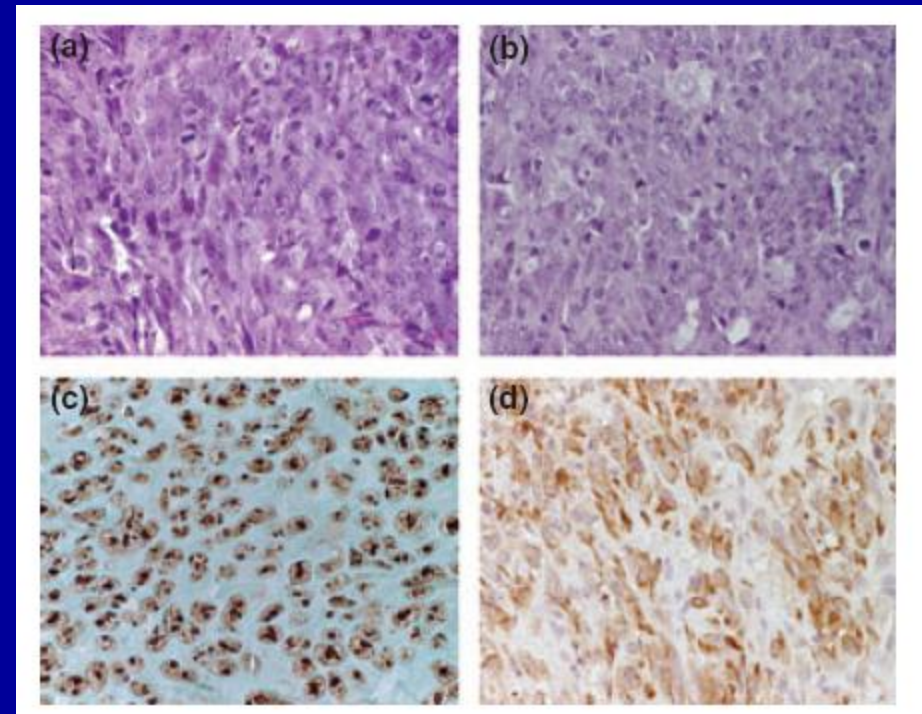
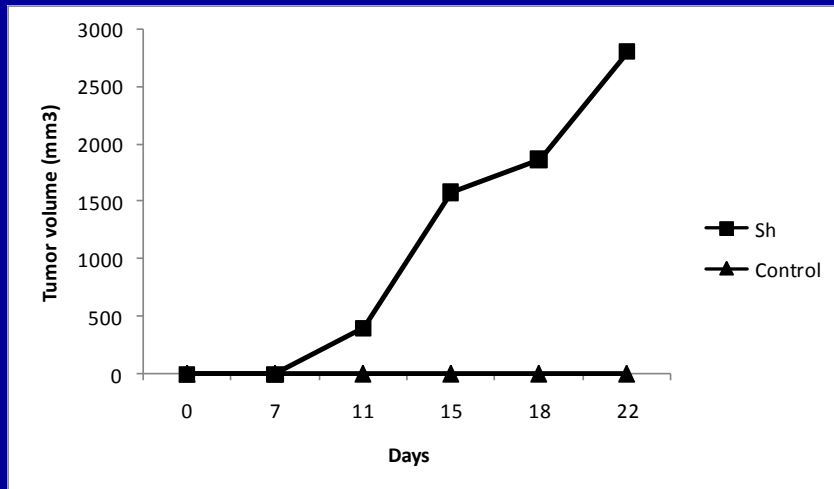
Sh-treated CHO cells



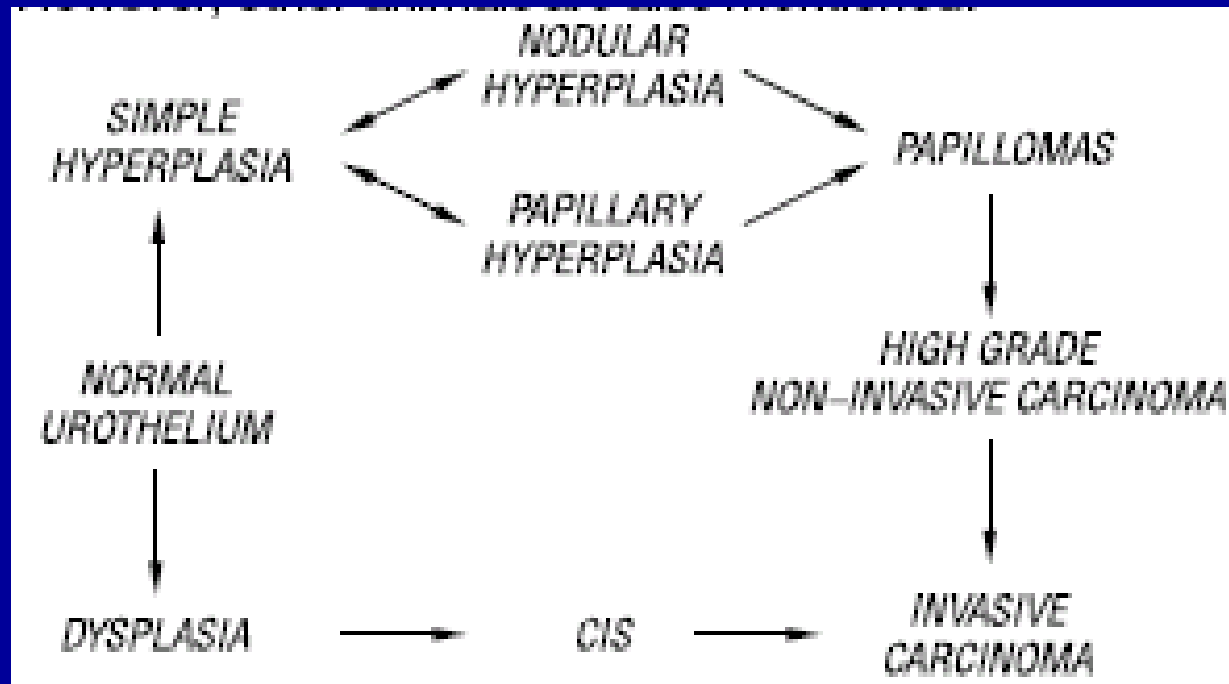
Tumour-Bearing Nude Mouse

2 weeks

# *S. haematobium* total antigen induces tumorigenesis

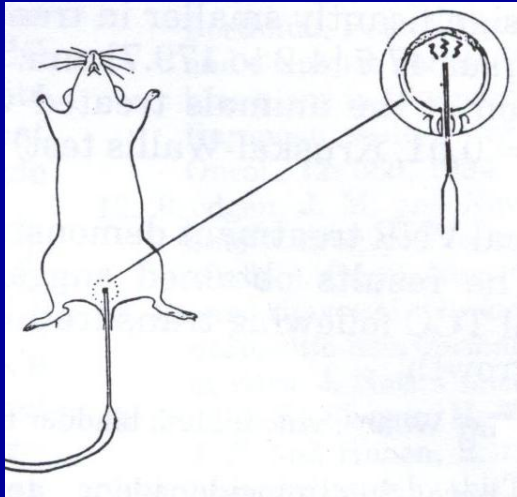


# *Schistosoma haematobium* total antigen induces dysplasia and *KRAS* gene mutations in CD-1 mice normal urothelium

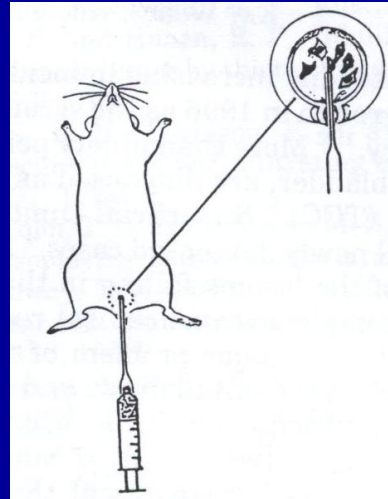
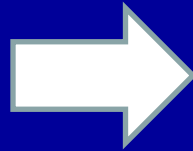


Pathogenic pathways of rat and mice urinary bladder carcinogenesis.

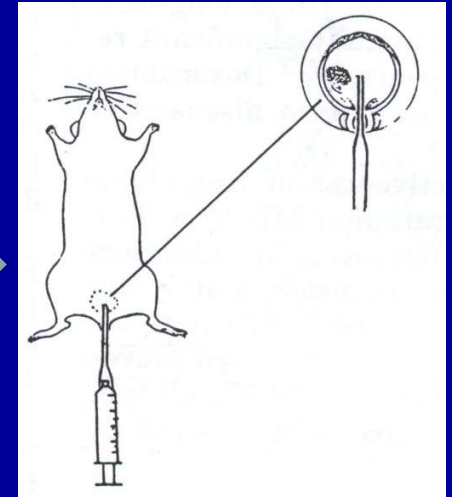
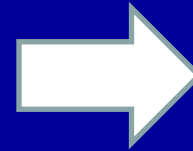
# Methodological Strategy



Transurethral  
Catheterization

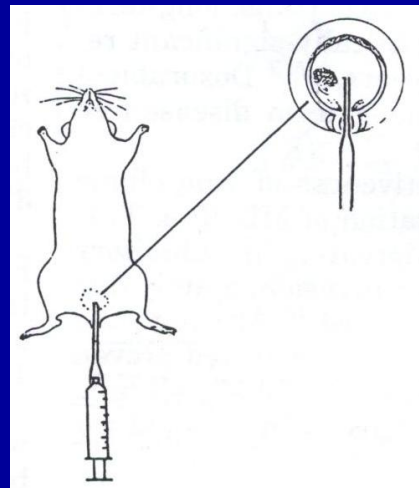


Urothelial  
Denudation



Sh-treatment  
/PBS  
1 hour

In situ fixation  
10% PPA

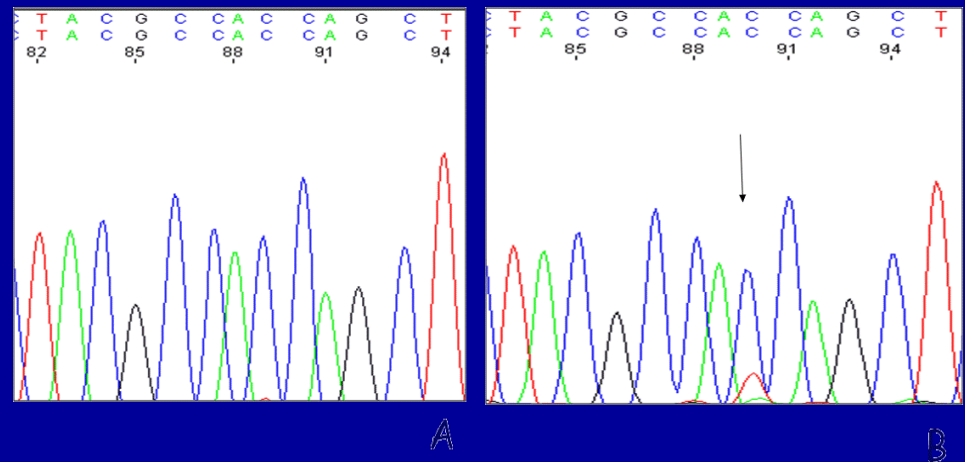
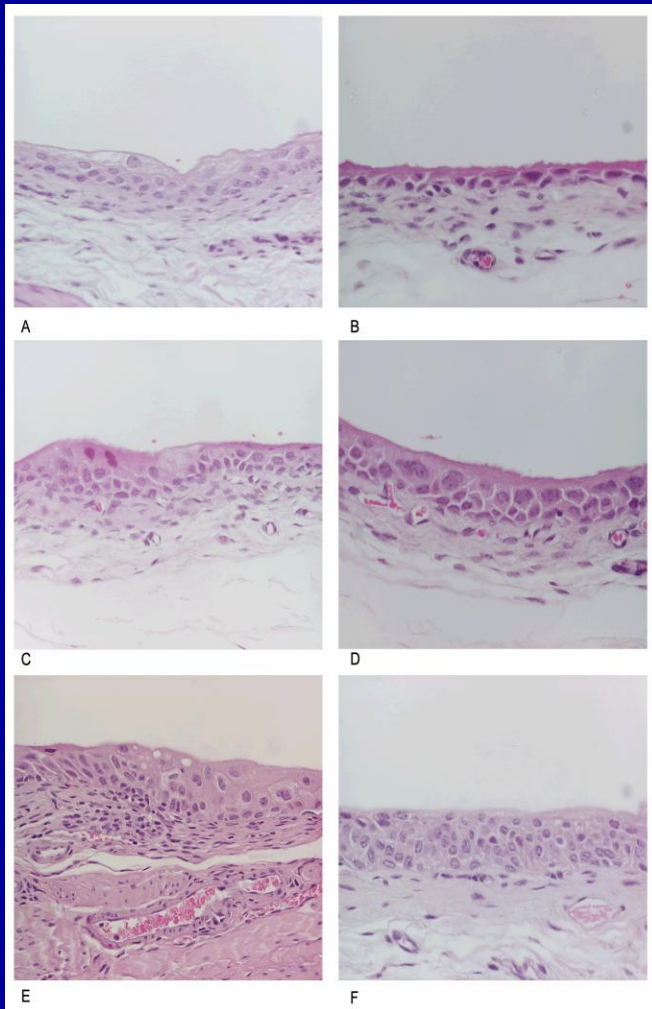


20 weeks  
40 weeks

# Methodological Strategy



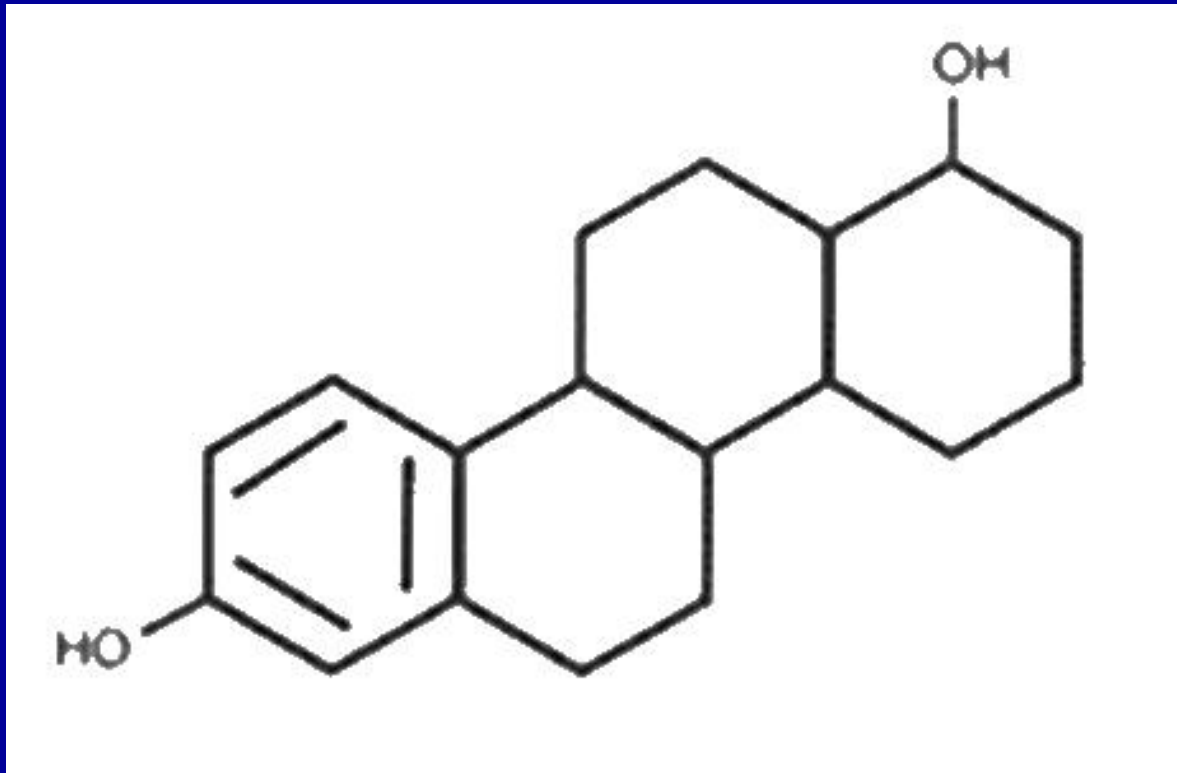
# *Schistosoma haematobium* total antigen induces dysplasia and *KRAS* gene mutations in CD-1 mice normal urothelium



Botelho *et al.* (Submitted)

# Schistosomiasis and host hormones

*Shistosoma haematobium* produces an estradiol-related molecule



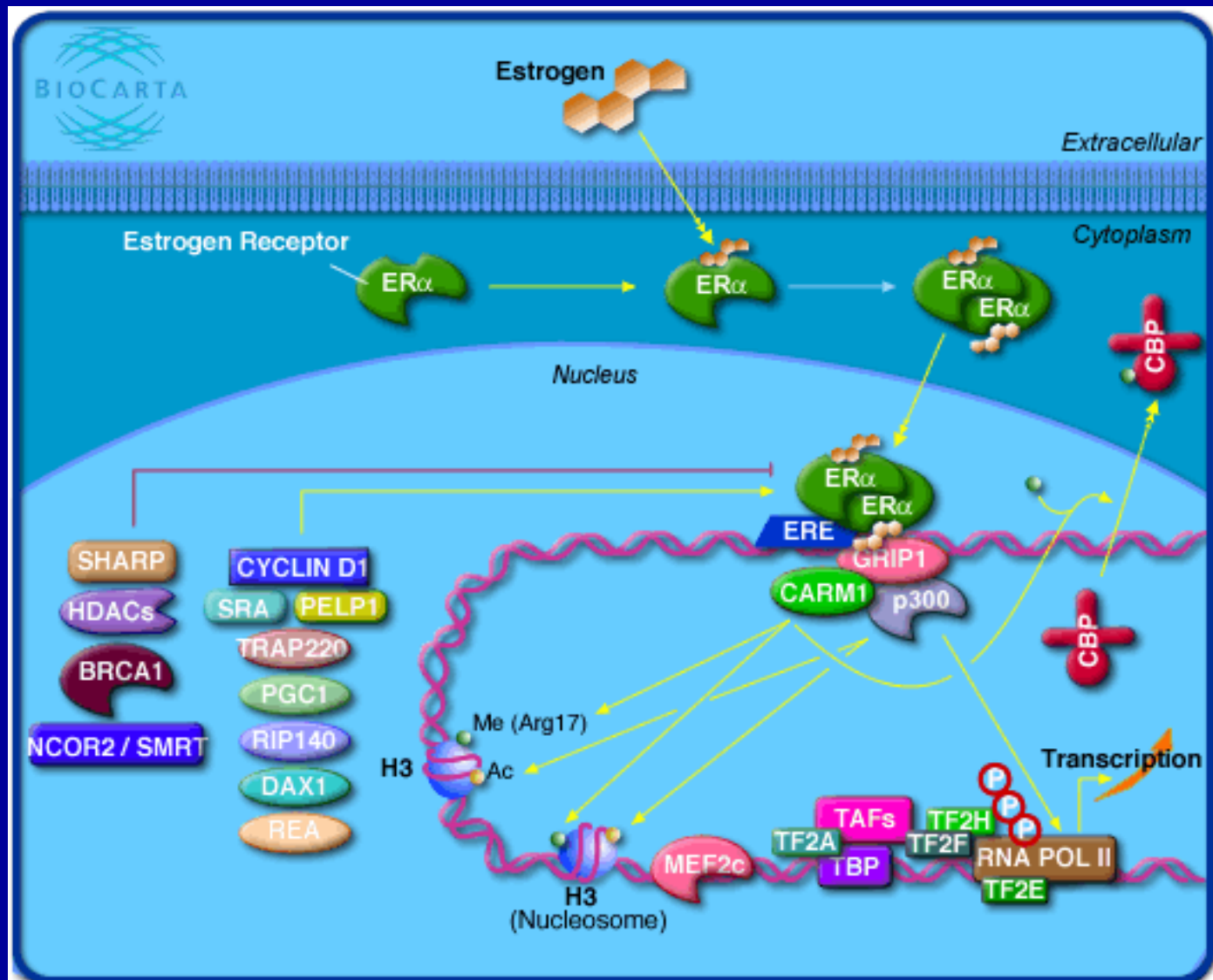
Estradiol (E2)

# *Shistosoma haematobium* produces an estradiol-related molecule

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

Antigenic preparations	E2 (pg/ml) ± SD
<i>S. haematobium</i>	14,84±0,14
<i>S. mansoni</i>	12,63±0,27
<i>F. hepatica</i>	<10
H <sub>2</sub> Od	<10
10 nM E2	1632,99±2,55

*S. haematobium* produces estrogenic molecules that are able to down-regulate ER alpha and ER beta and repress ER transcriptional activity



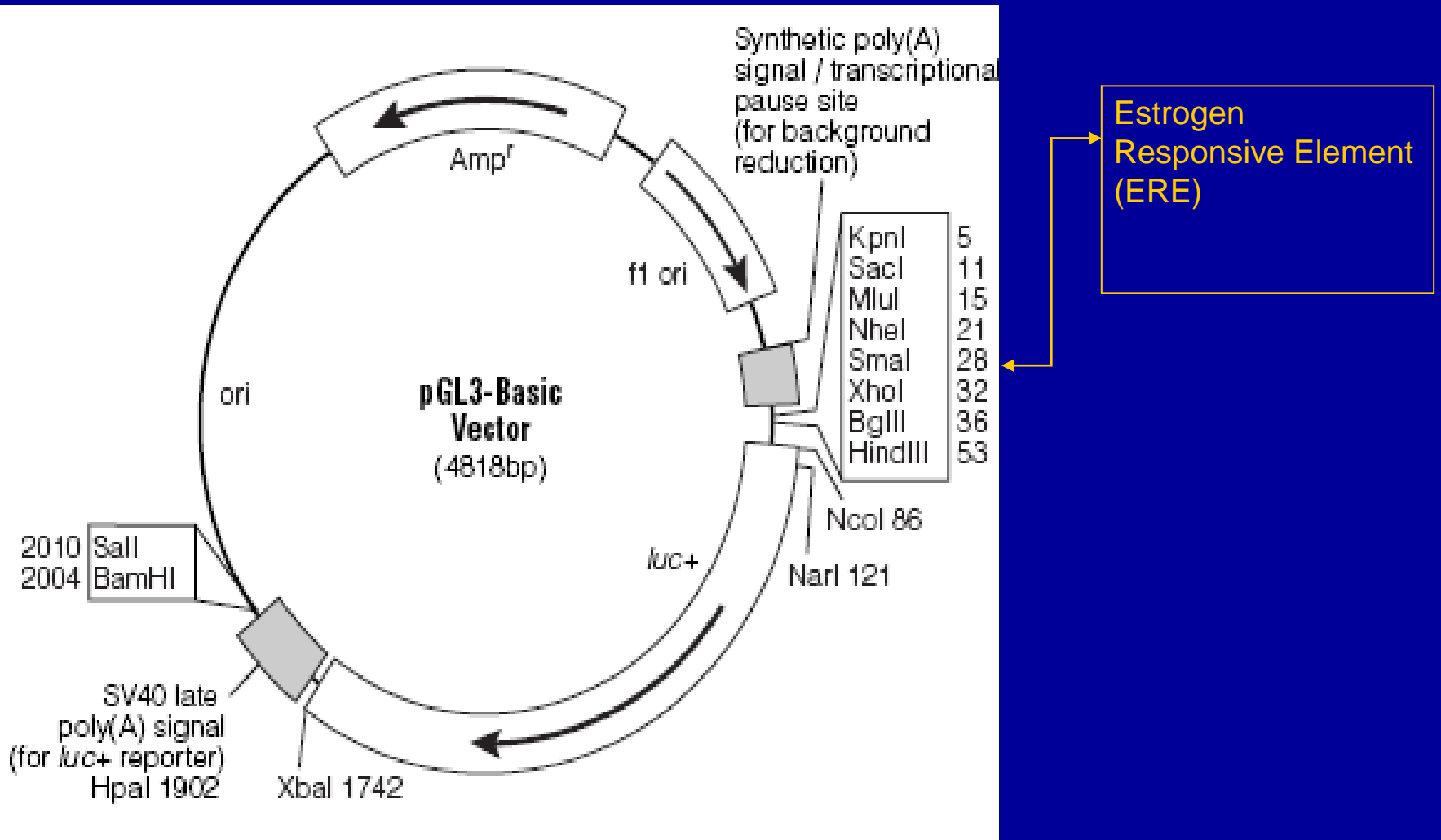
# Methodological Strategy

Gene expression Real-Time PCR



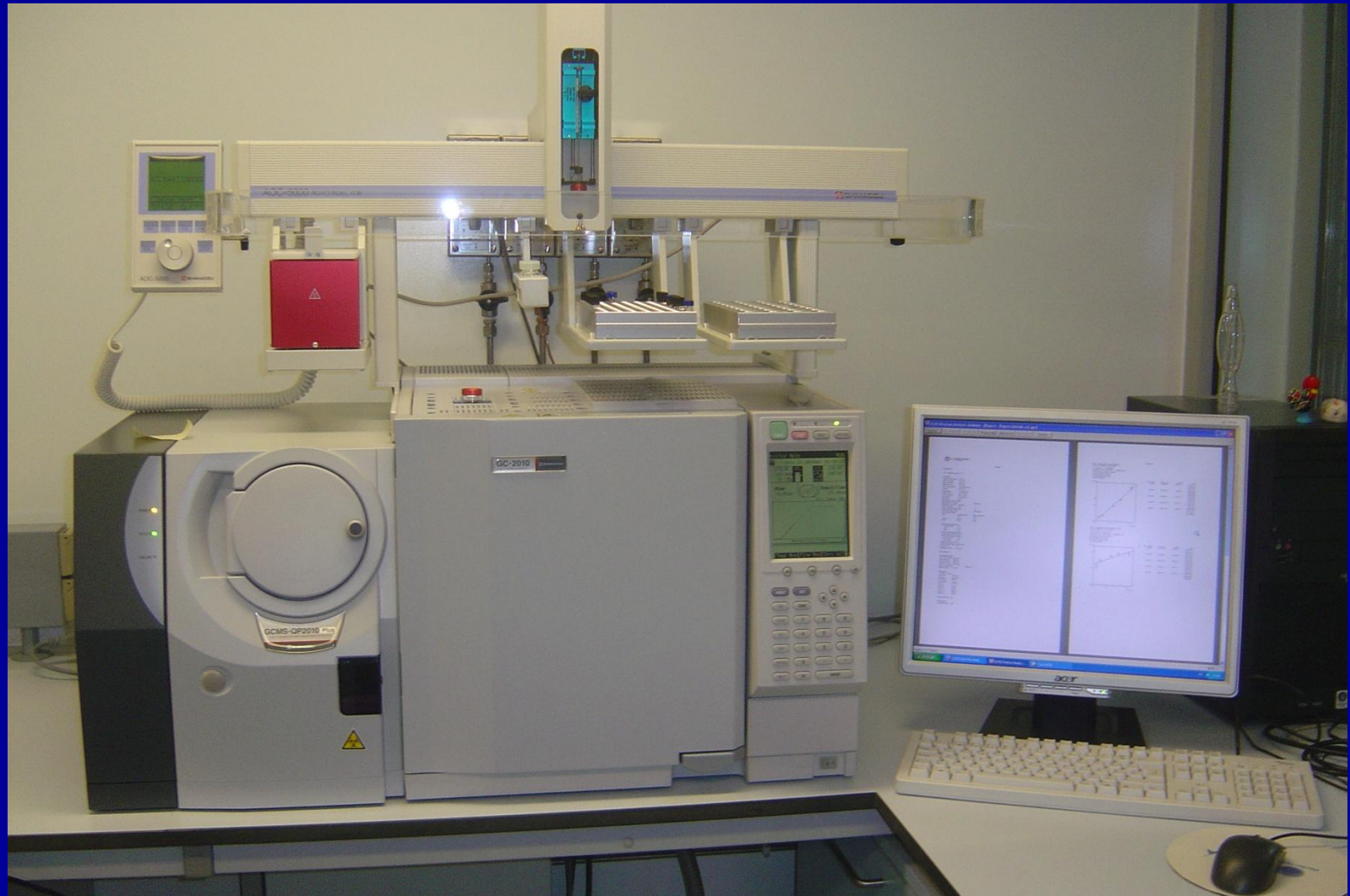
# Methodological Strategy

## Transfection pERE-Luc

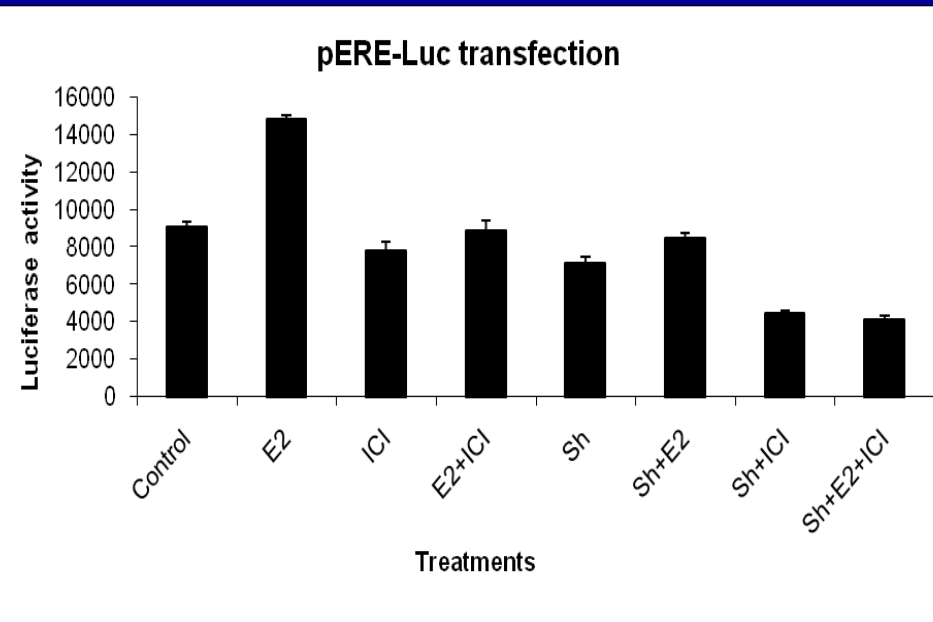
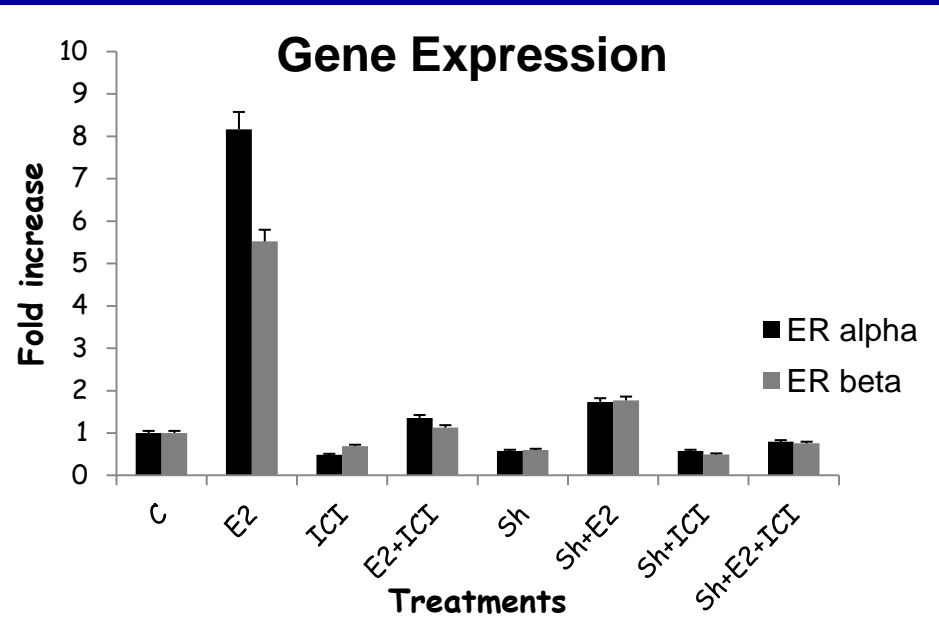


# Methodological Strategy

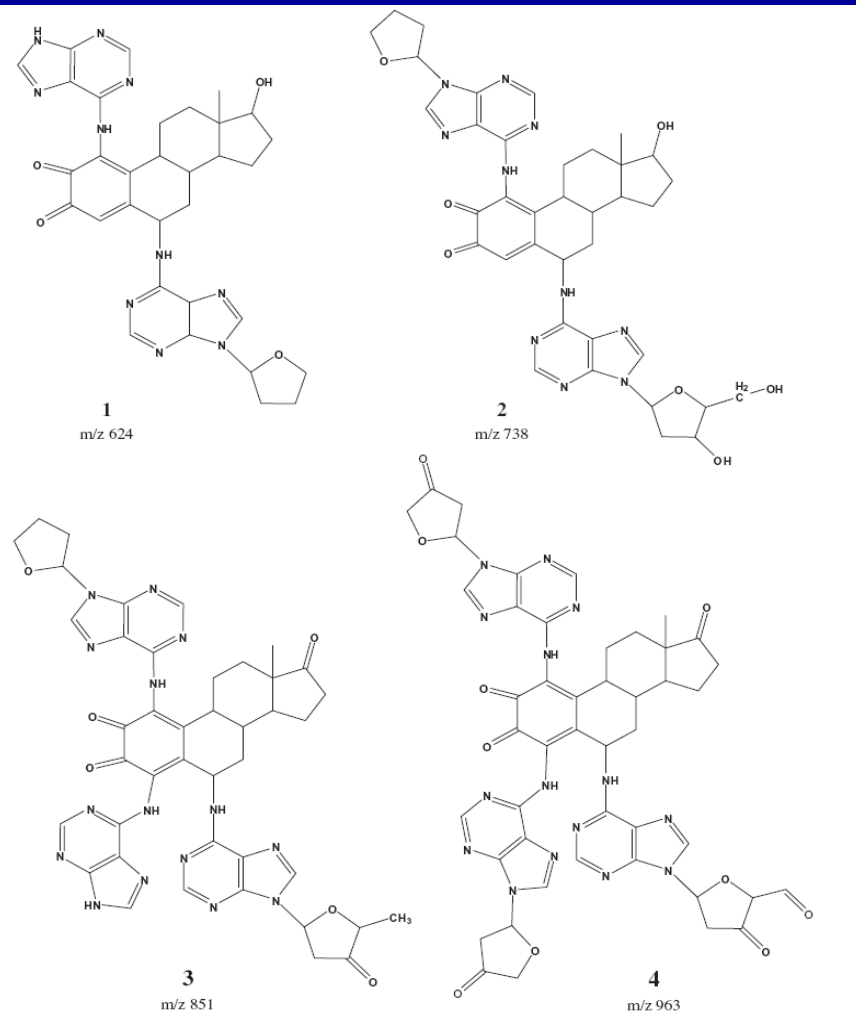
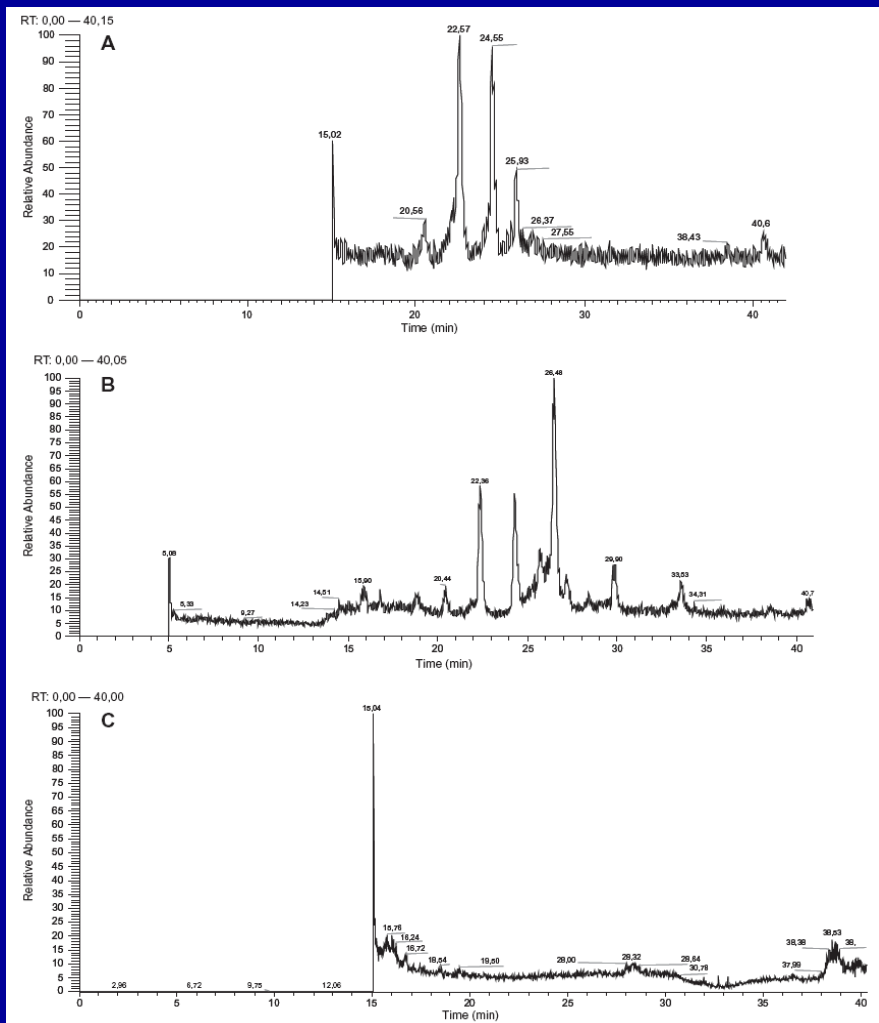
## Mass spectrometry LC-ESI-MS



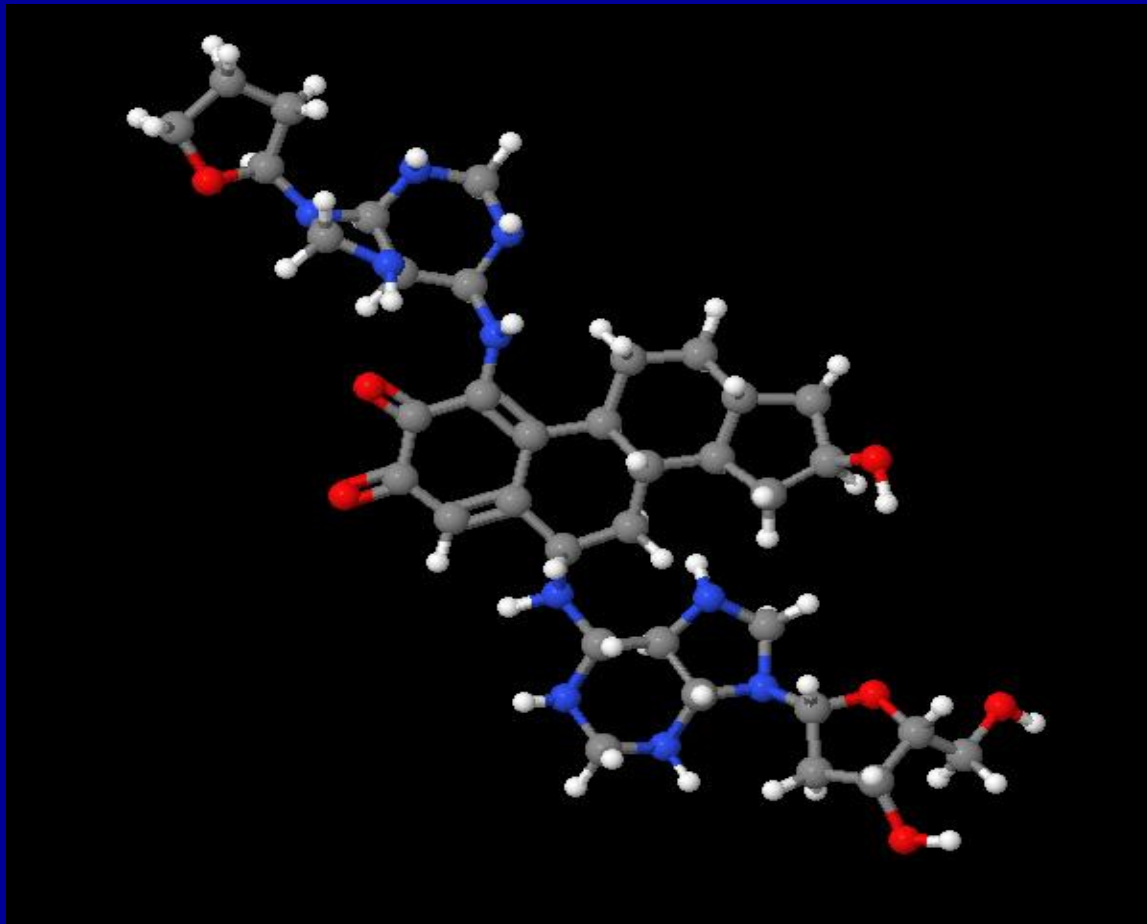
# *S. haematobium* produces estrogenic molecules that are able to down-regulate ER alpha and ER beta and repress ER transcriptional activity



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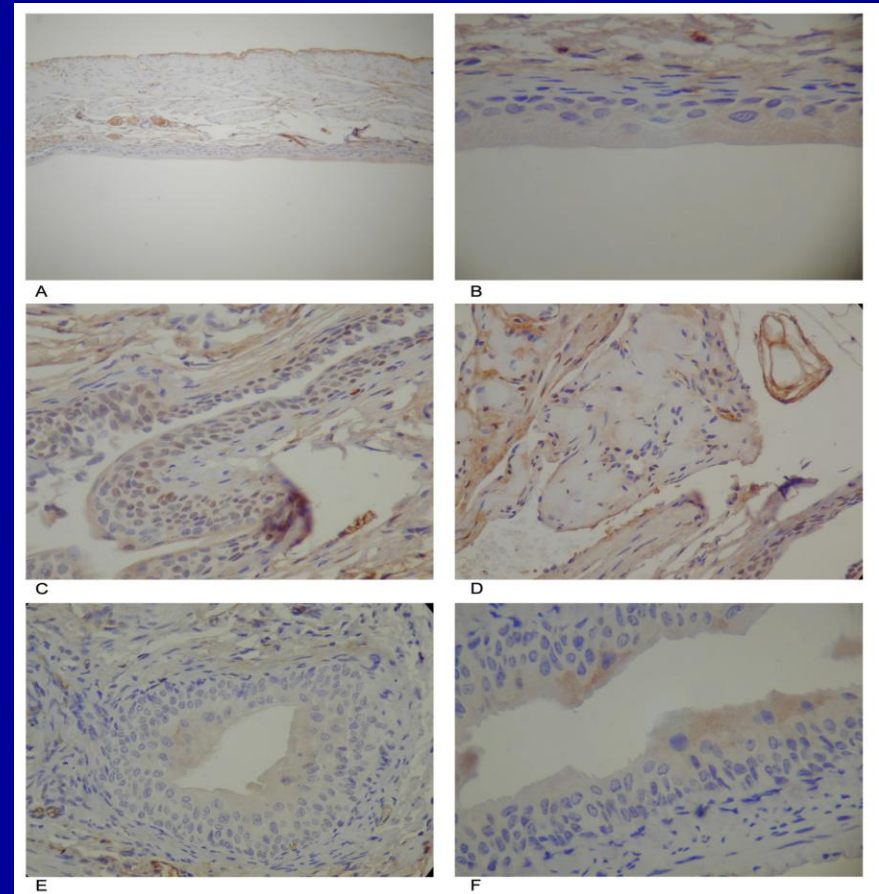
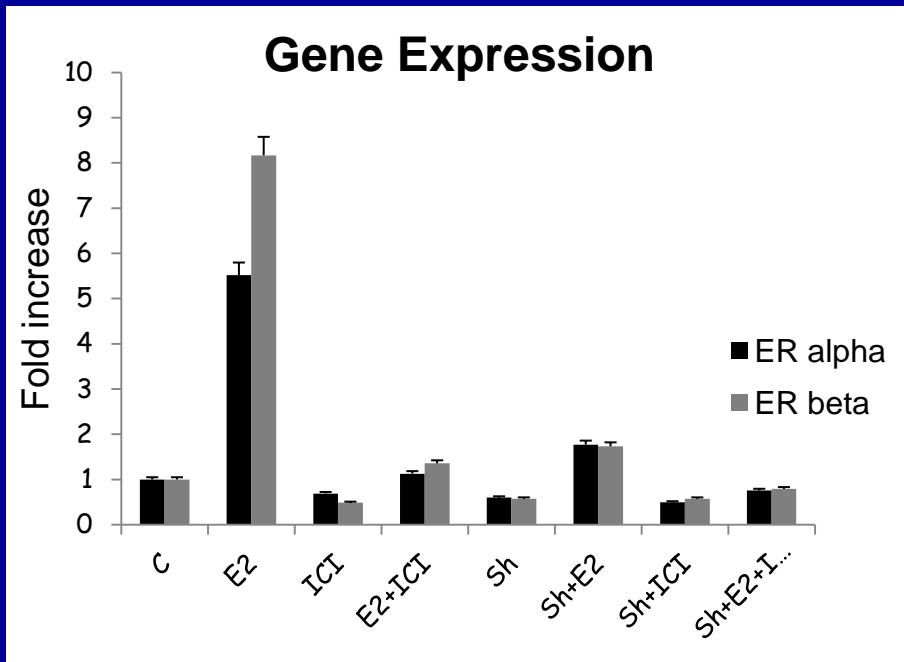


*S. haematobium* produces estrogenic molecules that are able to down-regulate ER alpha and ER beta and repress ER transcriptional activity



# Schistosomiasis, bladder cancer and host hormones

# *Schistosoma haematobium* total antigen down-regulates ER alpha and ER beta in HCV29 normal urothelial cells and down-regulates ER expression in the bladders of CD1 mice



# Conclusions

## *S. haematobium* and bladder cancer

*S. haematobium* total antigen induced increased proliferation, decreased apoptosis, and induced migration and invasion of normal epithelial cells in culture. In addition, the parasite extract has also the potential to induce tumour development, assessed by the use of a nude mice xenograft model.

According to our findings, *S. haematobium* total antigen in CD-1 mice normal bladders after intravesical instillation of the parasite antigen, induced alterations in the urothelium of these animals consistent with dysplasia and inflammation. In these animals, we also found that the parasite extract of *S. haematobium* has carcinogenic ability possibly through oncogenic mutation of *KRAS* gene.

# Conclusions

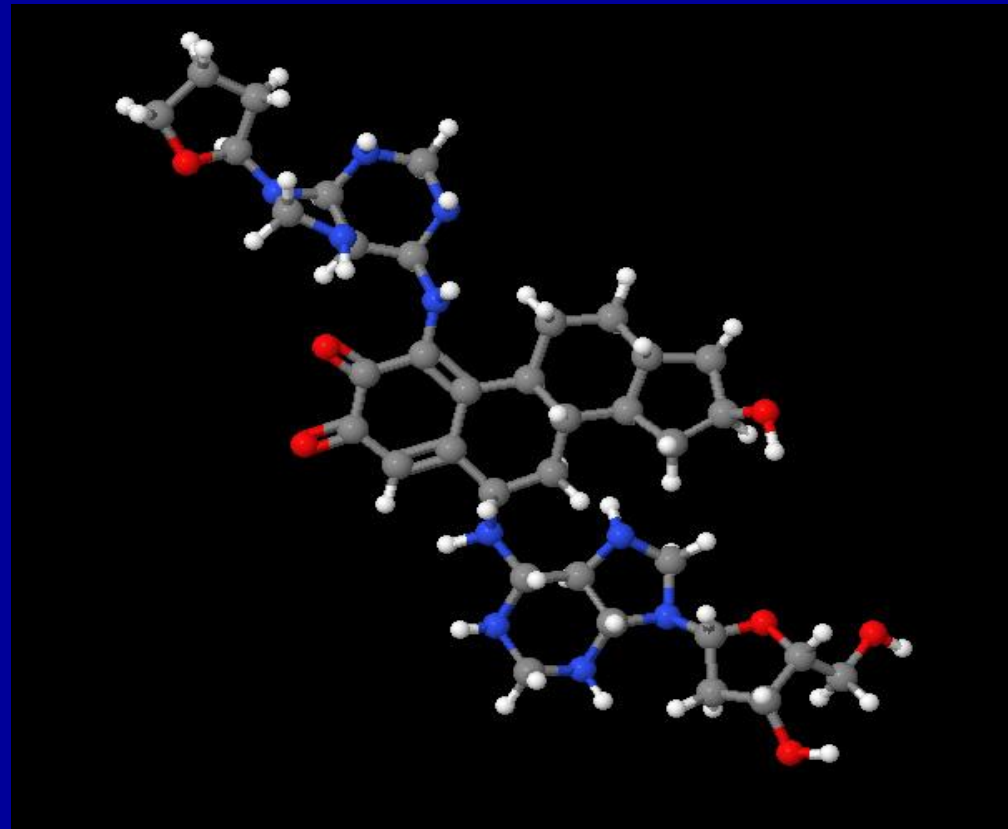
## *S. haematobium* and hormones

*S. haematobium* total antigen expresses estradiol-related molecules that down regulate Estrogen Receptor alpha and beta in estrogen responsive cells. These estrogens are also present in the sera of *Schistosoma*-infected individuals, and they have the ability to repress Estrogen Receptor transcriptional activity.

The estrogenic molecules present in *S. haematobium* extracts could have a carcinogenic effect possibly through estrogen adduct-mediated pathway and could further explain the link between this parasite and squamous cell carcinoma of the bladder..

## *OVERALL*

Therefore, these results may open potential new strategies for cancer diagnosis by using these estrogens as biomarkers in schistosomiasis-associated bladder cancer.





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Joana Gomes



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Professor Fernanda Travassos  
**Ligia Lourenço**

**ICBAS**

Professor Carlos Lopes



**FMUP**

Professor Raquel Soares



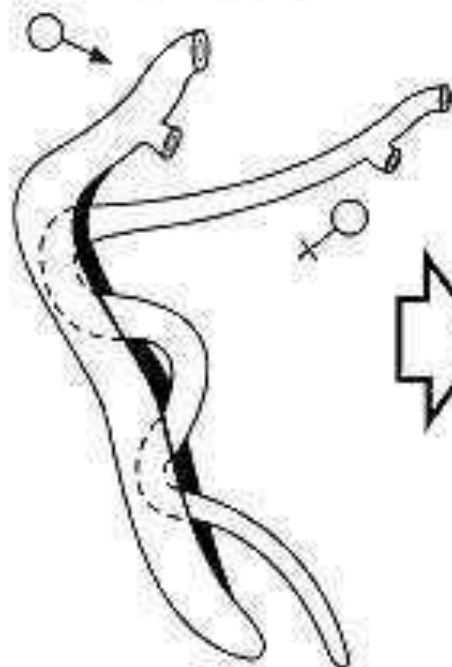
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Prof. Rui Medeiros  
Prof. Manuel Teixeira  
Dr. Ricardo Ribeiro  
Dr. Gabriela Martins  
Isabel Veiga  
Madalena Crespo  
Carlos Palmeira



Thank you for your attention



### Origin



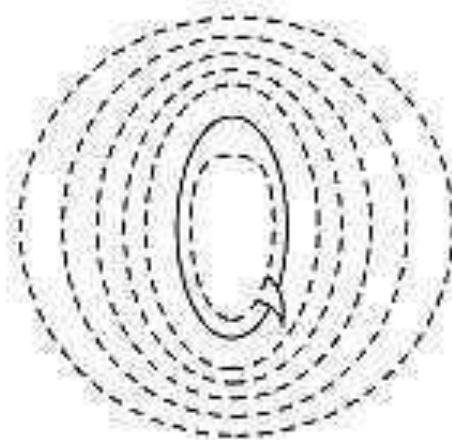
Adult schistosomes  
in blood vessels around  
small intestine

### Stimulus



Eggs laid by female  
are carried in blood  
vessels and trapped  
in liver

### Response



Hypersensitivity to antigens  
of larva inside egg cause  
formation of granuloma.  
Liver sinusoids become blocked,  
impeding blood flow

### Pathology

#### Fibrosis of liver

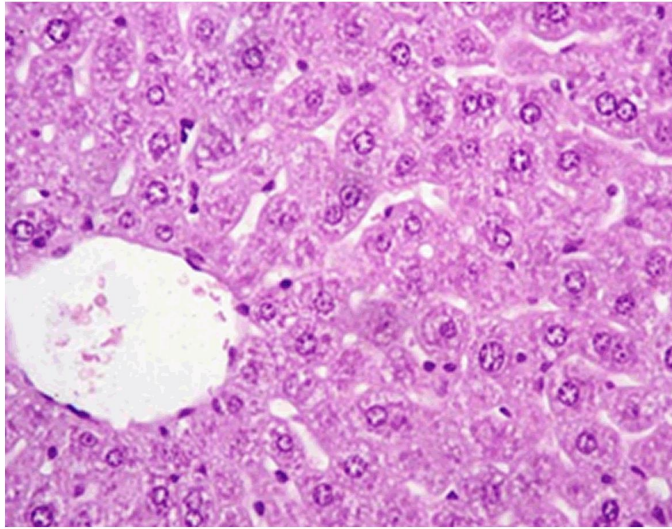
- Raised portal pressure
- Perihepatic shunting of blood
- Hepatomegaly
- Splenomegaly
- Formation of varices

# Histopathological data in the livers of infected animals.

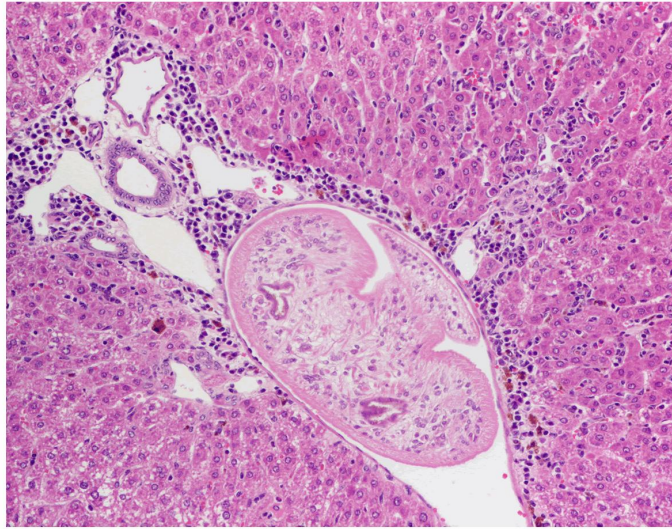
Group	Hamster	P	PH	F	LPI	BP	E	MT	R
Group1 (Control)	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
Group 2 (Infected)	3	+	++	+++	++	++	++	++	++
	4	+	++	++	++	+++	+++	++	++
	5	+	+++	+++	+++	+++	+++	+++	+++
Group 3 (Control)	6	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-
Group 4 (Infected)	8	+	++	++	++	++	++	+	+
	9	+	+++	+++	+++	++	++	+++	+++
	10	+	++	+++	+++	++	++	+++	+++

P, *S. haematobium* in histological section; PH, fibrous perihepatitis; F, lymphoid follicles; LPI, infiltration of lymphocytes and plasma cells; BP, bilharzial pigment; E, infiltration of eosinophils; MT, Masson Trichrome stain; R, Reticulin stain; -, absent; +, mild; ++, moderate; +++, severe.

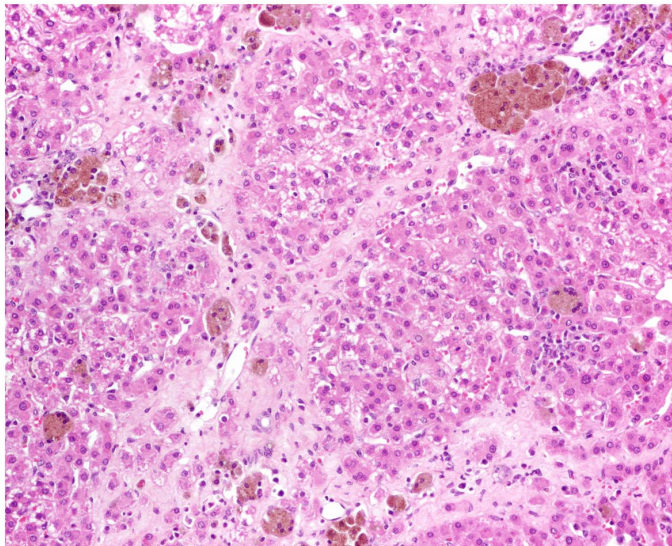
# Histological findings of livers of infected animals.



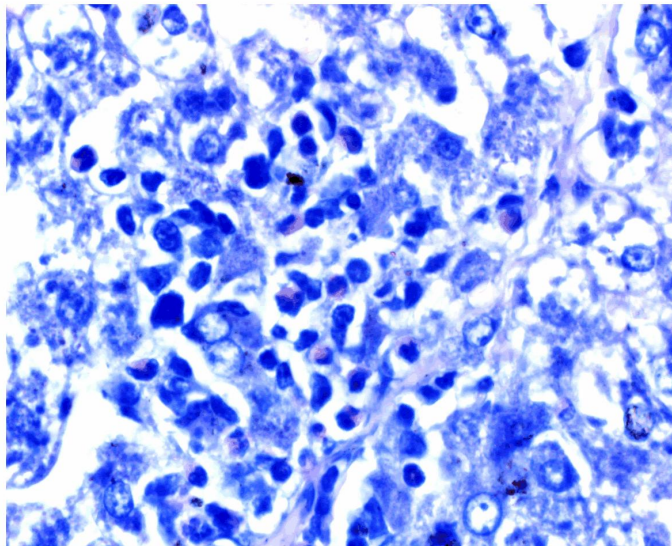
A



B

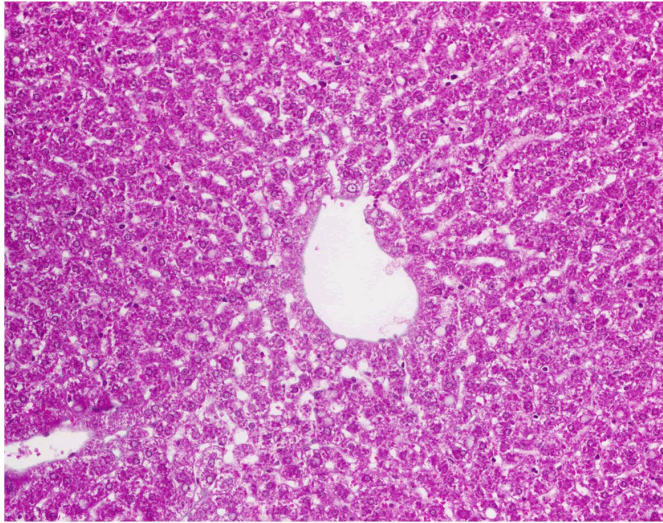


C

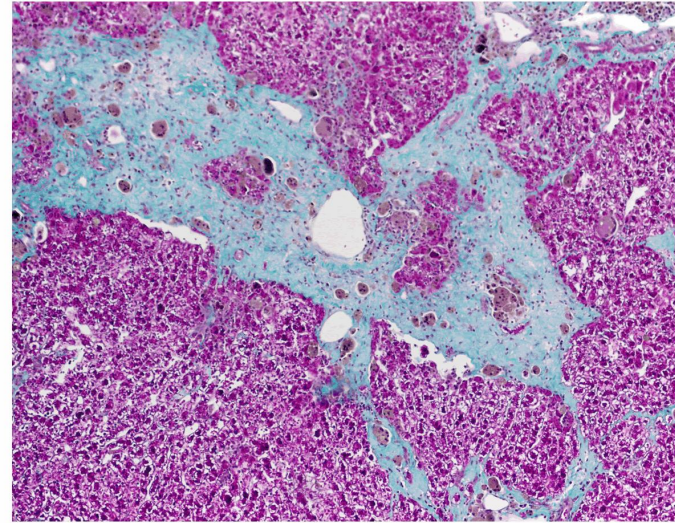


D

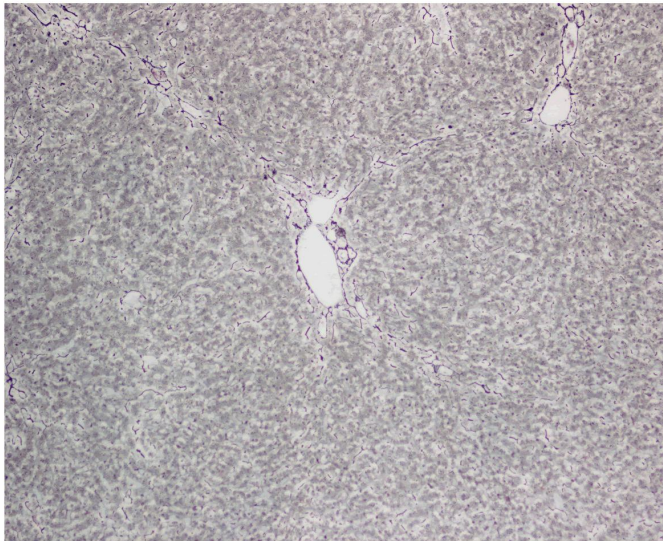
# Histological findings of livers of infected animals.



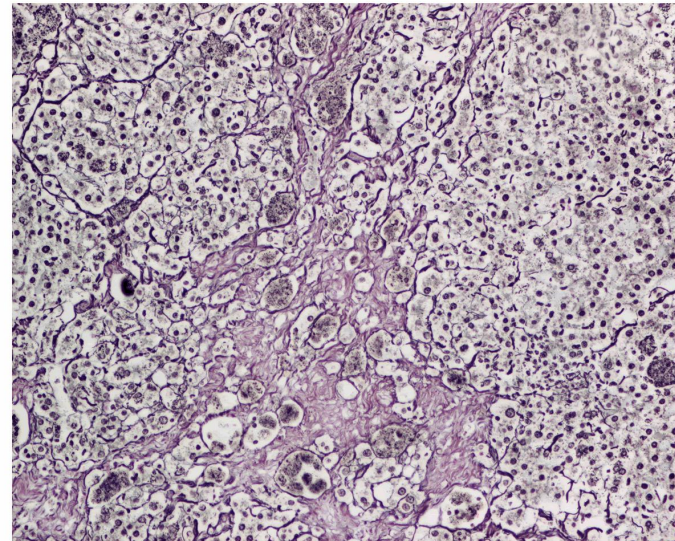
A



B



C



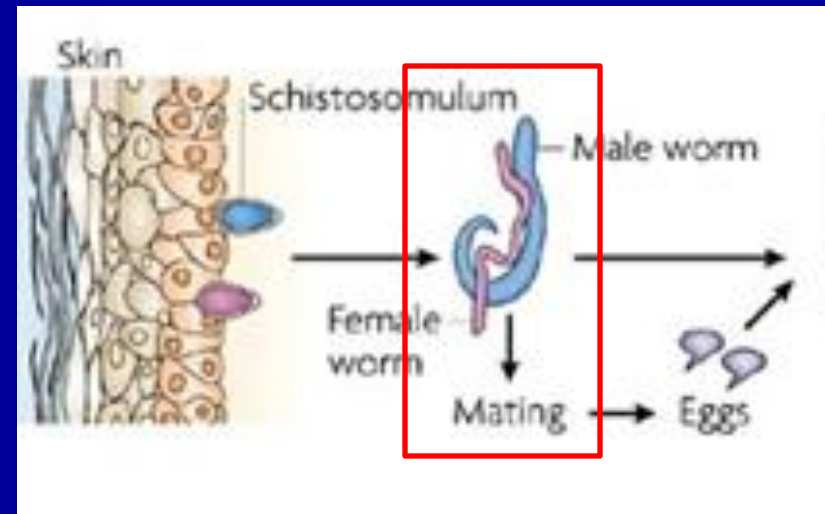
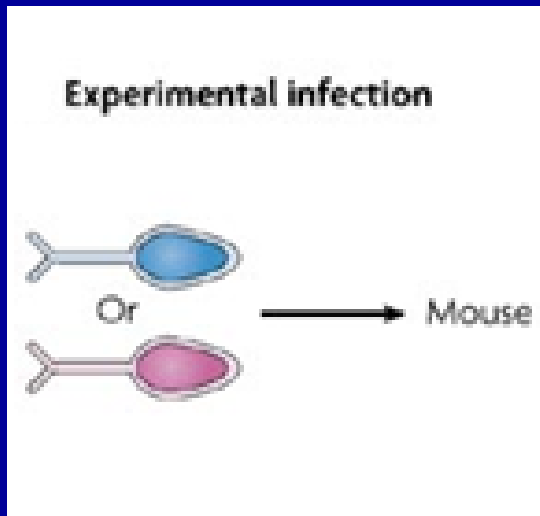
D

*Schistosoma haematobium* total antigen  
induces increased proliferation,  
migration and invasion, and decreases  
apoptosis of normal epithelial cells

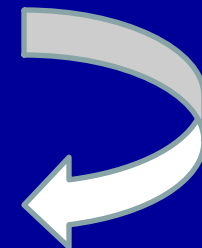
Monica Botelho, António Carlos Ferreira,  
Maria José Oliveira, José Carlos Machado  
and José Manuel Correia da Costa

(International Journal for Parasitology, 2009)

# Methodological Strategy

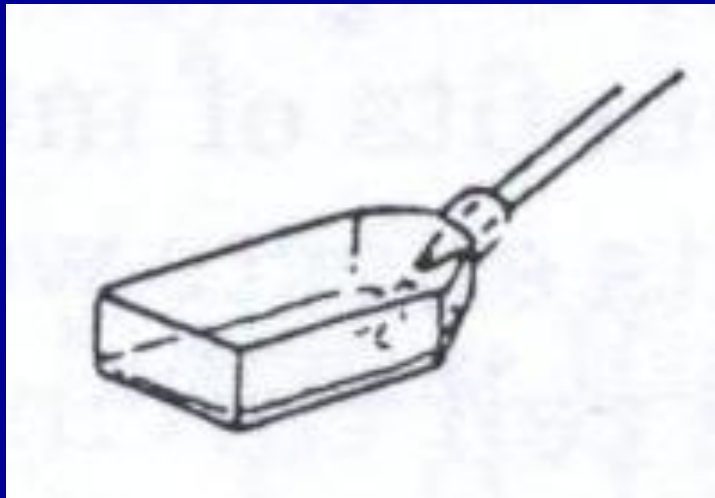


*S. haematobium*  
total antigen (Sh)



# Methodological Strategy

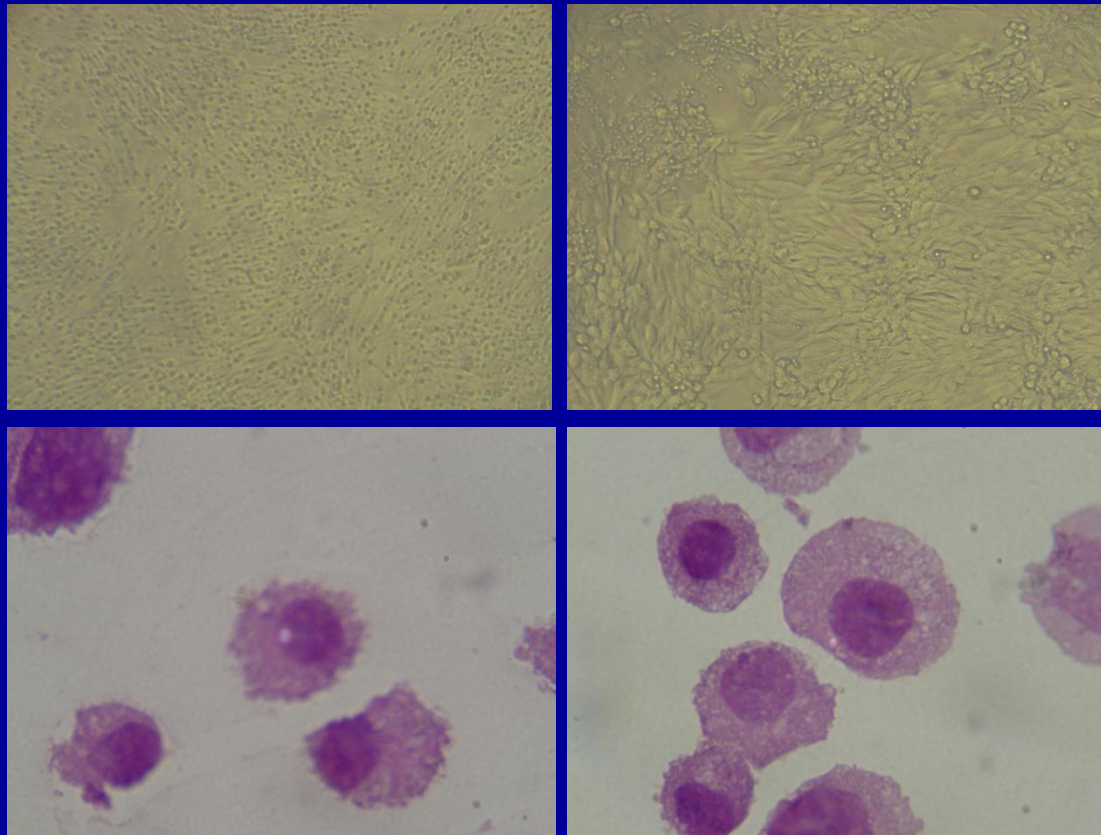
Chinese Hamster  
Ovary (CHO) cells



Sh-treatment 48 h



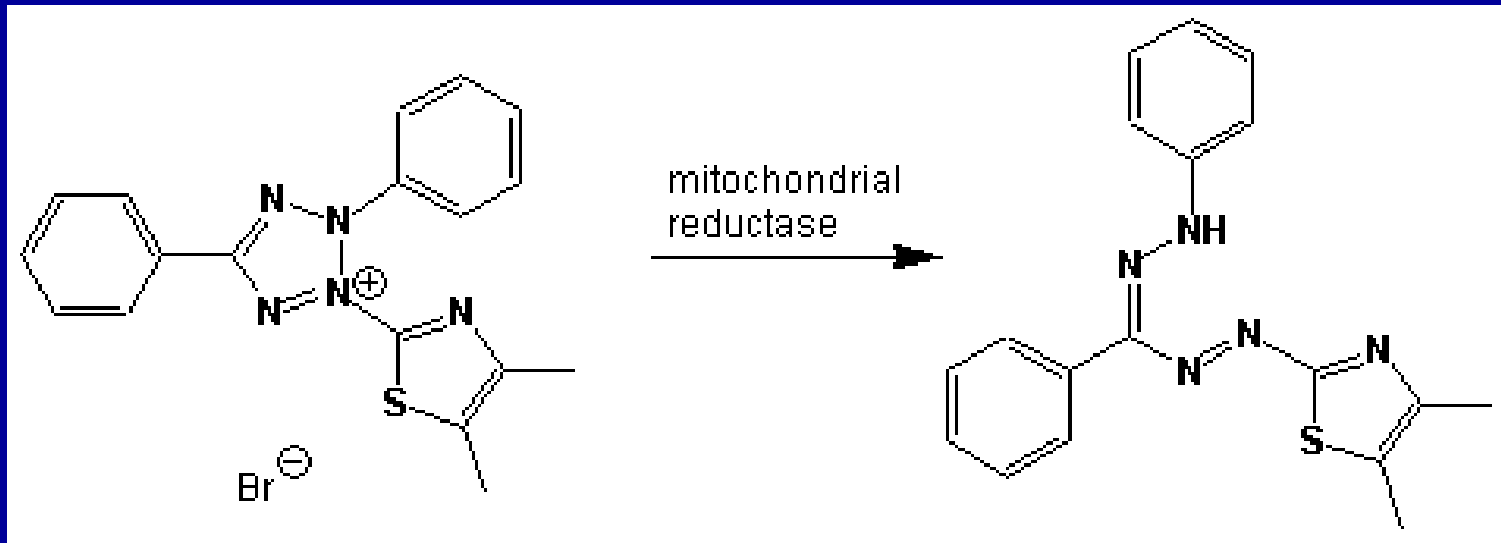
# *S. haematobium* total antigen induces alterations in the morphology of treated cells



Morphological differences of CHO cells in culture. Control in the left panels and Sh-treated cells in right panels.

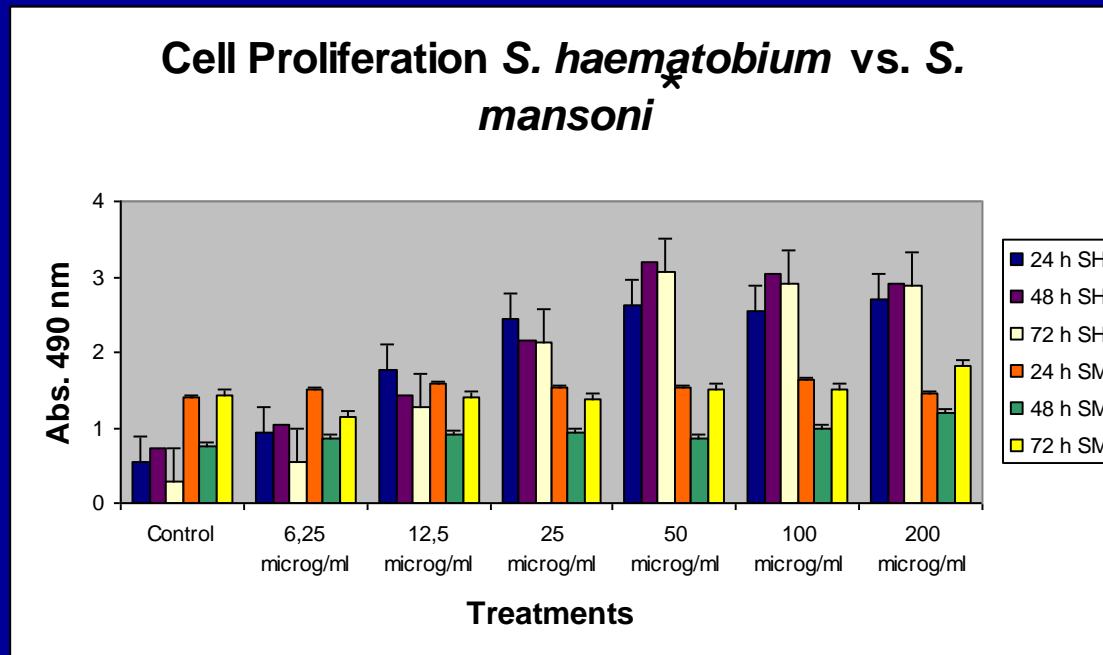
# Methodological Strategy

## Proliferation MTS assay



Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells.<sup>[1]</sup>

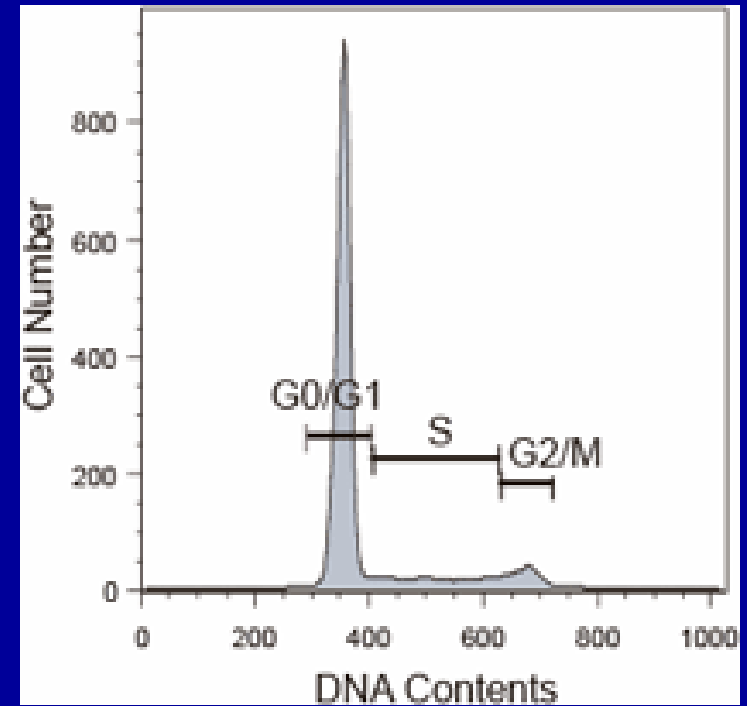
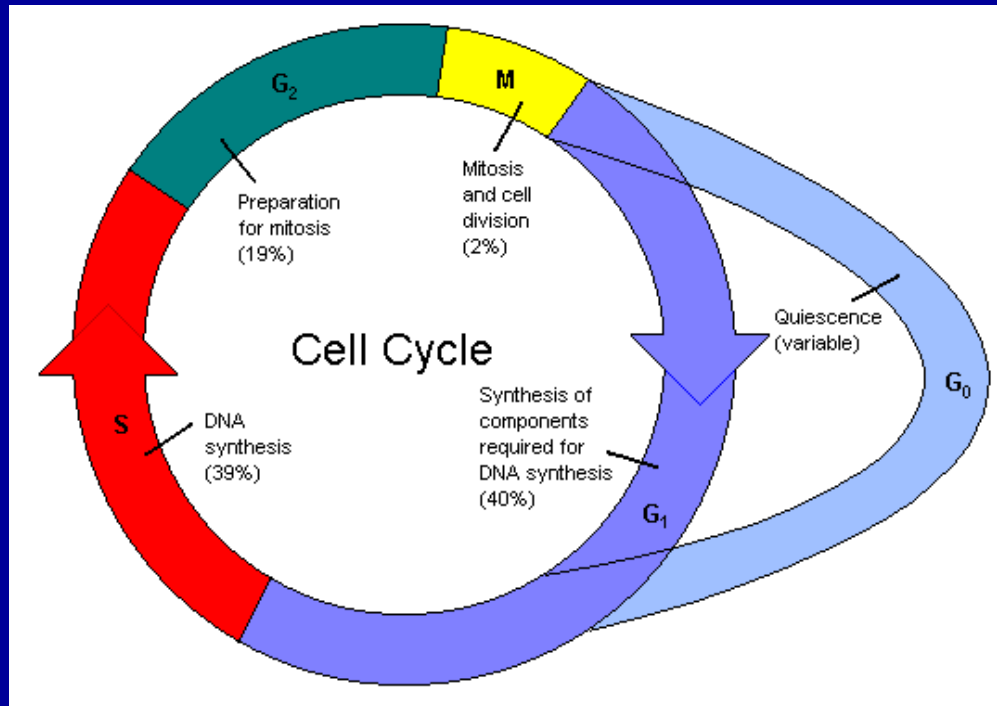
*S. haematobium* total antigen, but not *S. mansoni* total antigen, increased the proliferation of epithelial cells in vitro



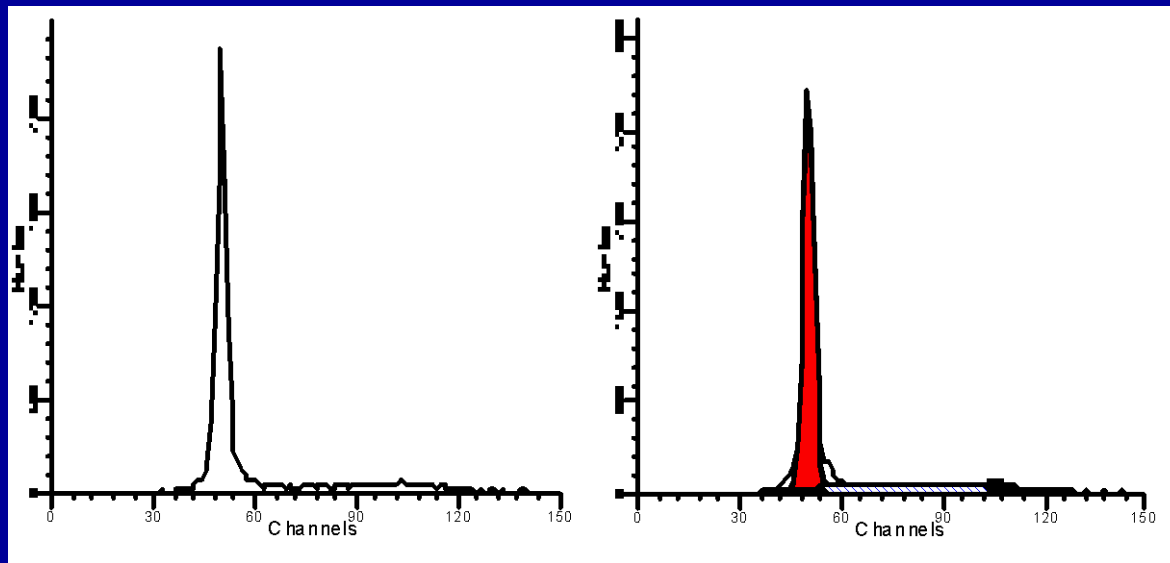
Comparison of proliferation between Sh and Sm treated cells (\* $p < 0,01$ ; Sh vs. Sm).

# Methodological Strategy

## DNA content Flow cytometry



*S. haematobium* total antigen altered the cell cycle distribution decreasing G0/G1 and increasing S- and G2/M-phase



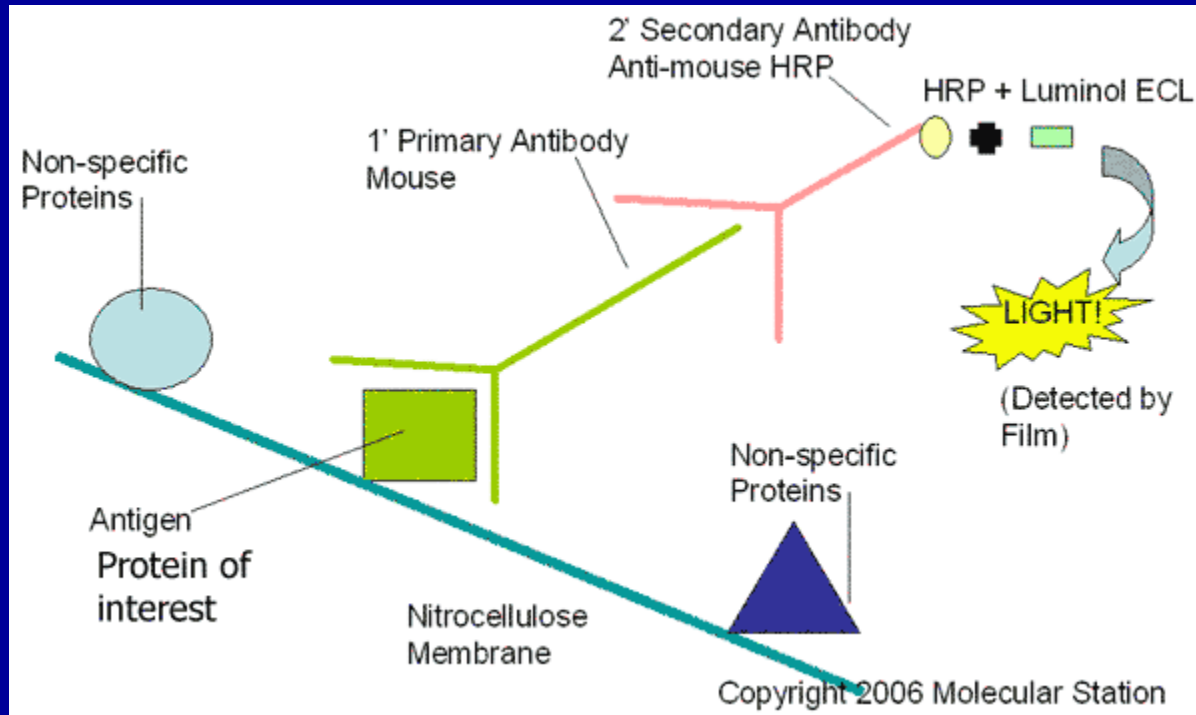
Control

Sh-treated

Groups	Cell Cycle					
	G0/G1		S		G2/M	
Control	80.61	3.39	16.67	2.73	2.72	0.92
Sh-treated	78.07	4.04	18.59	3.42	3.34	0.97

# Methodological Strategy

## Protein expression Western blot

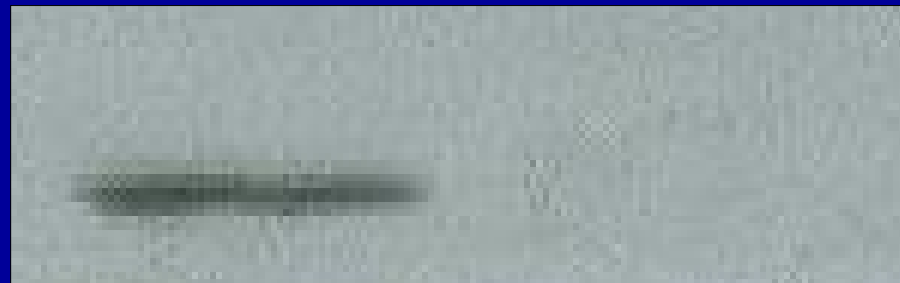


The **western blot** is used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins. The proteins are then transferred to a membrane where they are detected using antibodies specific to the target protein.

Down-regulation of p27, a classic tumor suppressor, *in vitro*, by *S. haematobium* total antigen



beta-Actin



p27

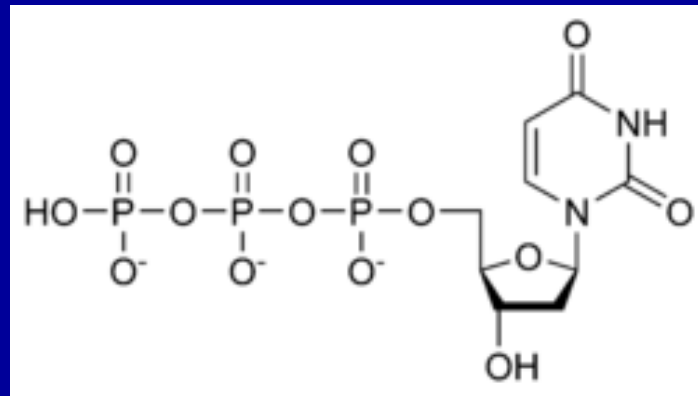
Control

Sh-treated

# Methodological Strategy

## Apoptosis

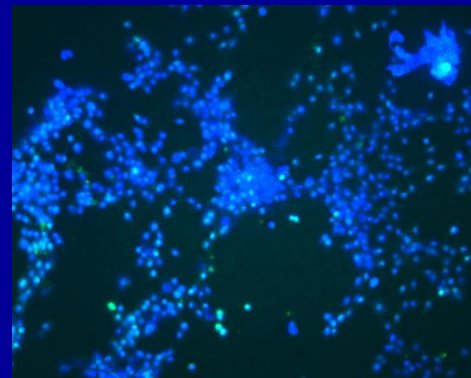
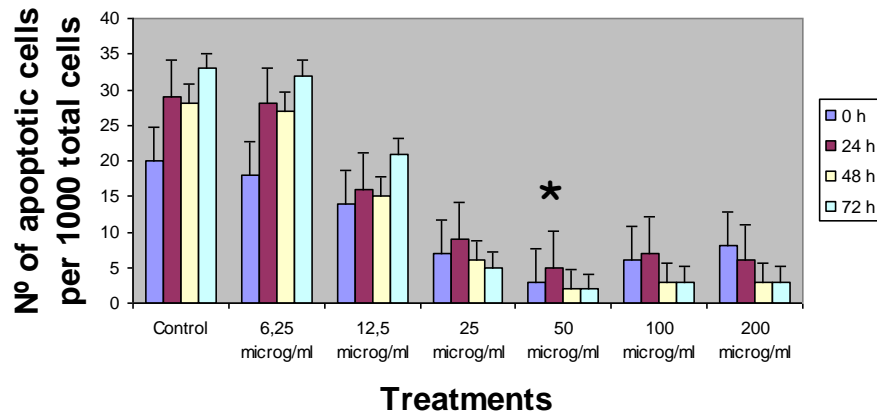
TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling)



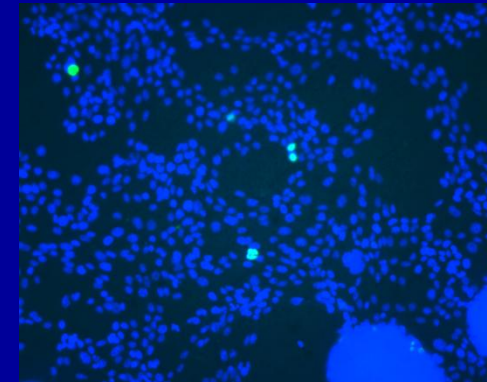
The assay relies on the presence of nicks in the DNA which can be identified by terminal deoxynucleotidyl transferase, an enzyme that will catalyze the addition of dUTPs that are secondarily labeled with a marker. It may also label cells that have suffered severe DNA damage.

# *S. haematobium* total antigen decreased the apoptosis of epithelial cells *in vitro*

## Apoptosis



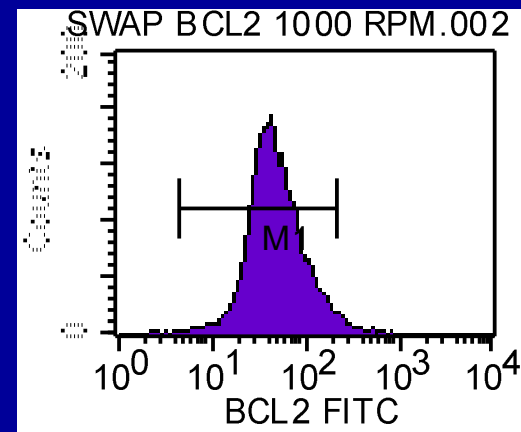
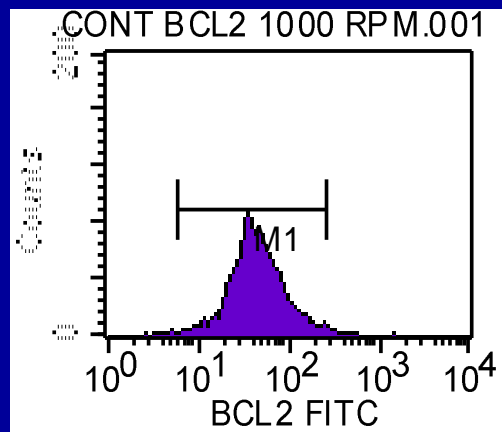
Control



Sh-treated

Apoptosis of Sh treated cells analysed by TUNEL. The experiments were done in triplicate (\* $p < 0,01$ ; control vs. 50 microg/ml).

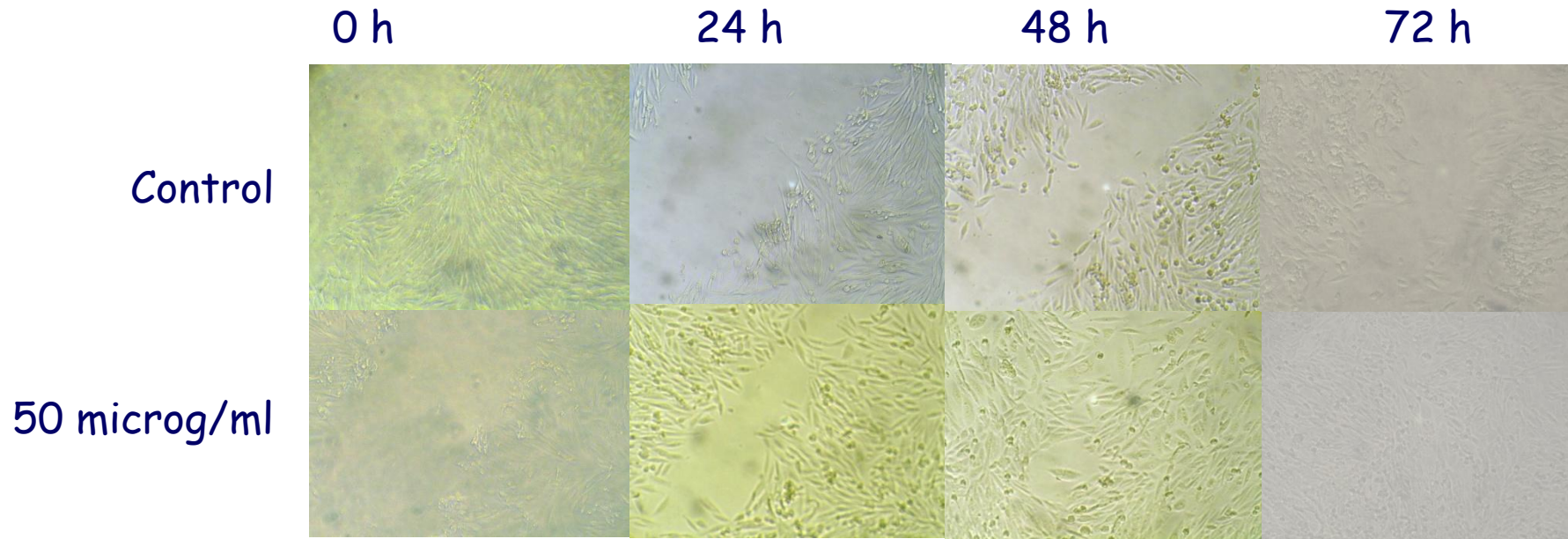
# Up-regulation of the anti-apoptotic protein Bcl-2, *in vitro*, by *S. haematobium* total antigen



Histogram of CHO cells expressing BCL-2 by FACS analysis. Control left panel and Sh treated cells right panel.

Groups	Bcl-2
Control	50,11 1,39
Sh-treated	52,99 2.04

# Induced migration of epithelial cells *in vitro* by *S. haematobium* total antigen

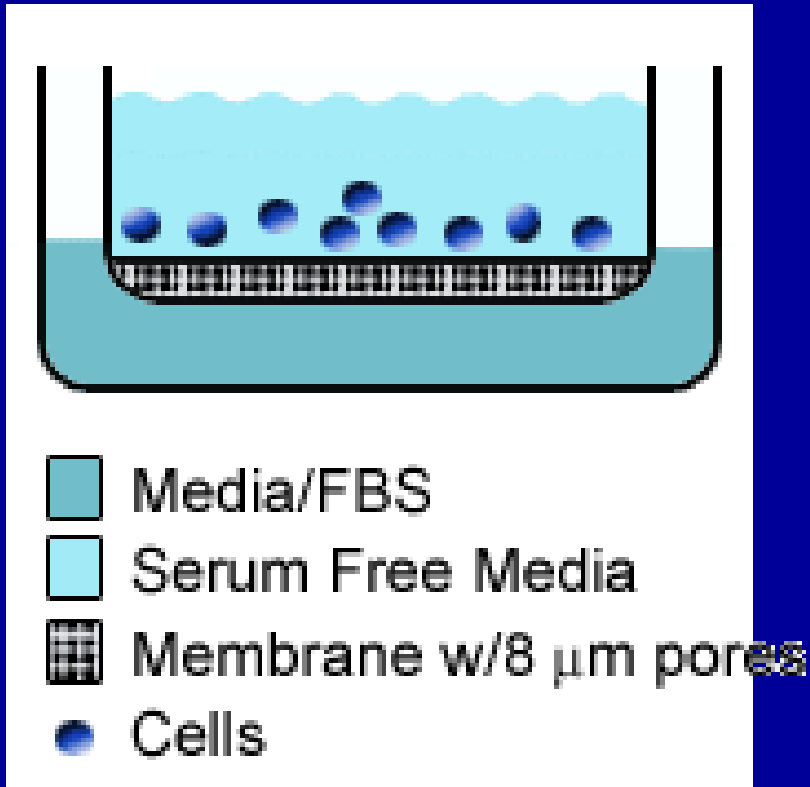


Wound closure assay

# Methodological Strategy

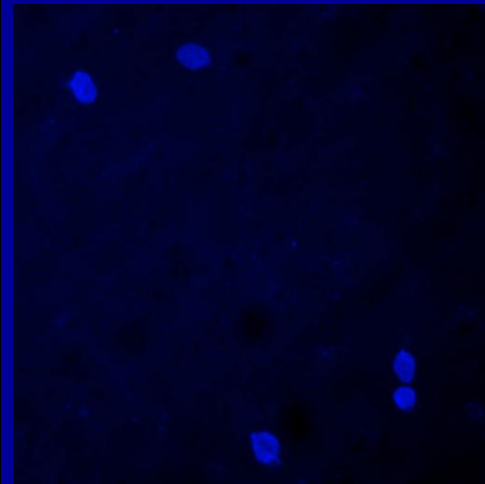
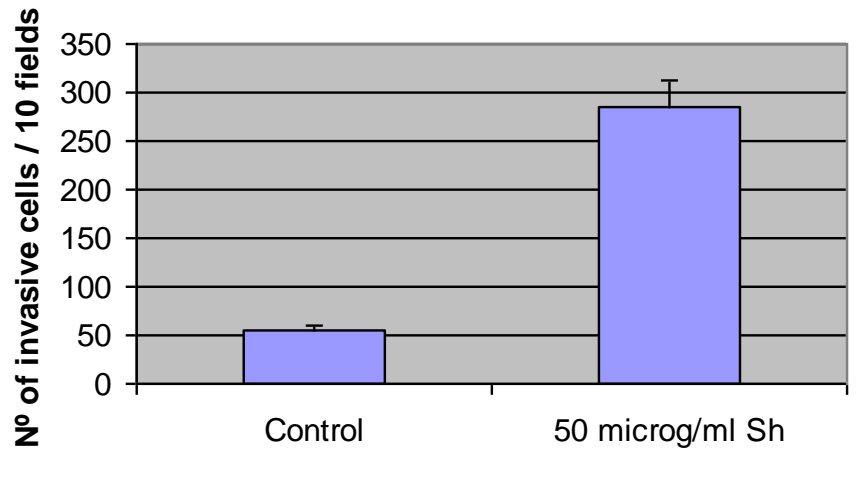
## Invasion

## Boyden chamber

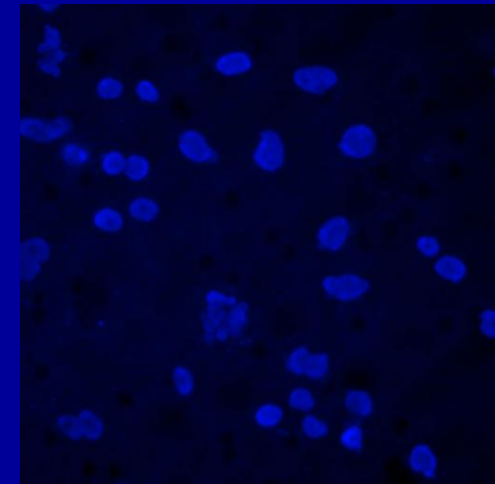


1. Cell suspension is placed in upper chamber and incubated for 24-72 hours at 37 C.
2. Invasive cells pass through the membrane and cling to the bottom of the polycarbonate membrane that is tissue culture-treated to enhance cell attachment.
3. Non-invading cells cannot pass through the membrane and stay in the upper chamber.

# Induced invasion of epithelial cells *in vitro* by *S. haematobium* total antigen



Control



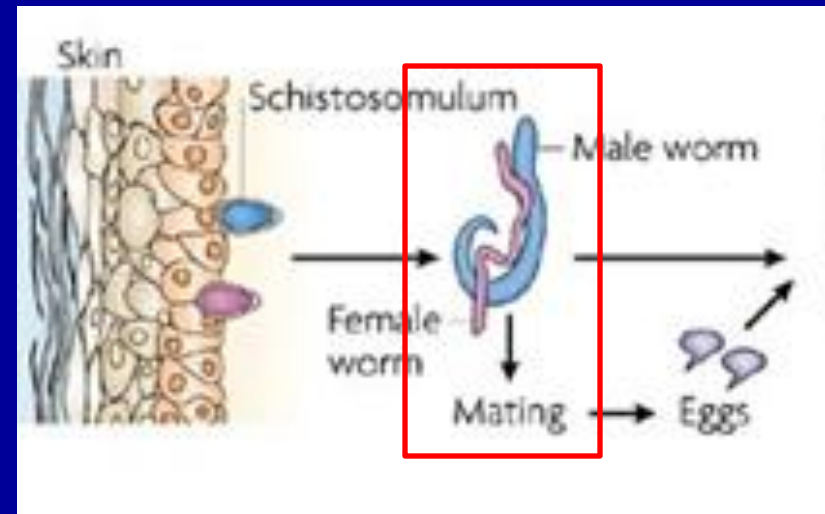
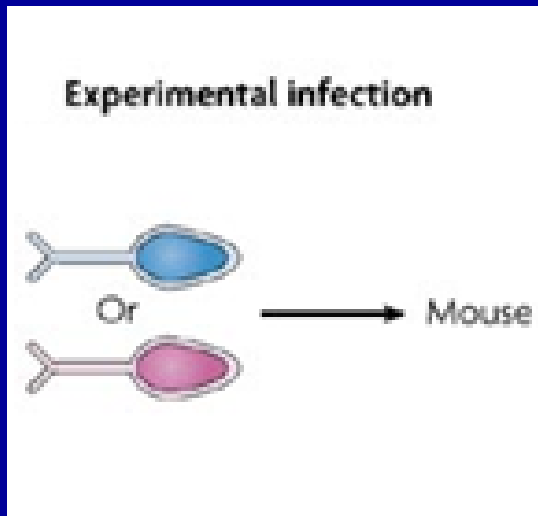
Sh-treated

Effect of Sh on cell invasion ( $p < 0,01$ ; control vs. Sh treated cells).

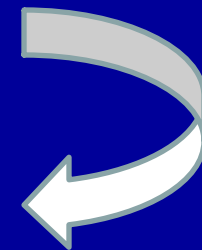
Tumorigenic effect of  
*Schistosoma haematobium* total  
antigen in normal mammalian cells

Botelho M, Oliveira PA, Gomes J,  
Gartner F, Lopes C, Correia da Costa  
JM, Machado JC  
(Int. J. Exp. Path., 2009)

# Methodological Strategy

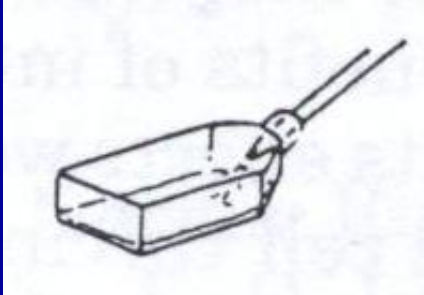


*S. haematobium*  
total antigen (Sh)

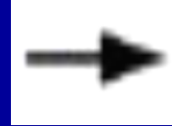
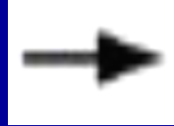


# Methodological Strategy

Chinese Hamster  
Ovary (CHO) cells



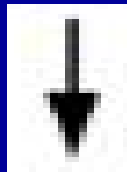
Sh-treatment 48 hours



Nude mice

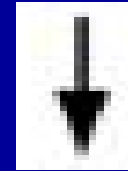


Control CHO cells



No Tumour

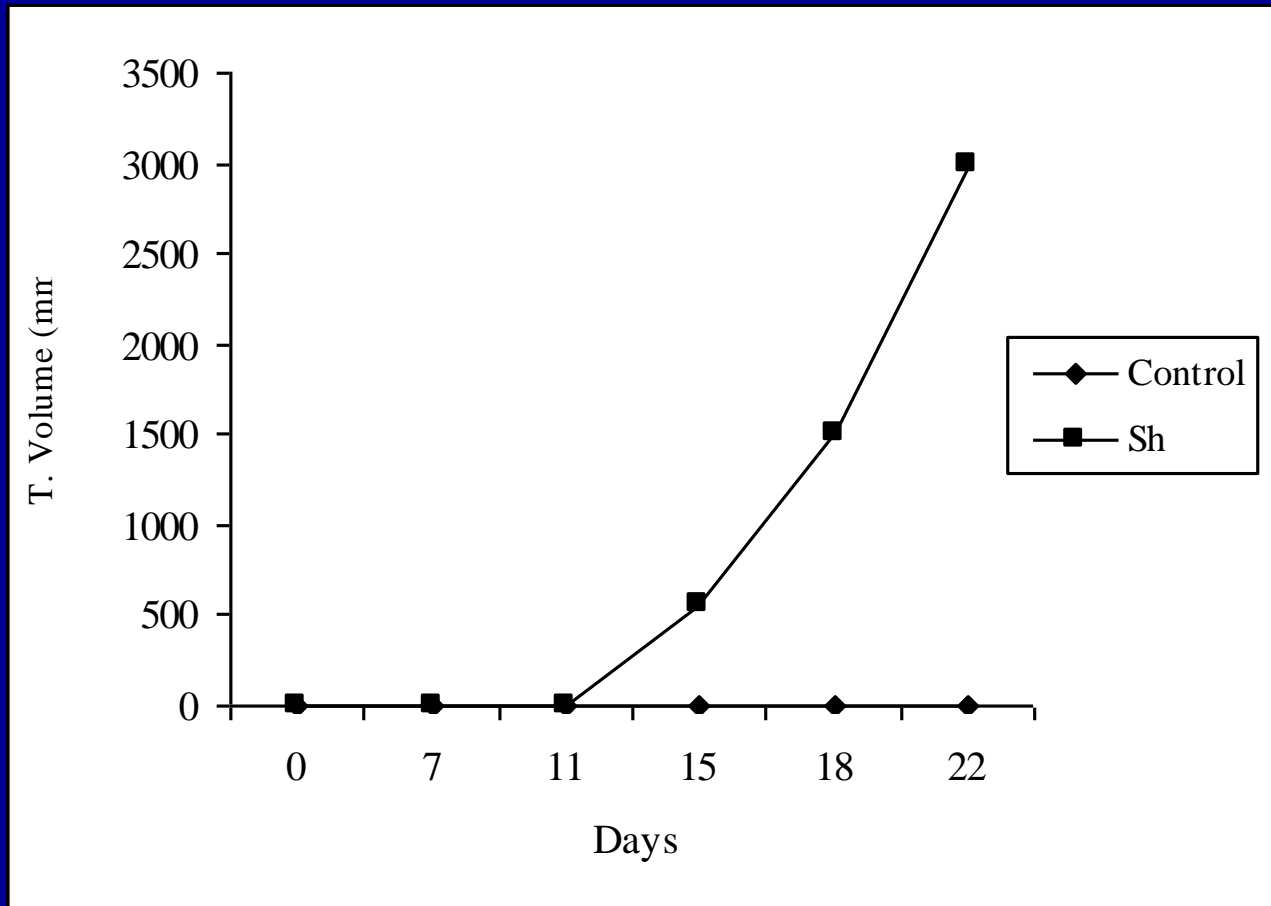
Sh-treated CHO cells



Tumour-Bearing Nude Mouse

2 weeks

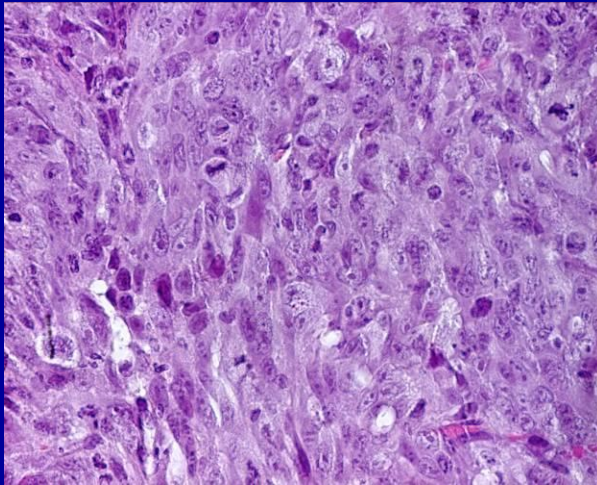
# Sh allows the vigorous growth of CHO xenografts in nude mice



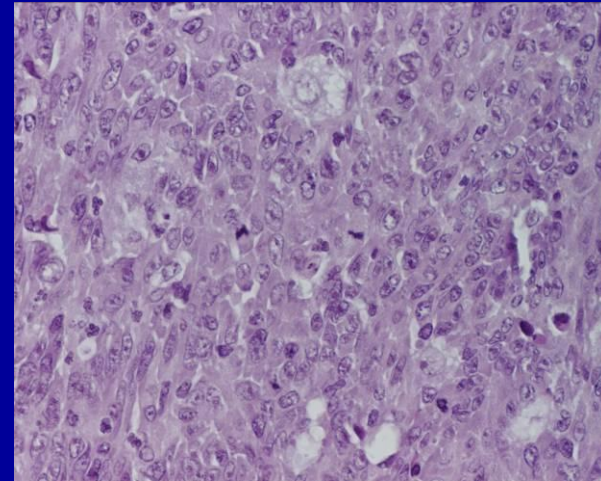
# Immunohistochemistry and histochemical staining

Group	Tumour	Immunohistochemistry		Histochemistry
		Cytokeratin	Vimentin	AgNORs
1(Sh)	Sarcoma	-	+	1,97 1,09
1(Sh)	Sarcoma	-	+	1,94 1,06
1(Sh)	Sarcoma	-	+	1,72 0,9
1(Sh)	Sarcoma	-	+	1,88 1,09
1(Sh)	Sarcoma	-	+	1,86 1,12
2(Ctrl)	-	-	-	-
2(Ctrl)	-	-	-	-
2(Ctrl)	-	-	-	-

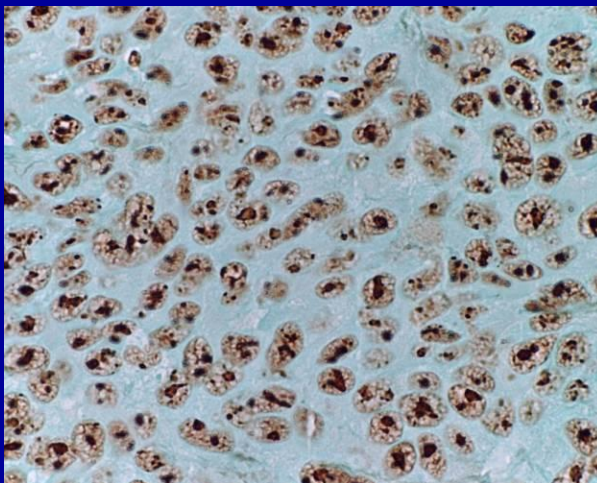
# Histological findings of the tumour mass produced by inoculation of CHO cells exposed to Sh



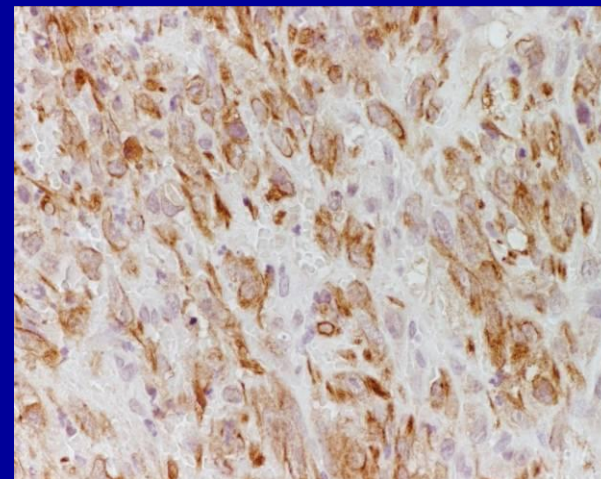
A



B



C



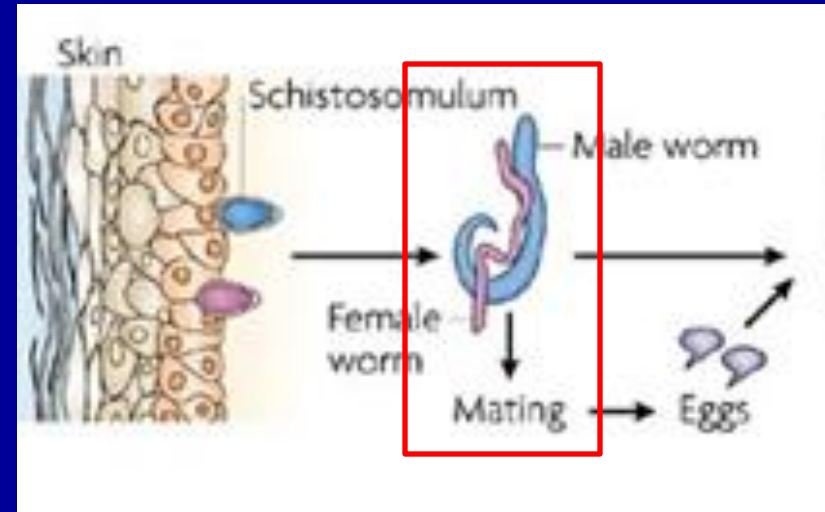
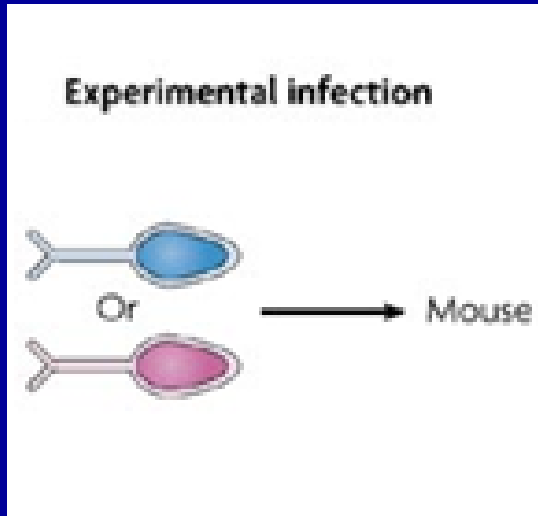
D

Induced dysplasia and  
inflammation of *Schistosoma*  
*haematobium* total antigen on  
normal urothelium of CD-1 mice

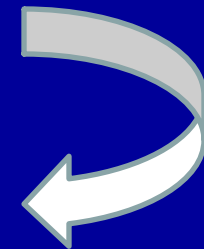
Botelho M , Oliveira PA, Lopes C,  
Correia da Costa JM and Machado  
JC

(Urologic Oncology: Seminars and Original  
Investigations, 2009)

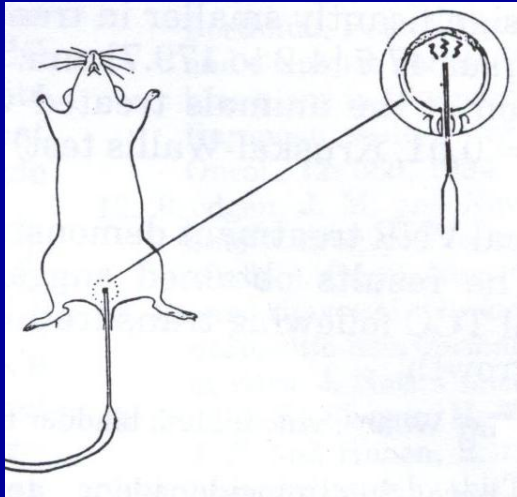
# Methodological Strategy



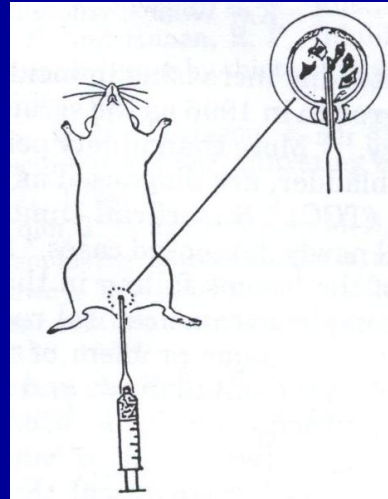
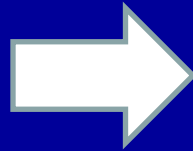
*S. haematobium*  
total antigen (Sh)



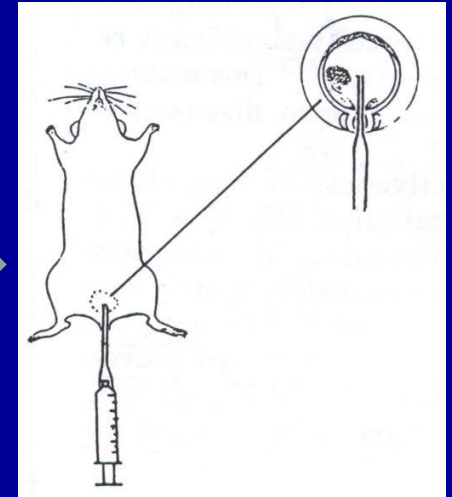
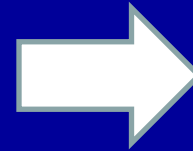
# Methodological Strategy



Transurethral  
Catheterization

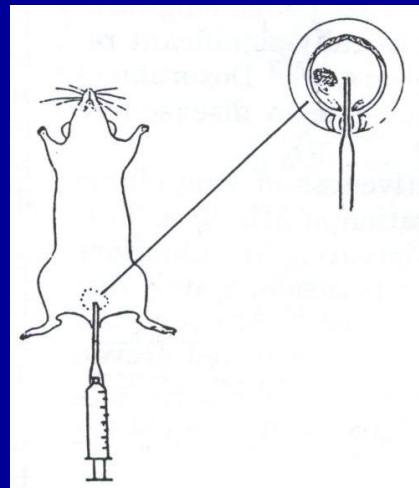


Urothelial  
Denudation



Sh-treatment  
/PBS  
1 hour

In situ fixation  
10% PPA



20 weeks  
40 weeks

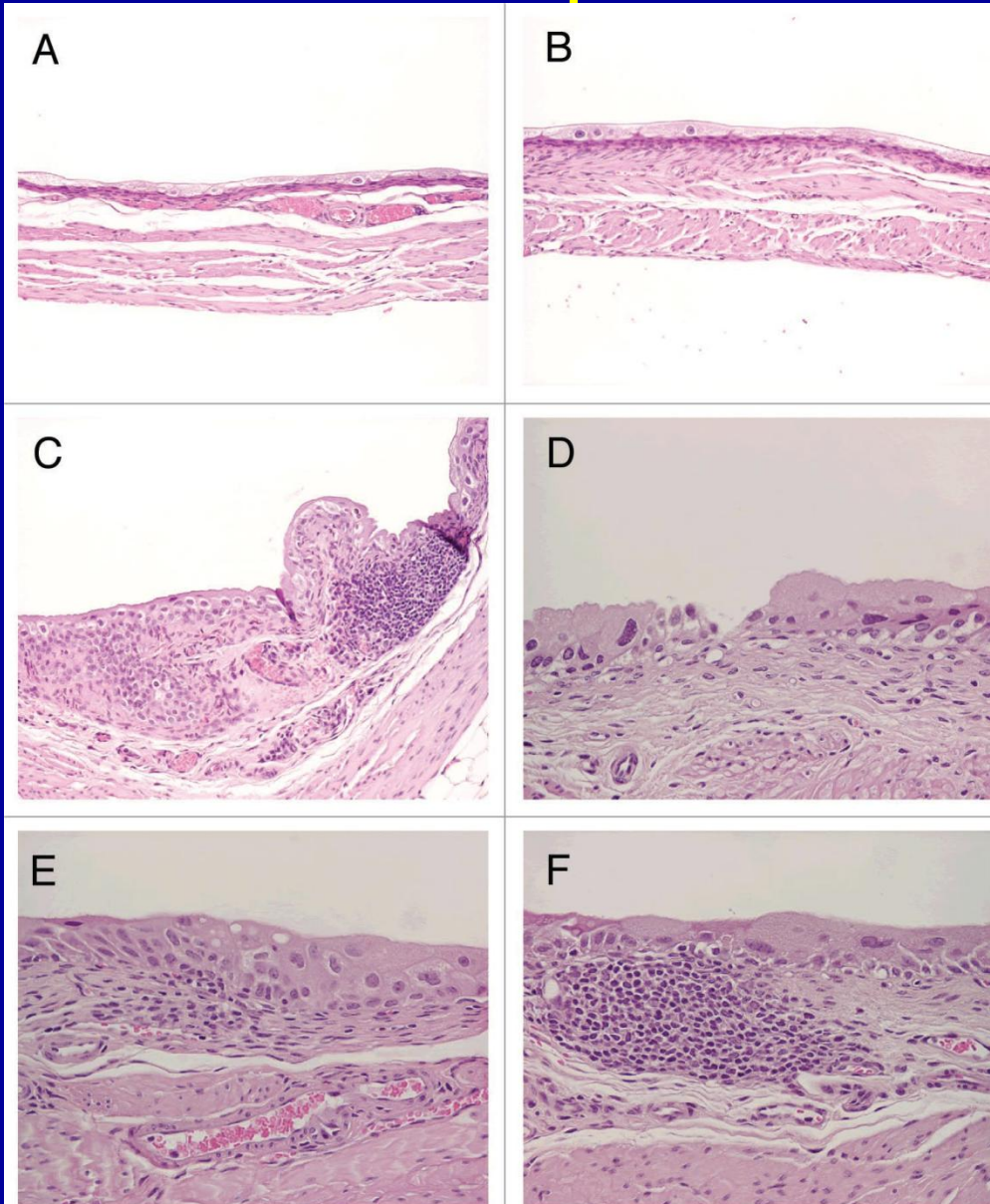
# Methodological Strategy



# Summary of the histopathological data in CD-1 mice urothelium exposed to Sh

	Group	Umbrella cells	Inflammation	Dysplasia
20 weeks	Sh	(5/10) 50%	(9/10) 90%	(3/10) 30%
	Control	(9/10) 80%	(2/10) 20%	(0/10) 0%
40 weeks	Sh	(0/10) 0%	10/10 (100%)	(7/10) 70% P=0,001
	Control	(10/10) 100%	3/10 (30%)	(0/10) 0%

# Histological findings of CD-1 mice urothelium exposed to Sh

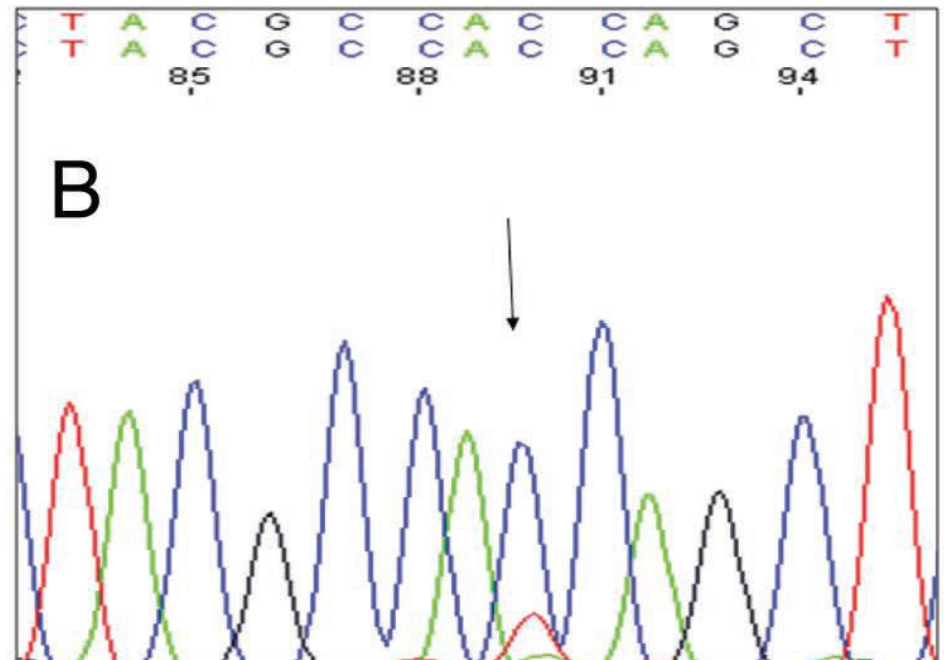
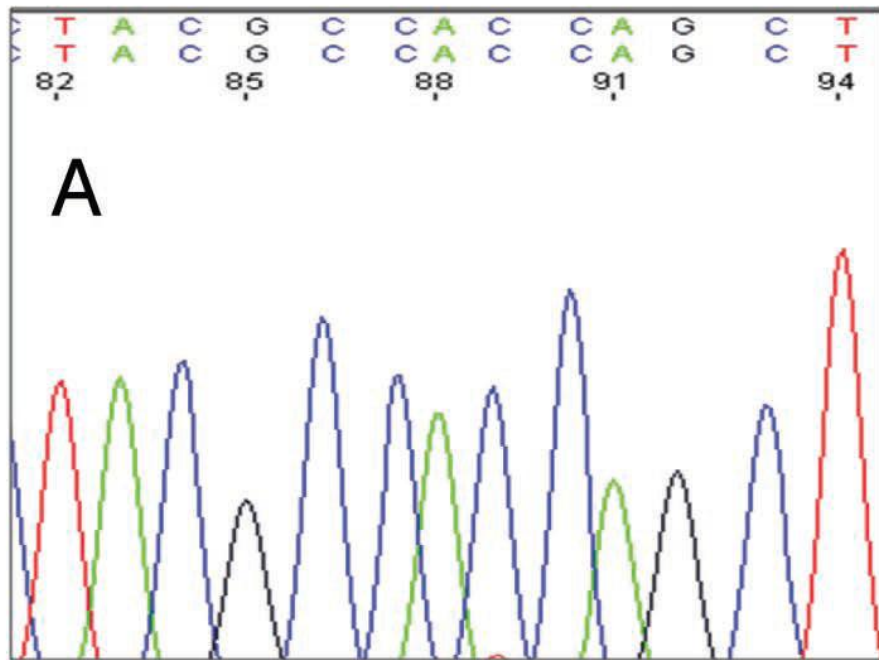


**Carcinogenic ability of *S.*  
*haematobium* through oncogenic  
mutation of KRAS gene**

Mónica Catarina Botelho, José Carlos  
Machado and José Manuel Correia da  
Costa

(Virulence, 2010)

# KRAS exon 2 sequencing results

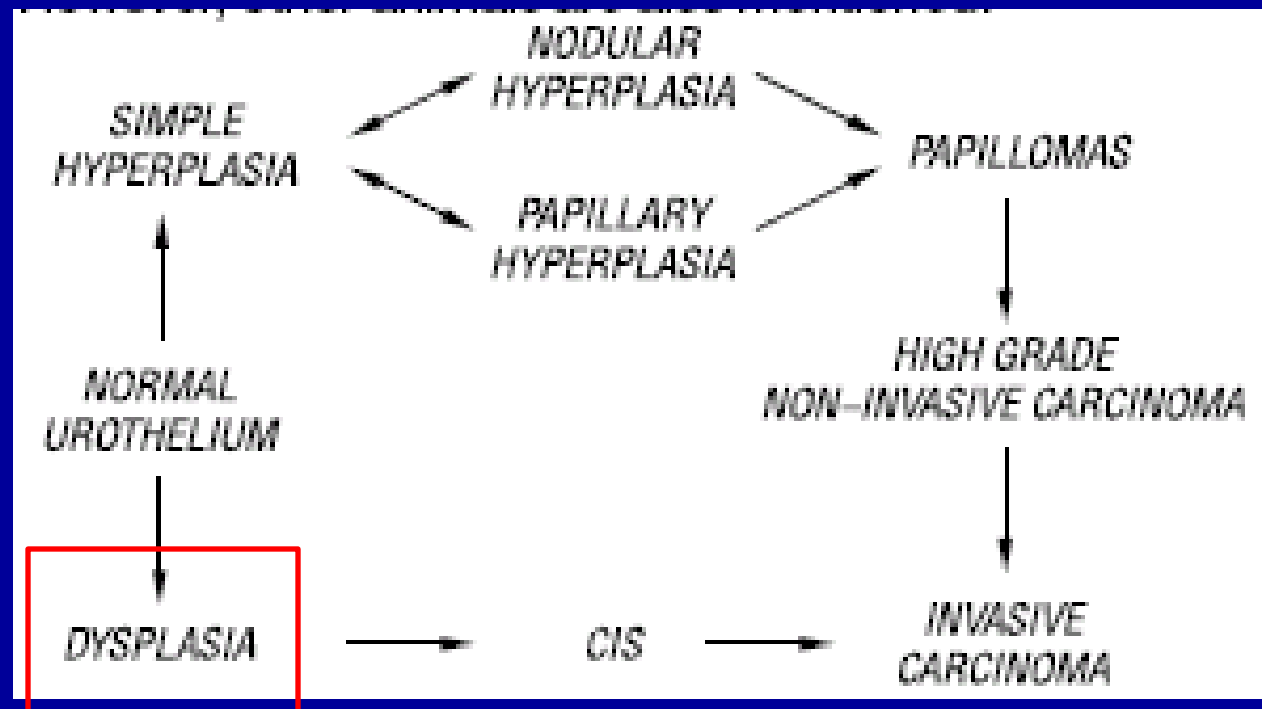


- Is there a role for *S. haematobium* in bladder cancer?

- Is there a role for *S. haematobium* in bladder cancer?
- Yes!

- Is there a role for *S. haematobium* in bladder cancer?

- Yes!



Pathogenic pathways of rat and mice urinary bladder carcinogenesis.

# Take home message I

- *S. haematobium* total antigen induced increased proliferation of normal epithelial cells in culture.
- *S. haematobium* alters the cell cycle of treated cells as demonstrated by increased S-phase and down-regulation of the CDKI p27 expression of normal epithelial cells in culture.
- p27 causes cell cycle arrest in G1 phase and abrogating its function results in uninhibited cell proliferation and tumorigenesis.

# Take home message II

- *S. haematobium* dramatically decreased the apoptosis of epithelial cells by up-regulating Bcl-2 protein.
- Treated cells with *S. haematobium* total antigen had increased migration than control cells.
- *S. haematobium* induced the invasion of treated cells 5 times more than control cells.

# Take home message III

- *S. haematobium* total antigen has the potential to induce tumour development, assessed by the use of a nude mice xenograft model.
- *S. haematobium* total antigen in CD-1 mice normal bladders after intravesical administration of the parasite antigen, induced alterations in the urothelium of these animals consistent with dysplasia and inflammation .
- The parasite extract of *S. haematobium* has carcinogenic ability possibly through oncogenic mutation of KRAS gene.

# Credits

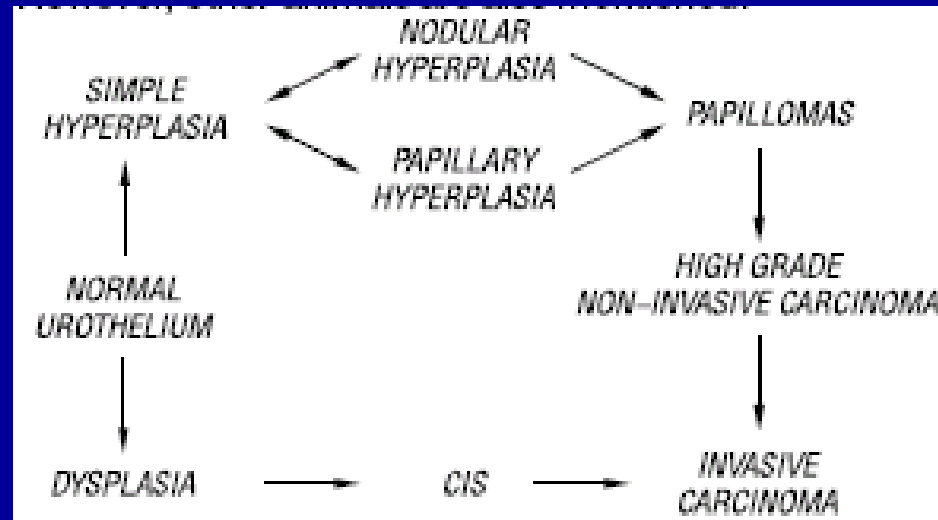
- **CIBP-INSA Porto**
  - José Manuel Costa
  - Paulo Vieira
  - Lurdes Delgado
- **IPATIMUP**
  - José Carlos Machado
  - Maria José Oliveira
  - António Carlos Ferreira
  - Fatima Gartner
  - Joana Gomes
- **FMUP**
  - Raquel Soares
- **UTAD**
  - Paula Oliveira
- **ICBAS**
  - Carlos Lopes
- **IPO Porto**
  - Gabriela Martins
  - Carlos Palmeira
  - Manuel Teixeira
  - Isabel Veiga

# Specific Aim

- To study schistosoma-associated SCC of the bladder through cancer pathways using *in vitro* and *in vivo* approaches.

# Ongoing work

- Animal studies with experimental infections



Pathogenic pathways of rat and mice urinary bladder carcinogenesis.

# *S. haematobium* and host hormones

- Male hypogonadism is a well known consequence of schistosomiasis (Saad, J Egypt Soc Parasitol, 1999).
- It has been shown that schistosomes synthesizes steroid hormones (Nirde et al, FEBS Letters, 1983).
- Schistosomes produce hormone-like signals (Mendonça et al, Parasitology Today, 2000).
- Existence of receptors able to bind the molecules of estradiol (Barrabes, Ann Parasitol Hum Comp, 1986; Mendonça et al, Parasitology Today, 2000).

# *S. haematobium* and host hormones

- Recent experimental evidence suggests that schistosomes can not only evade immune responses actively but also exploit the hormonal microenvironment within the host to favor their establishment, growth and reproduction (Escobedo et al, Trends in Parasitology, 2005).

Estrogen

Extracellular

Estrogen Receptor

ER $\alpha$

ER $\alpha$

ER $\alpha$

Cytoplasm

Nucleus

CBP

ER $\alpha$

ER $\alpha$

ERE

GHIP1

CARM1

p300

CBP

SHARP

HDACs

BRCA1

NCOR2 / SMRT

CYCLIN D1

SRA

PELP1

TRAP220

PGC1

RIP140

DAX1

REA

Me (Arg17)

Ac

H3

H3 (Nucleosome)

MEF2c

TAFs

TF2A

TBP

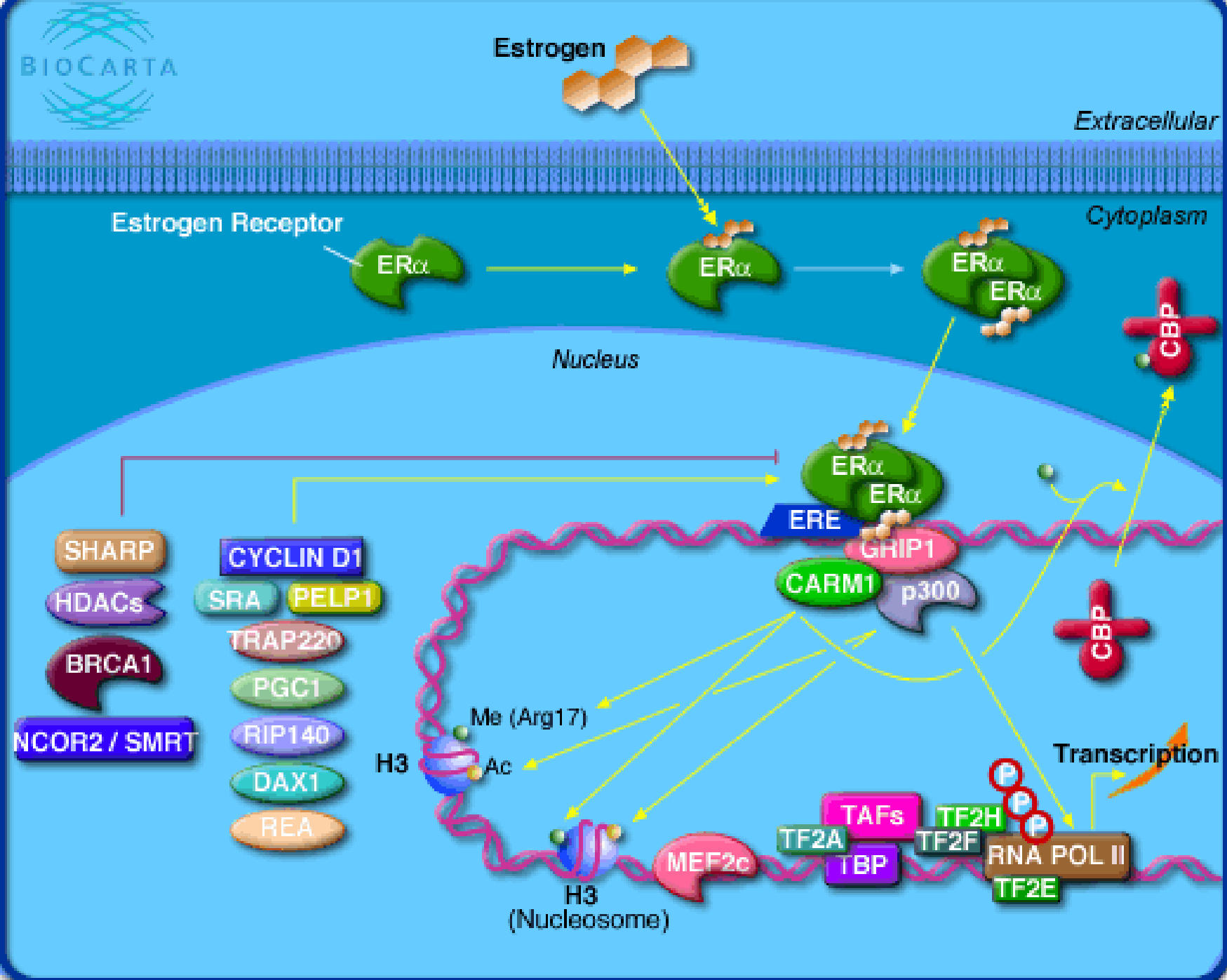
TF2H

TF2F

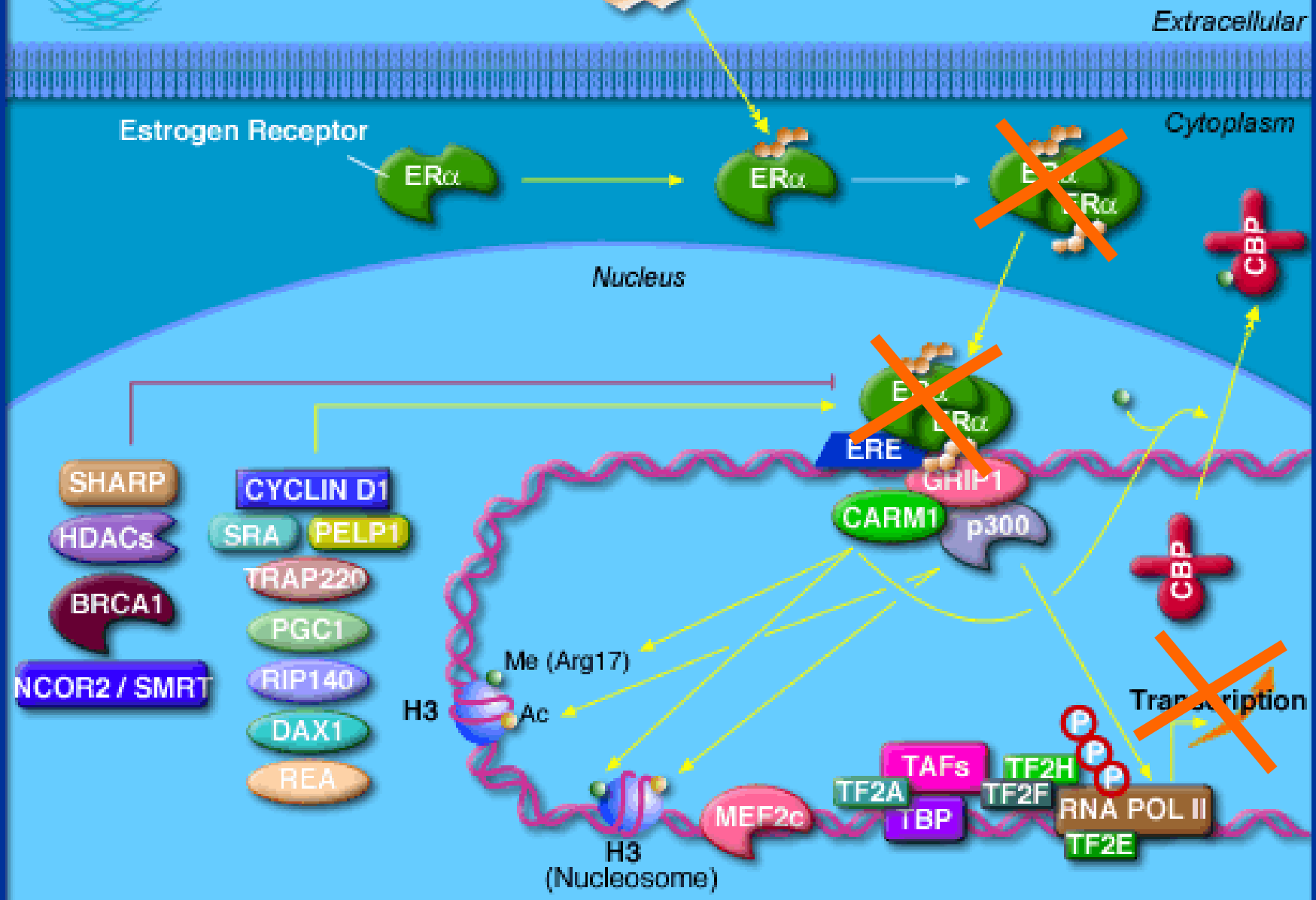
RNA POL II

TF2E

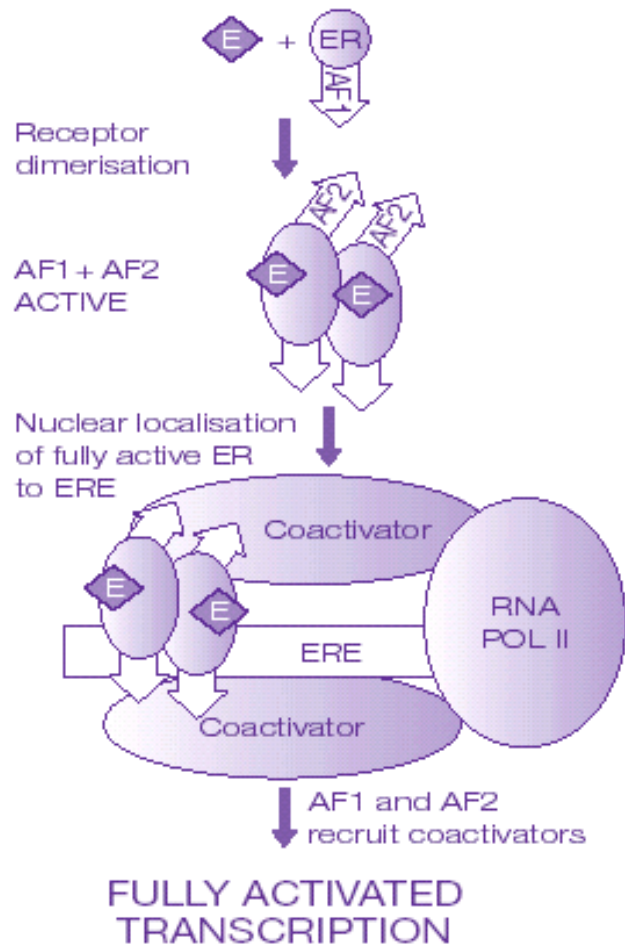
Transcription



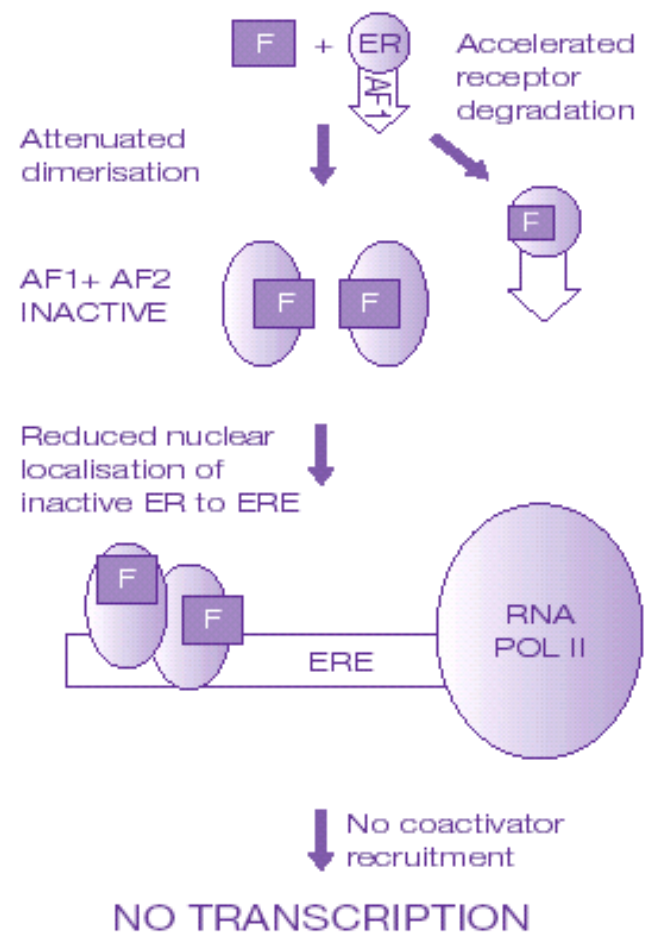
ICI 182,780



### ESTRADIOL



### 'FASLODEX'



Key:

ER = estrogen receptor, ERE = estrogen response element, AF = activation function, RNA POL II = RNA polymerase II

# *S. haematobium* infected patients have increased serum levels of estradiol

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

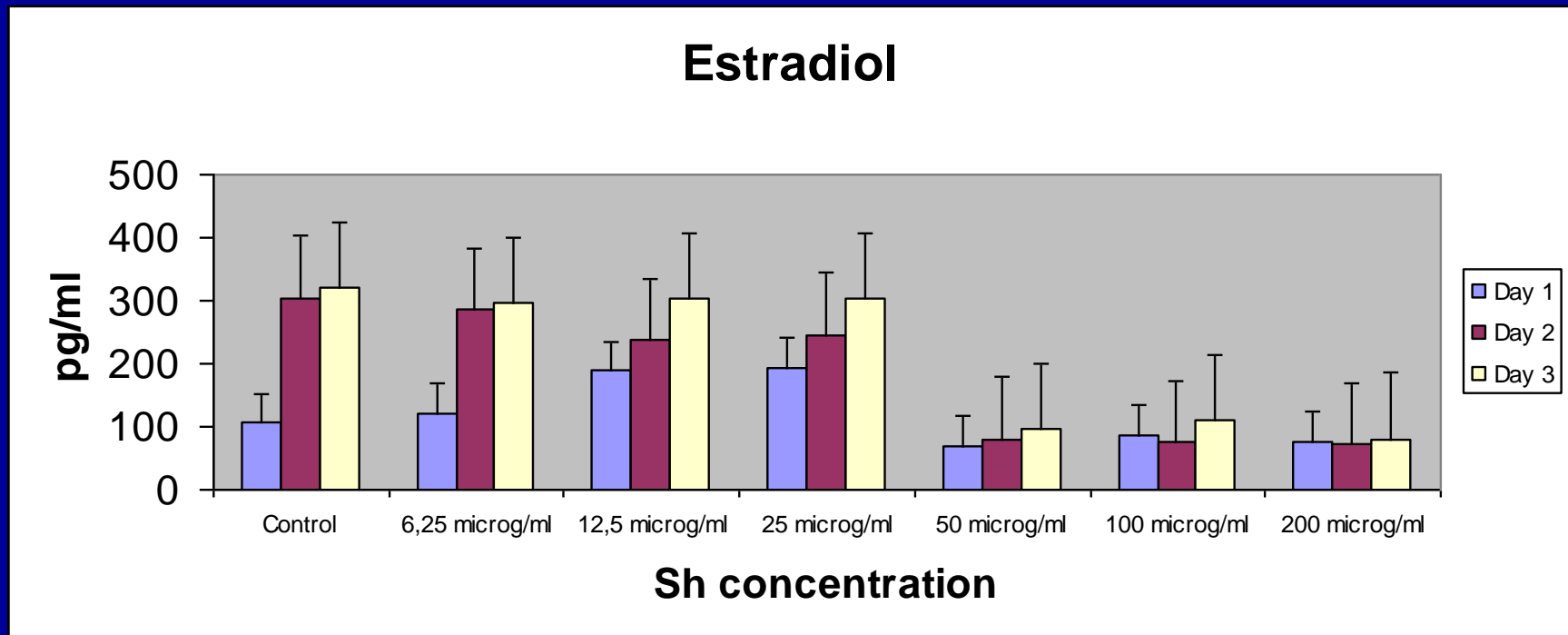
Hormones

Botelho M et al. Journal  
of Parasitology (In

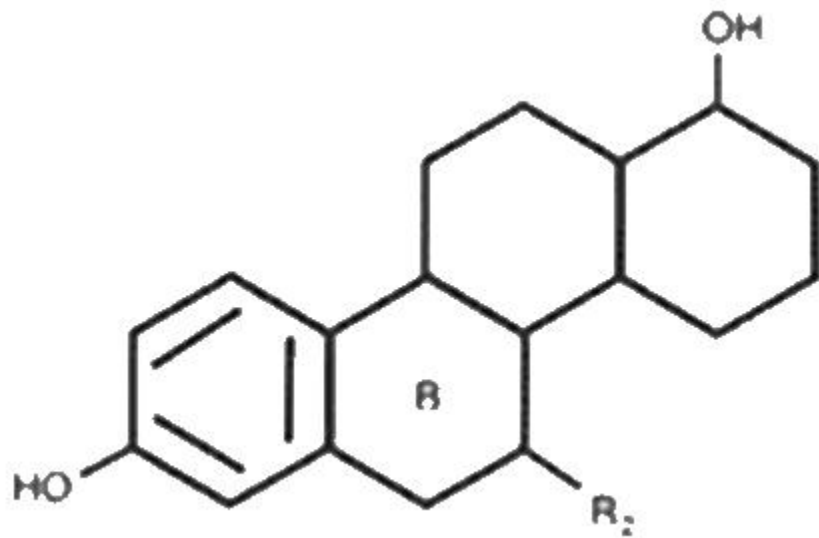
*S. haematobium* and *S. mansoni* express an estradiol analogue but not *Fasciola hepatica*

Antigenic preparations	E2 (pg/ml)	SD
<i>S. haematobium</i>	14,84	0,14
<i>S. mansoni</i>	12,63	0,27
<i>F. haepatica</i>	<10	
H <sub>2</sub> Od	<10	
10 nM E2	1632,99	2,55

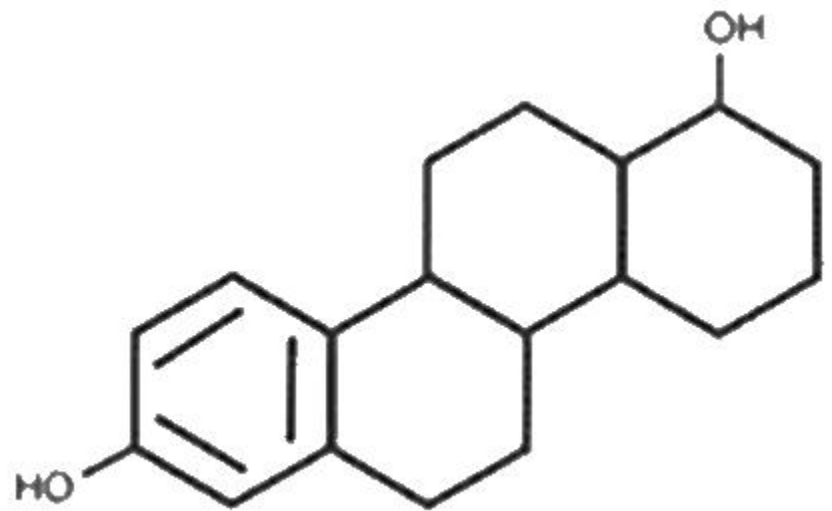
# *S. haematobium* total antigen decreases estradiol production of CHO cells *in vitro*



Estradiol production in culture. The experiments were done in triplicate ( $p < 0,01$ ; control vs. 50 microg/ml).

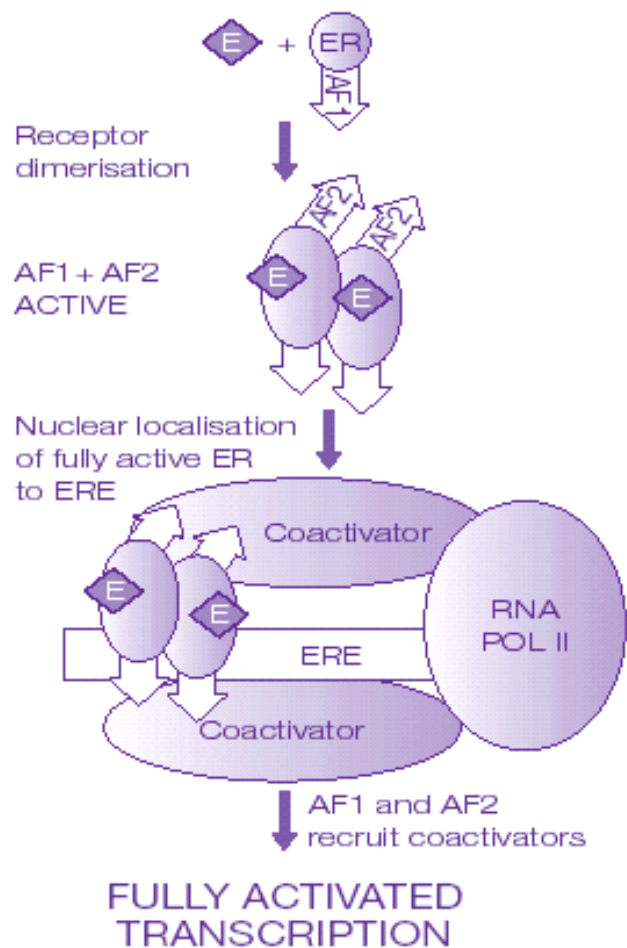


ICI 182,780

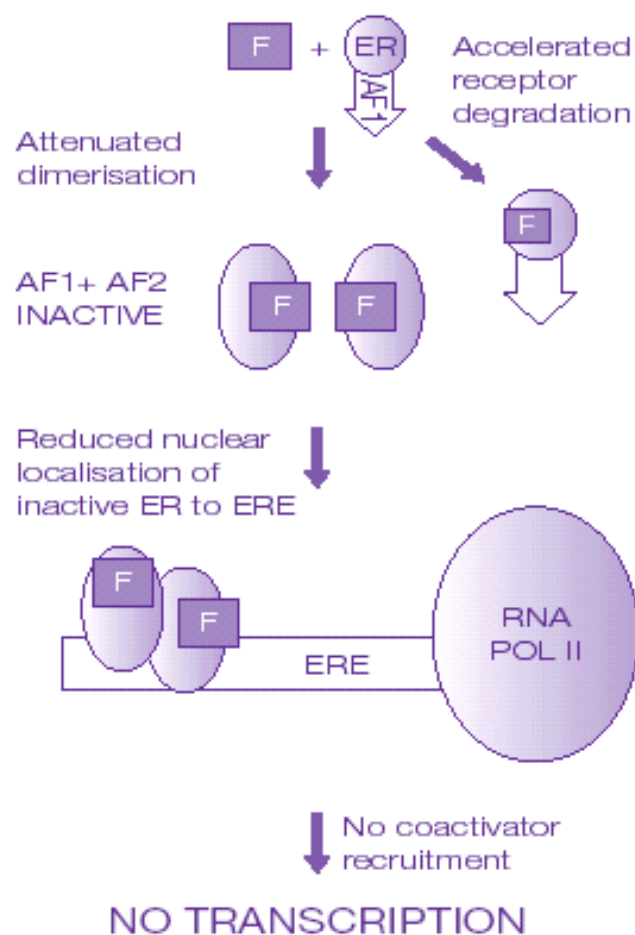


Estradiol (E2)

**ESTRADIOL**



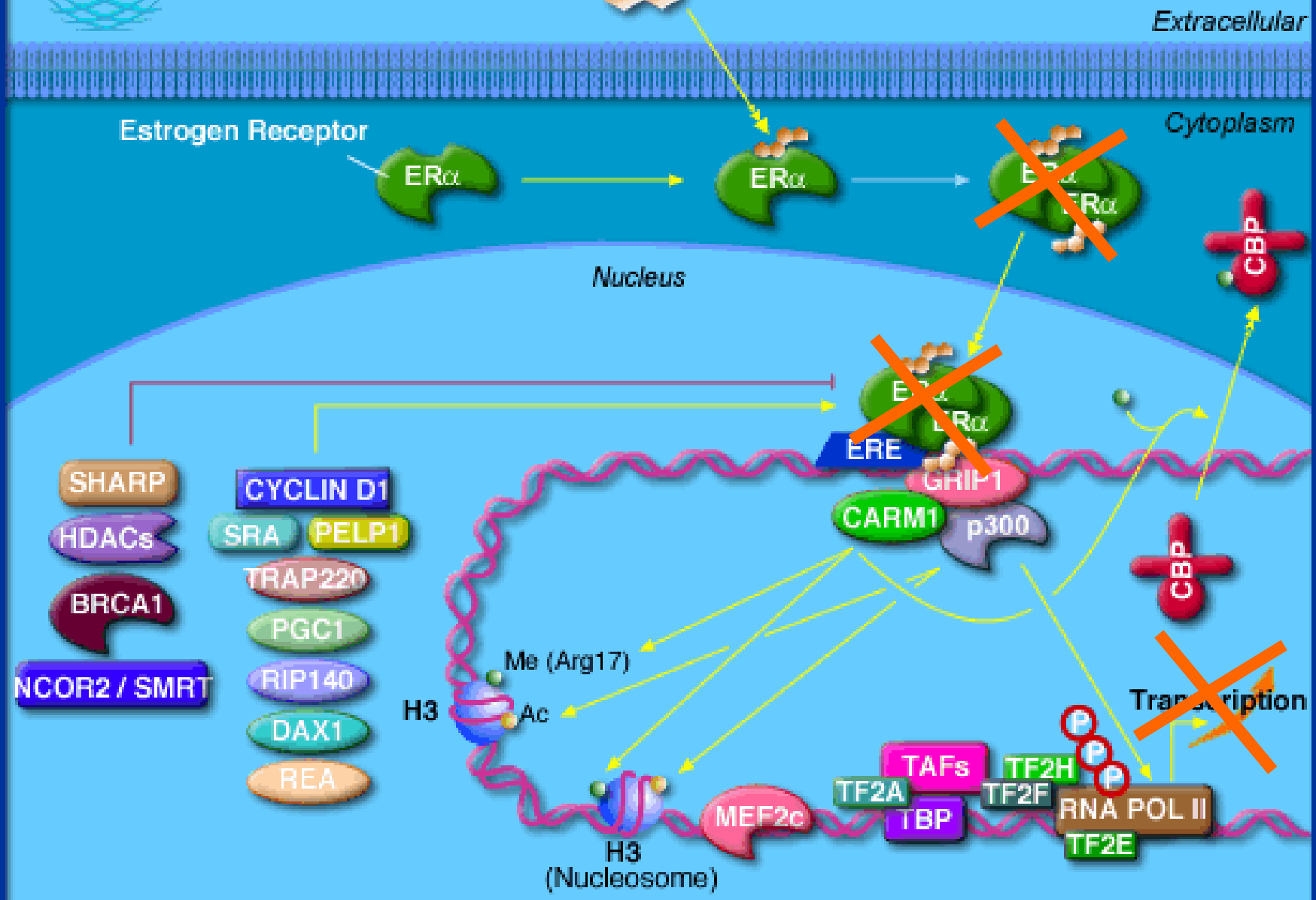
**'FASLODEX'**



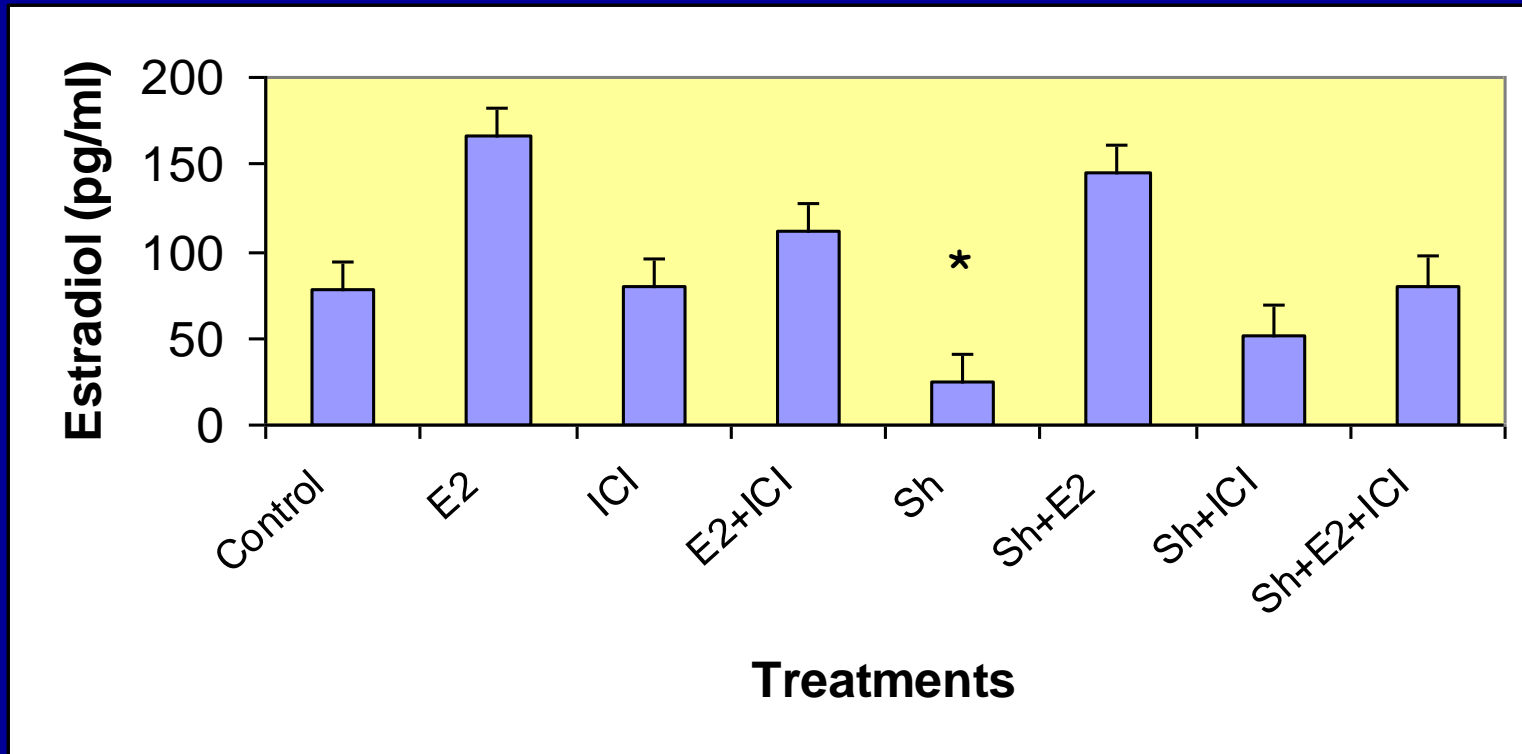
Key:

ER = estrogen receptor, ERE = estrogen response element, AF = activation function, RNA POL II = RNA polymerase II

ICI 182,780

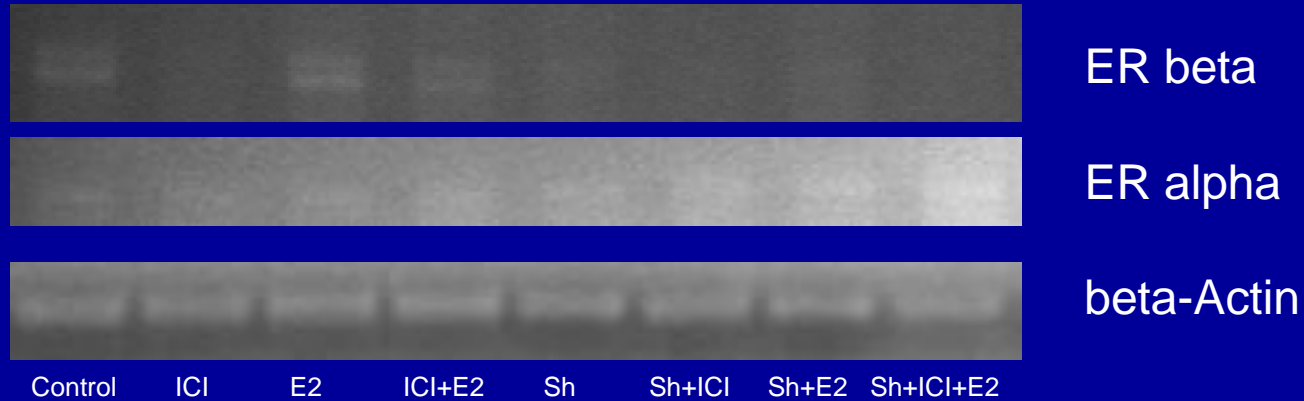


# *S. haematobium* total antigen is an antagonist of estradiol

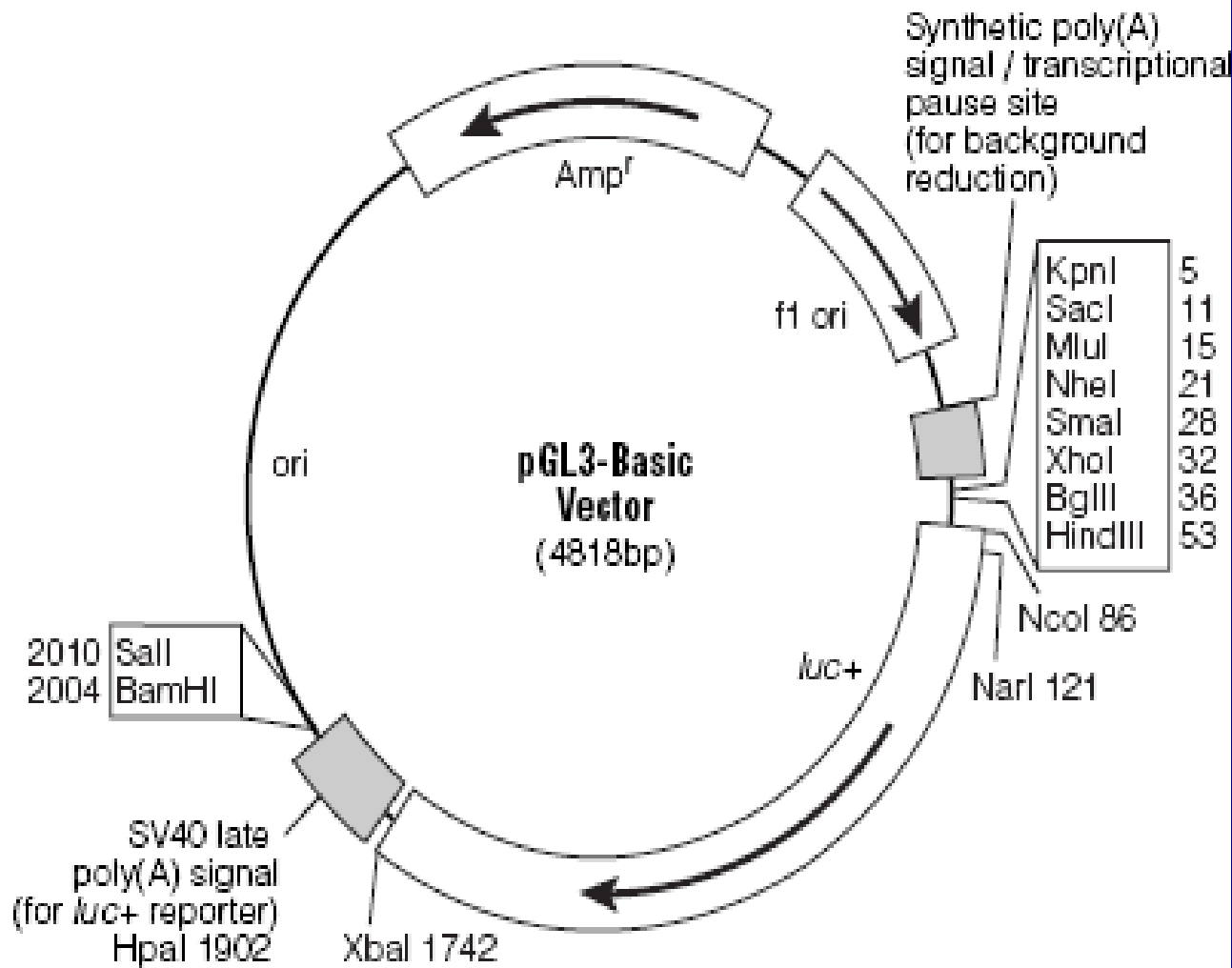


Estradiol production in supernatant of treated cells. Data represent means SEM from 3 independent experiments (\*  $p < 0,05$  control vs. Sh).

# *S. haematobium* total antigen down-regulates ER beta



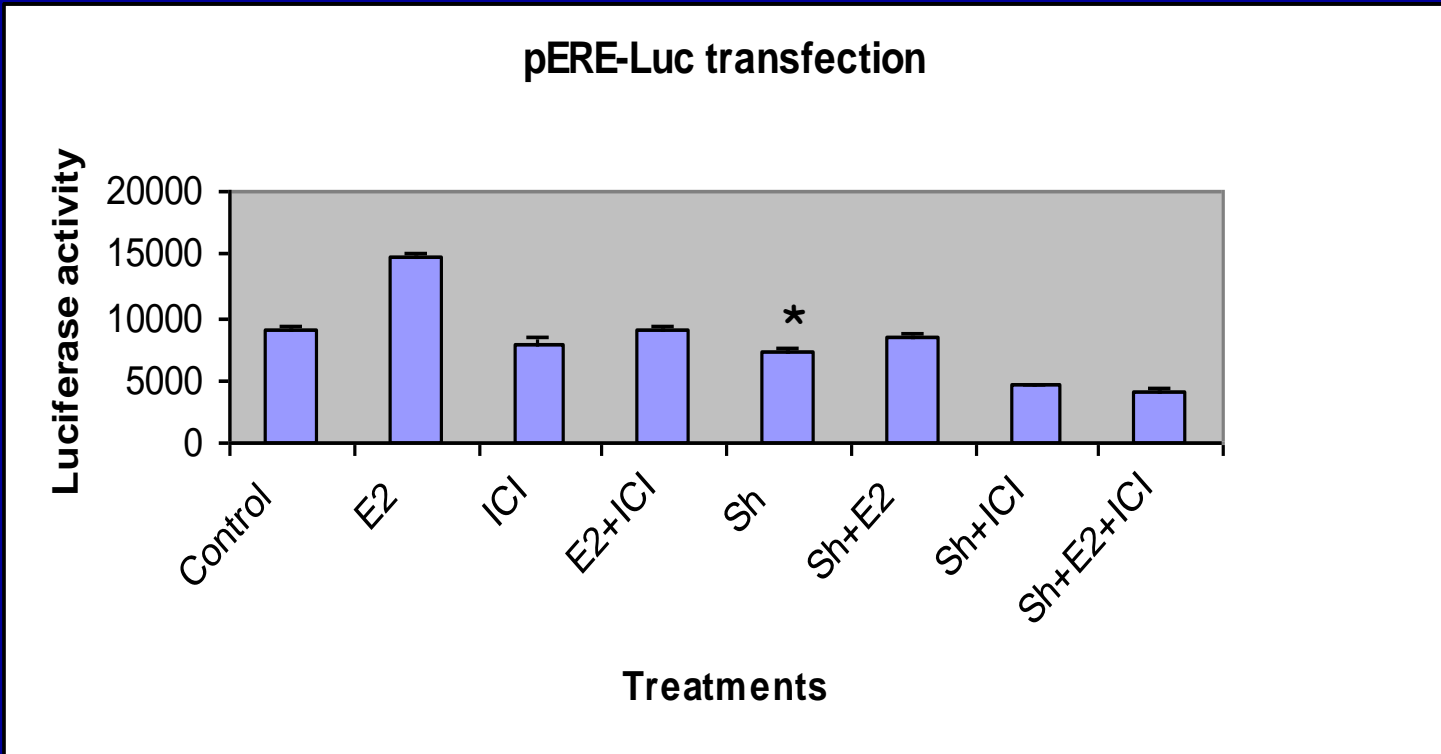
RT-PCR for ER alpha and ER beta in CHO cells.



Estrogen Responsive Element (ERE)

Hormones

# Inactivation of Estrogen Receptor signaling pathway by *S. haematobium* total antigen



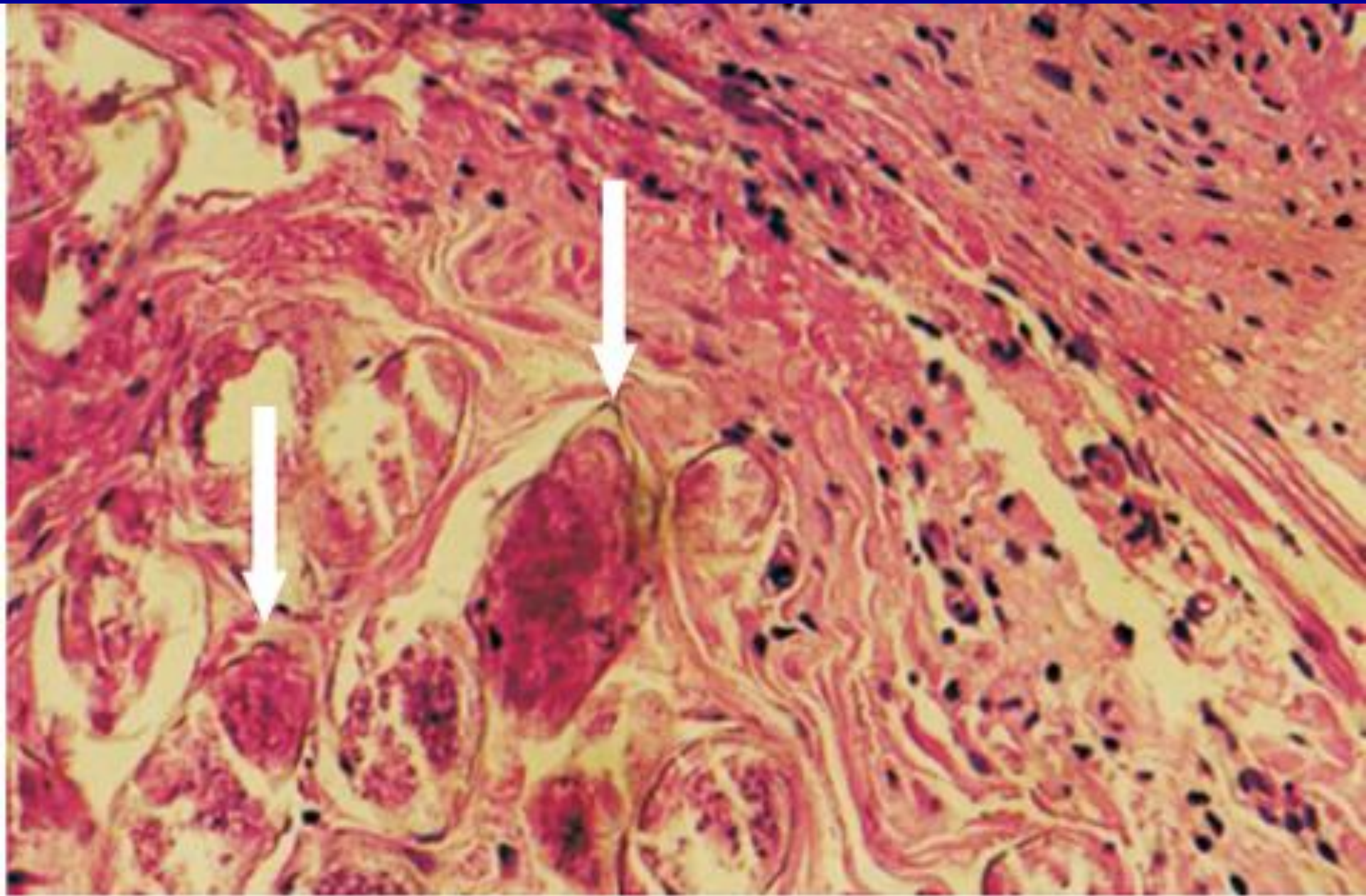
Role of Sh on inactivation of Estrogen Responsive Element (ERE) assessed by Luciferase Assay. Incubation with Sh resulted in a statistically significant decrease in luciferase activity compared to control (\*,  $P < 0.05$ ).



Egg with miracidium



Calcified egg



Hormones

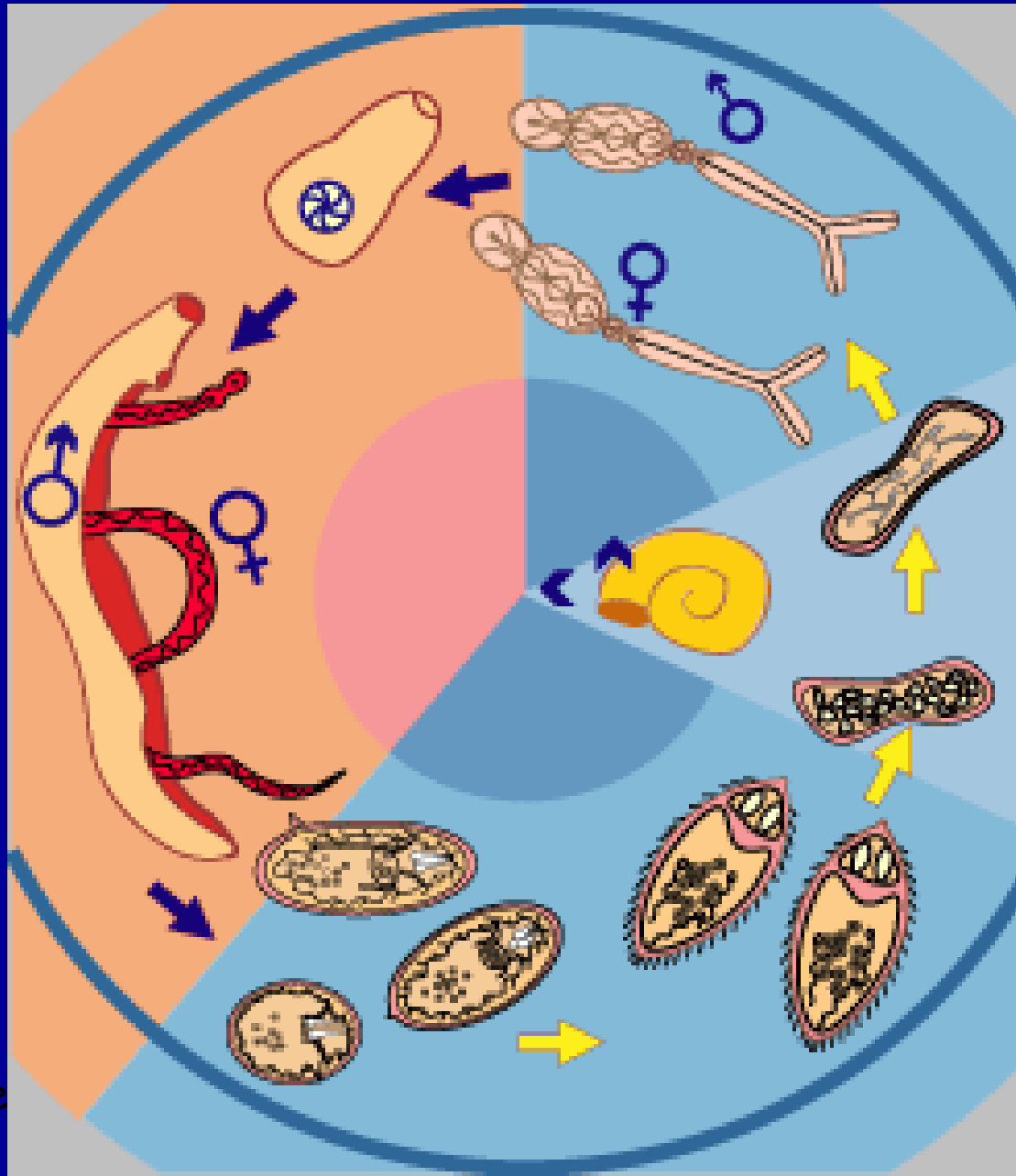


## Schistosomula

Adult worms in:  
blood vessel  
around rectum  
(mansoni)  
bladder  
(haematobium)  
mesenteric  
veins and  
pulmonary  
arteries  
(japonicum)

Egg production  
(10-30 per day)

Hormone



Cercaria (500  
per year per  
snail)

Sporocysts  
(several per  
miracidium)

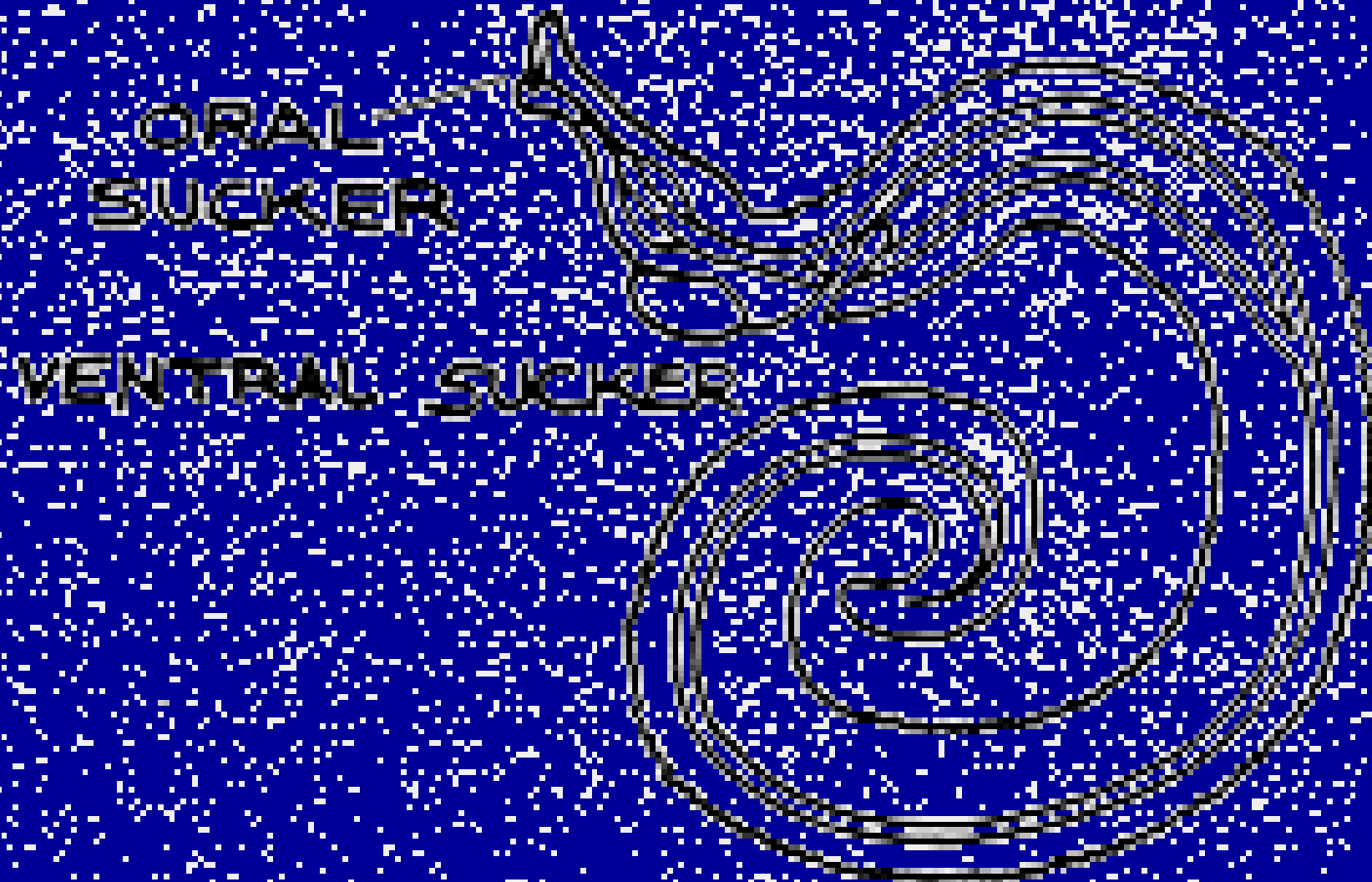
Miracidium in  
water



Hormones



Photo  
John Walker



oral sucker

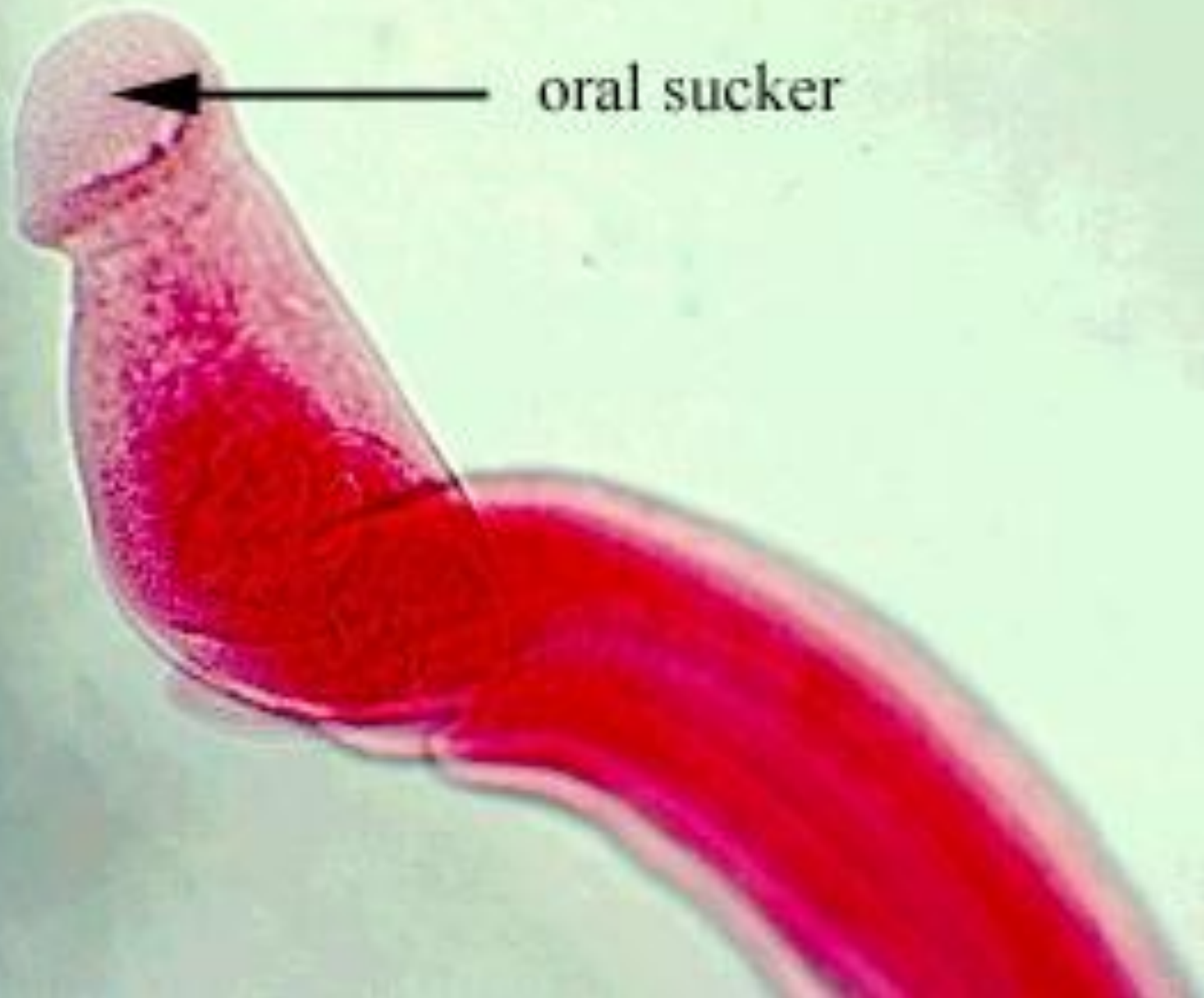


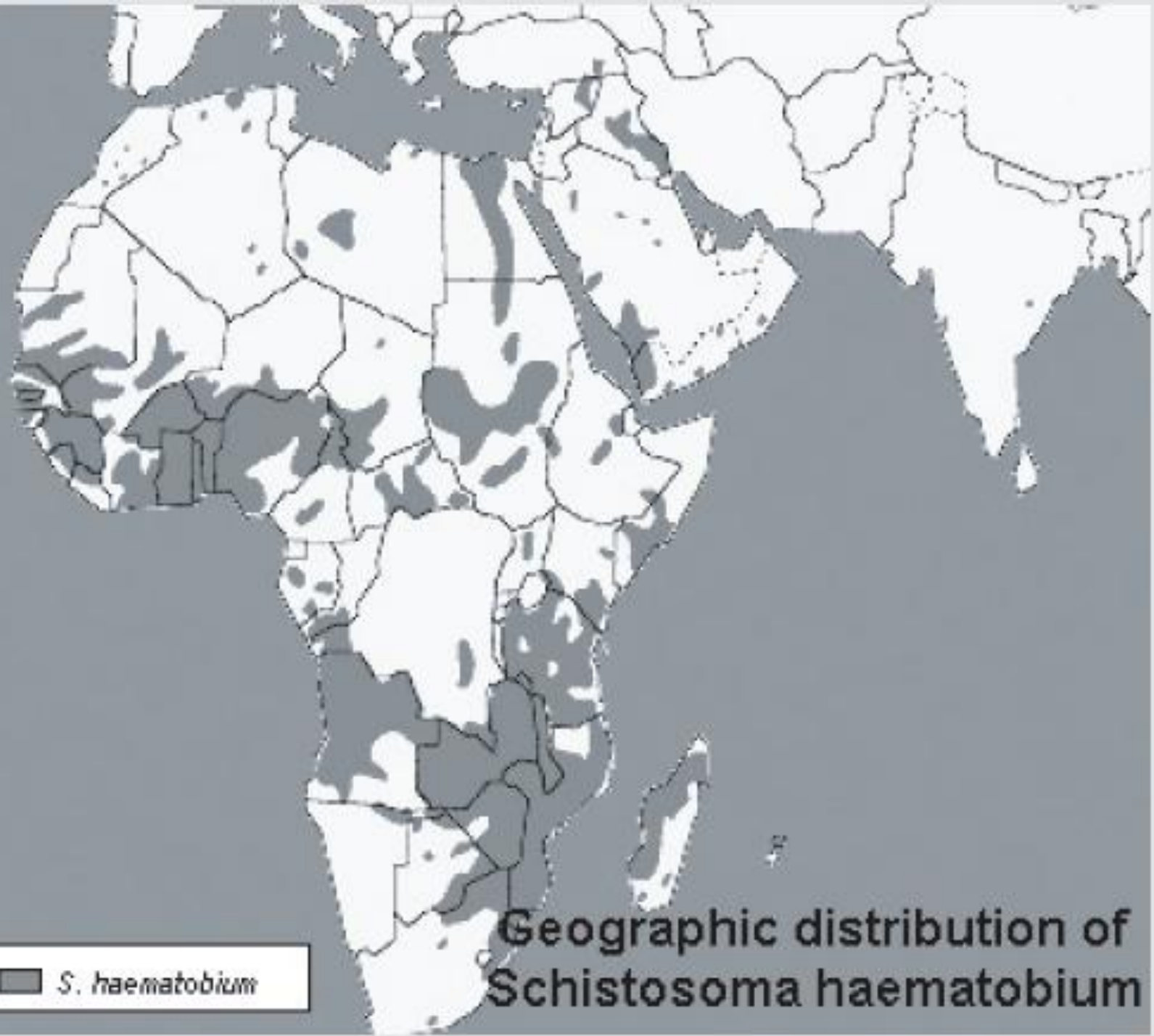


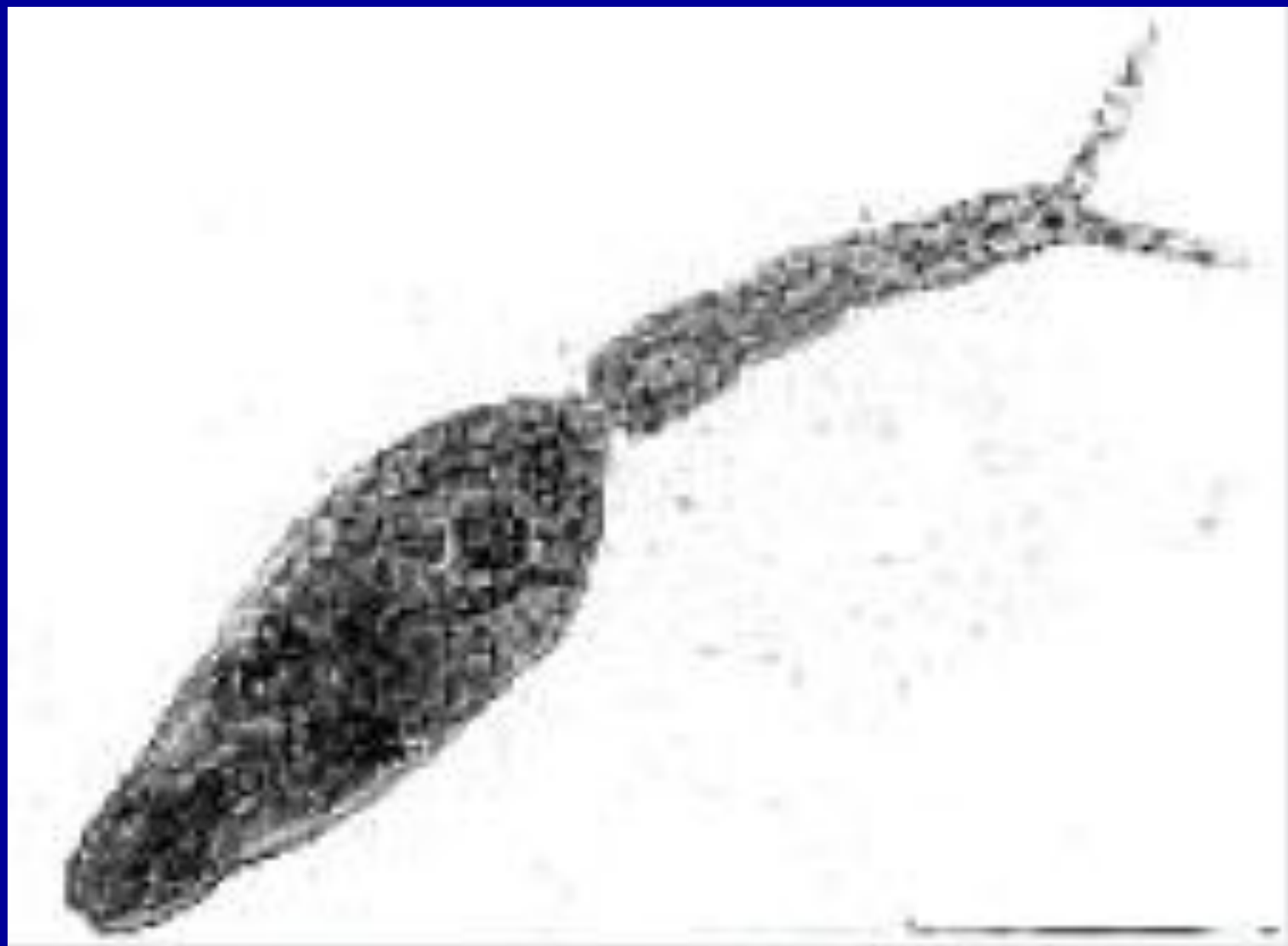
Photo  
John Walker

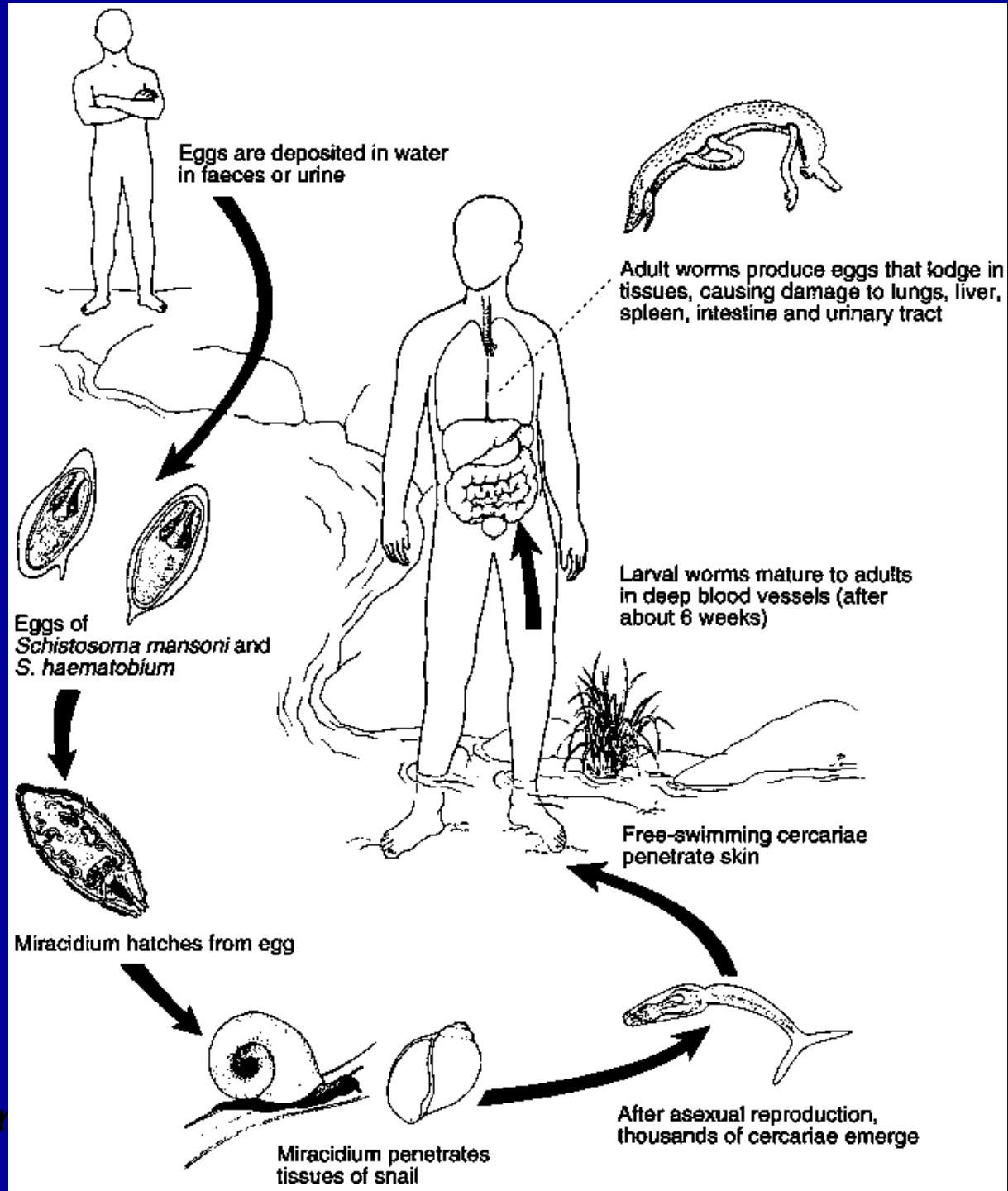


Photo  
John Walker

Hormones



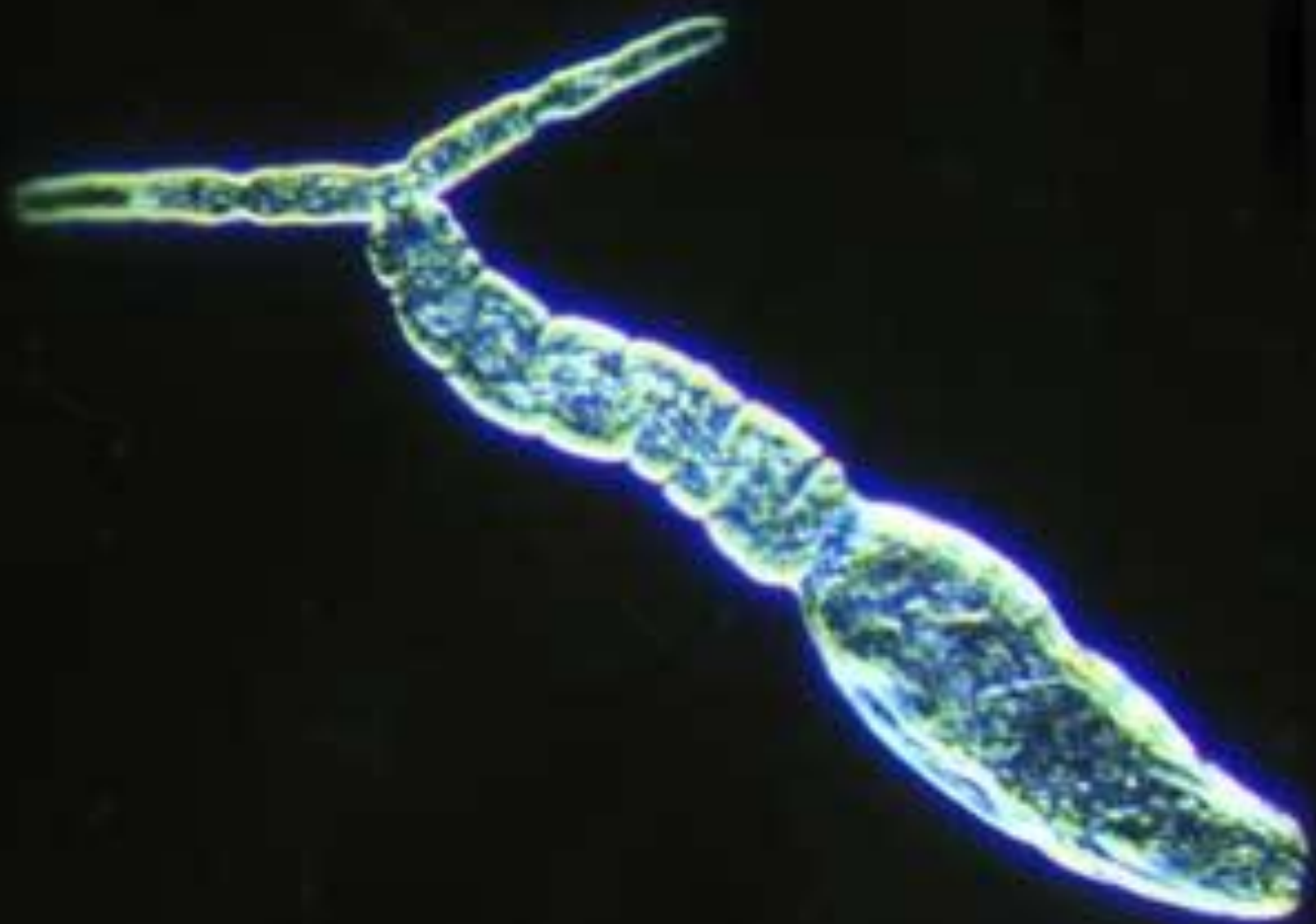


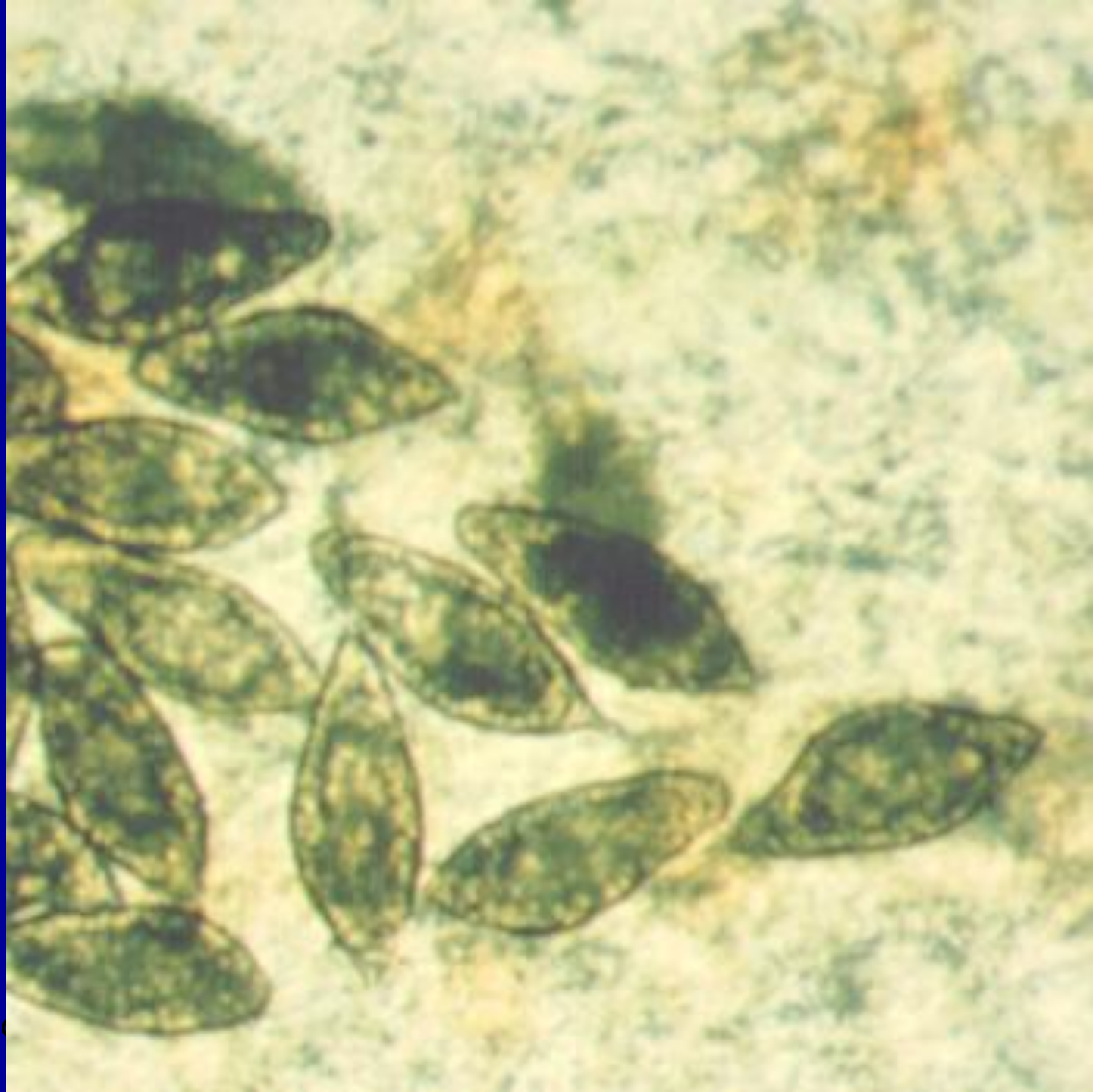


Hormon



Hormones



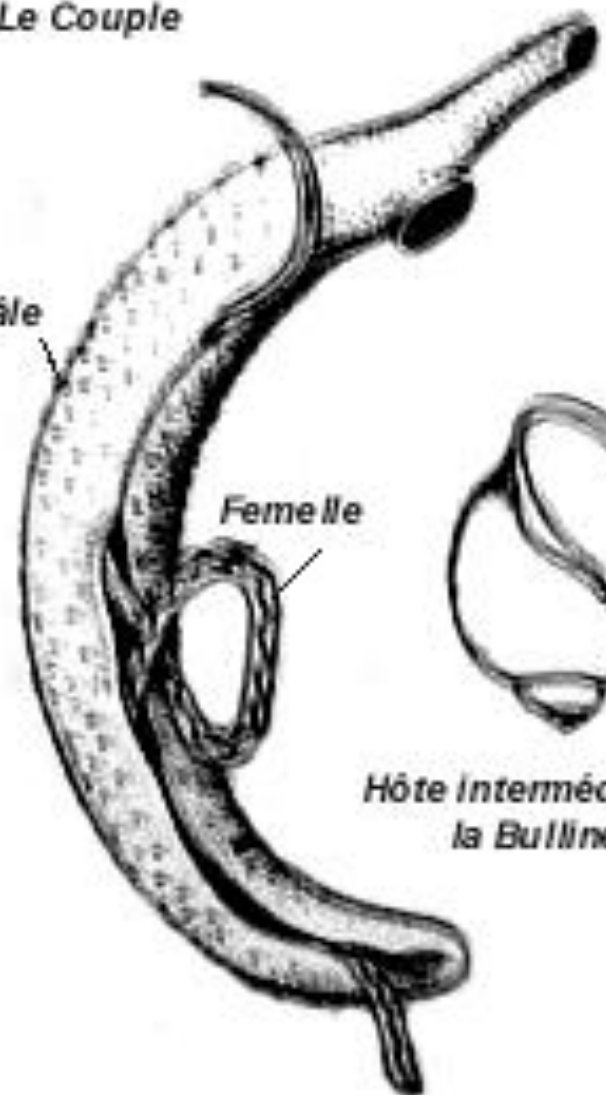


H

Le Couple

Mâle

Femelle



Hôte Intermédiaire,  
la Bulline



Cercaire

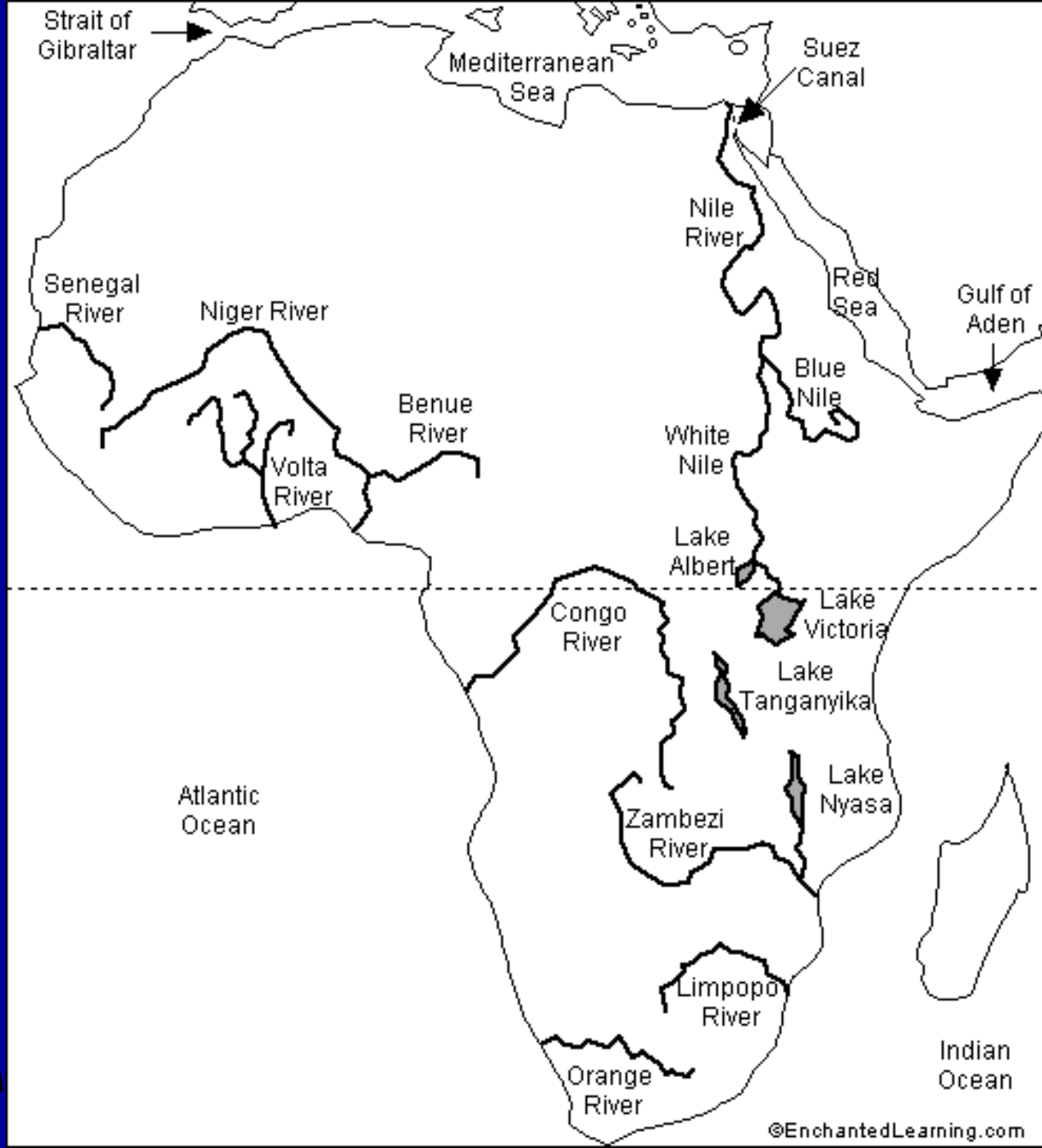


Oeuf



Horri

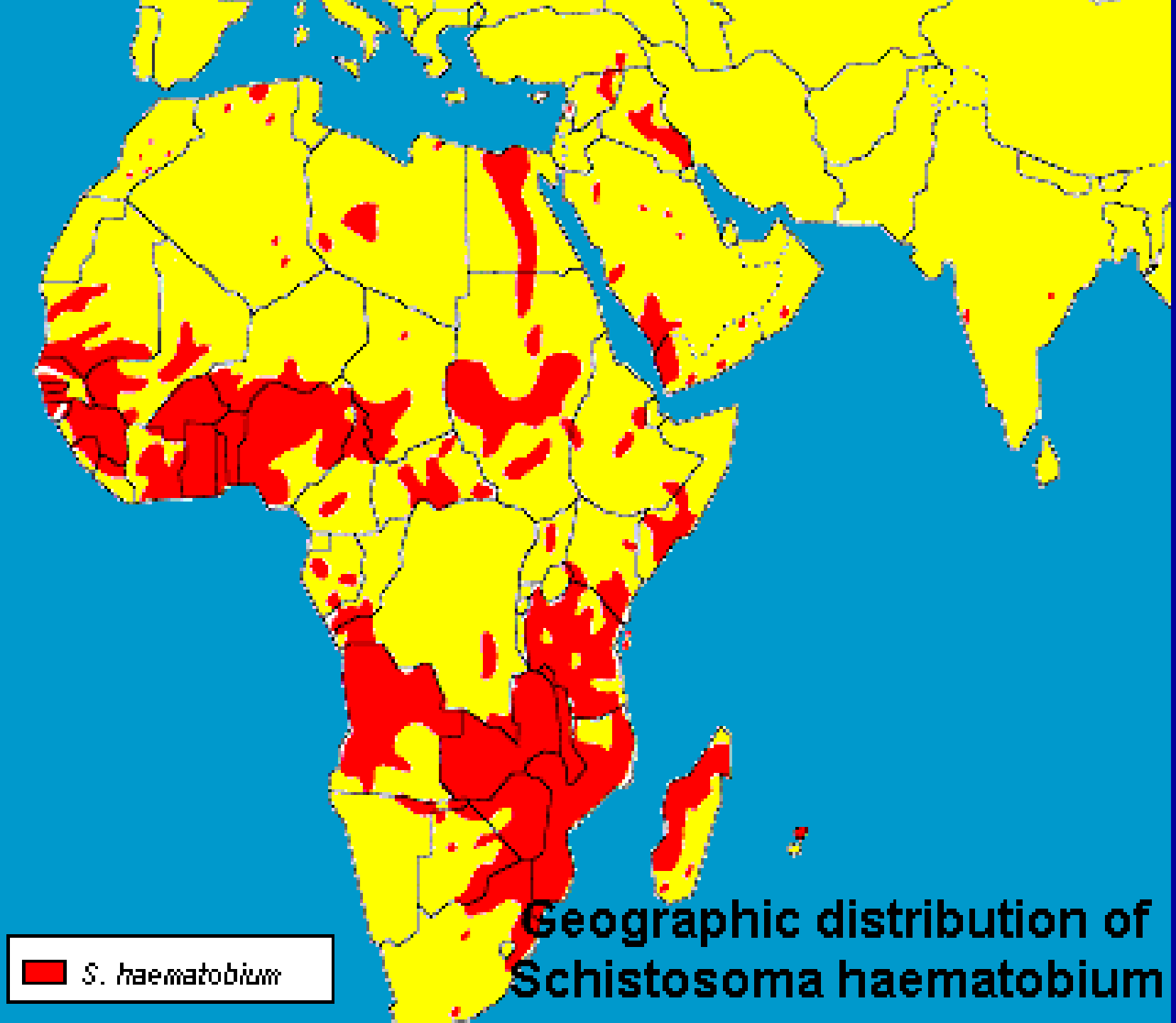
*Schistosoma Haematobium*



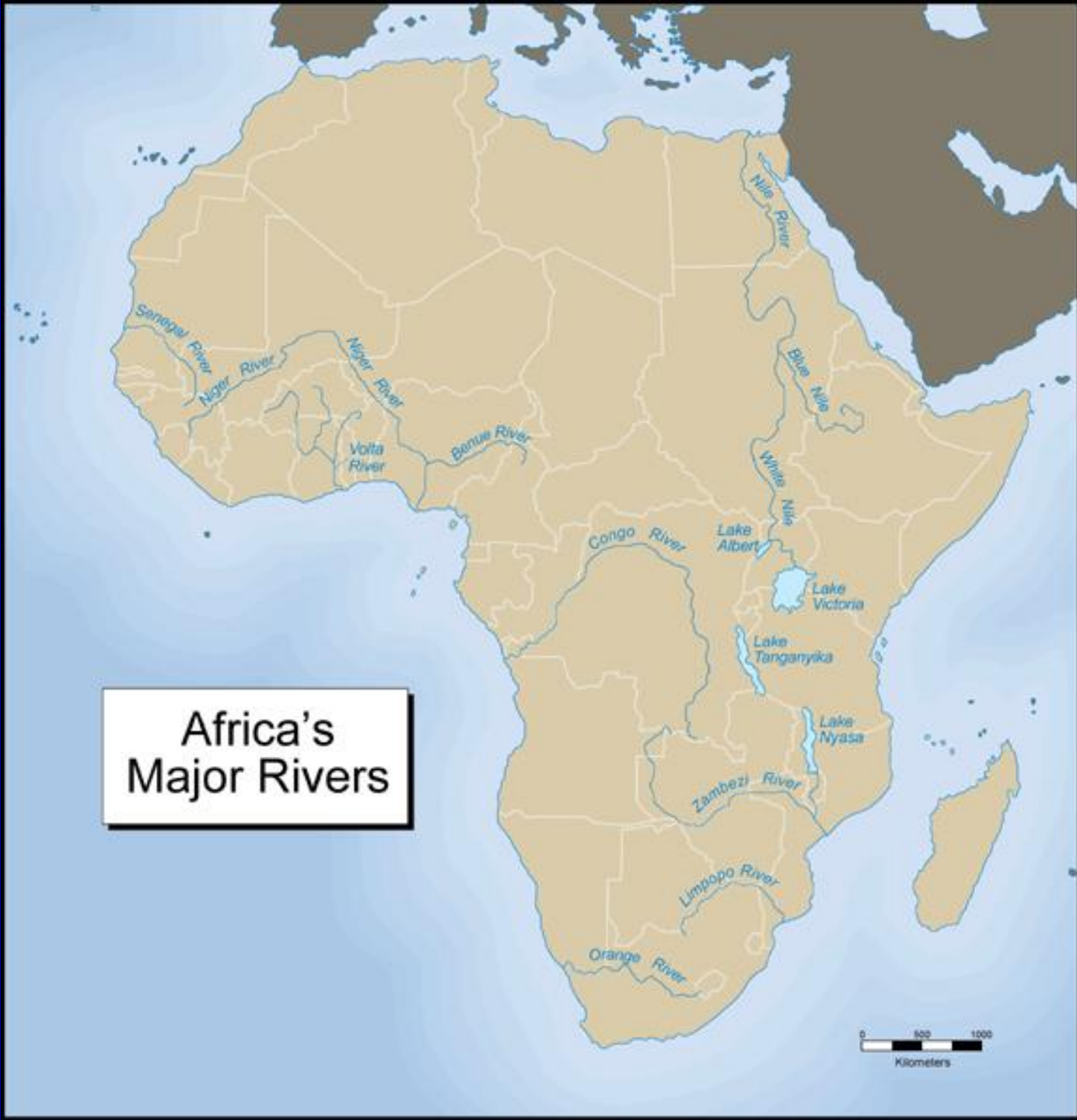
Horm



Ho



# Africa's Major Rivers



"Welcome to the fabulous world  
of parasites"

Mónica Botelho

06/07/07

- What is a parasite?

- **Parasitism**, wherein one organism, usually physically smaller of the two (the **parasite**) benefits and the other (the **host**) is harmed.

**Parasitism** is one version of symbiosis ("living together"), a phenomenon in which two organisms which are phylogenetically unrelated co-exist over a prolonged period of time, usually the lifetime of one of the individuals.

# List of parasitic organisms

## • Protists (Protozoa)

- *Giardia lamblia* (the most common intestinal protozoan in the United States)
- *Naegleria fowleri* (facultative parasite causing amoebic meningitis)
- *Entamoeba histolytica* (causes Amebiasis, common in developing countries)
- *Trypanosoma* (sleeping sickness or Chagas disease)
- *Plasmodium* (malaria)
- *Toxoplasma* (toxoplasmosis)

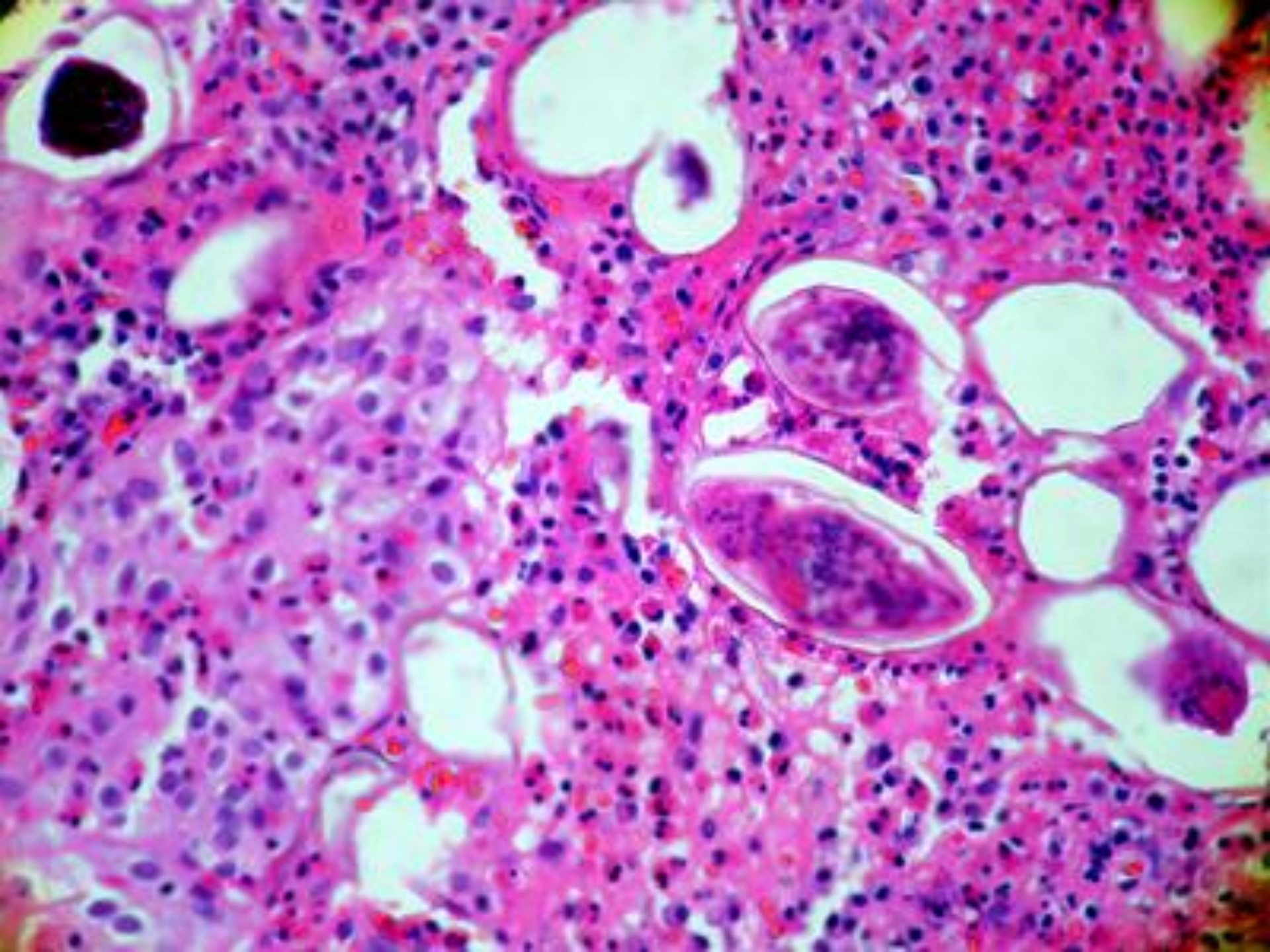
## • Helminths

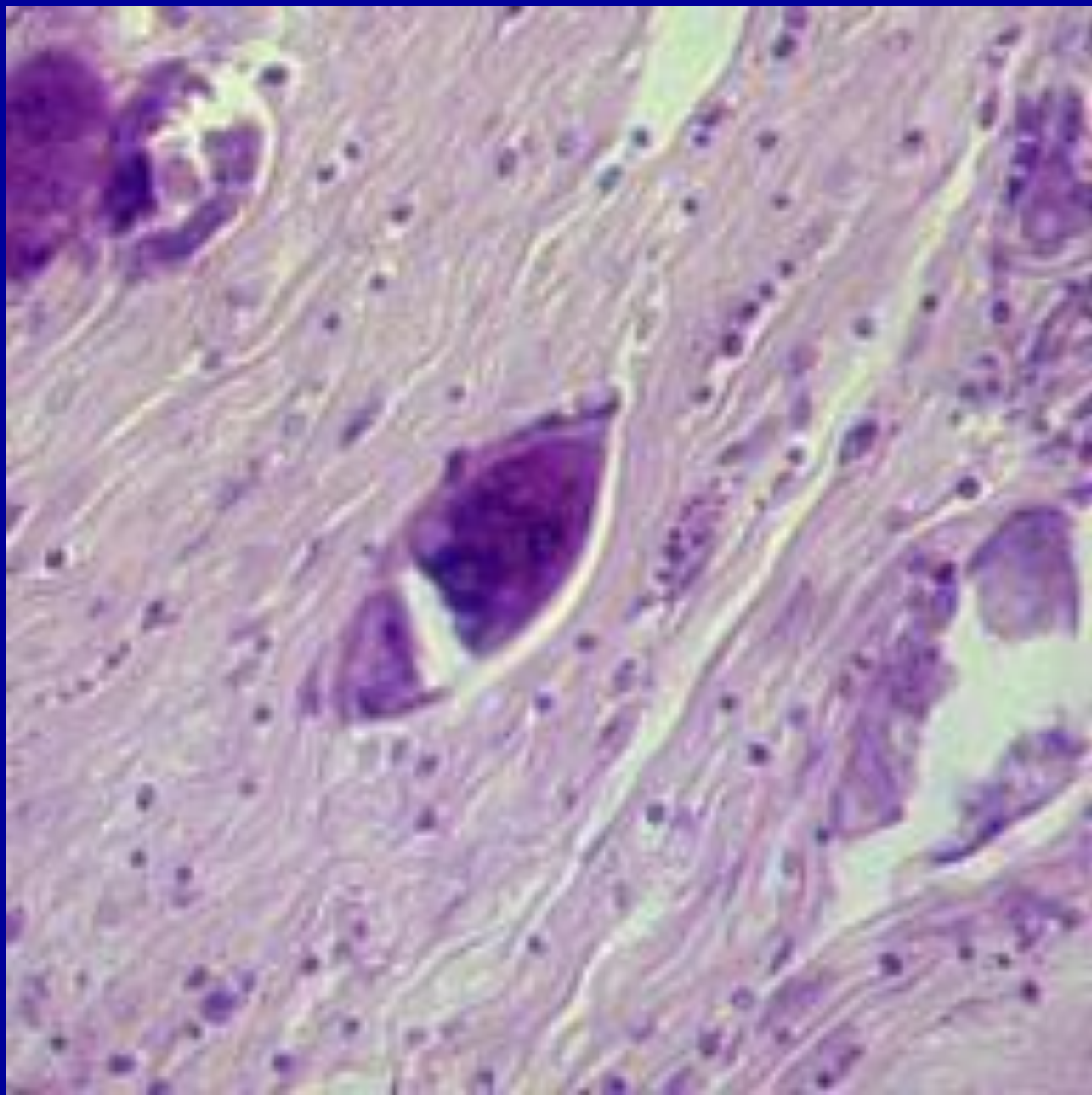
- *Ascariasis* (roundworms)
- Cestoda (tapeworms) including: *Taenia saginata* (human beef tapeworm), *Taenia solium* (human pork tapeworm)
- *Enterobius vermicularias* (pinworm)
- Filariasis
- Schistosomiasis or Bilharziosis

- *Schistosoma haematobium*



Hormones





# Tumorigenic effect of *S. haematobium* total antigen on normal epithelial cells

<b>Nude Mice</b>	<b>Dia 15 (mm3)</b>	<b>Dia 20 (mm3)</b>	<b>Dia 30 (mm3)</b>	<b>Dia 90 (mm3)</b>
<b>Sh1</b>	0	400	4416	Sacrif.
<b>Sh2</b>	0,125	6375	Sacrif.	-
<b>Sh3</b>	0,125	1584	Sacrif.	-
<b>Sh4</b>	2808	7200	Sacrif.	-
<b>Sh5</b>	0	0	1870	Sacrif.
<b>C1</b>	0	0	0	0,125
<b>C2</b>	0	0	0	0
<b>C3</b>	0	0	0	0

# Tumorigenic effect of *S. haematobium* total antigen on normal epithelial cells

- Histopathology
  - Inflammatory tumors
  - Lymphoid tissue

# Take home message II

- *S. haematobium* infected patients have increased serum levels of estradiol, most likely *Schistosoma* derived.
- *S. haematobium* total antigen expresses an analog of estradiol.
- *S. haematobium* total antigen decreased estradiol levels in culture, acting as an estradiol antagonist.
- *S. haematobium* total antigen down-regulates Estrogen Receptor beta.
- In agreement with these data, we found that *S. haematobium* total antigen was able to inactivate ER transcription through the use of a pERE-Luc.

**Granulomatous-like immune reaction and  
hepatic fibrosis induced by *Schistosoma  
haematobium* immature worms**

Botelho MC, Oliveira PA, Vieira P,  
Delgado ML, Lourenço L, Lopes C,  
Machado JC and Correia da Costa JM  
(Virulence, )

