Schistosoma haematobium e cancro da bexiga: a verdade escondida

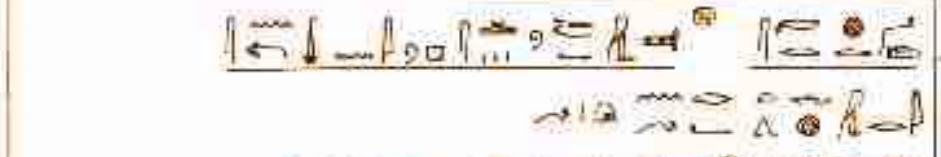
Mónica Botelho

14/6/2013

- What is schistosomiasis?

№ 831

№ 832



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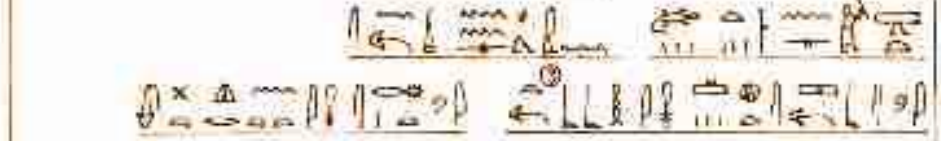
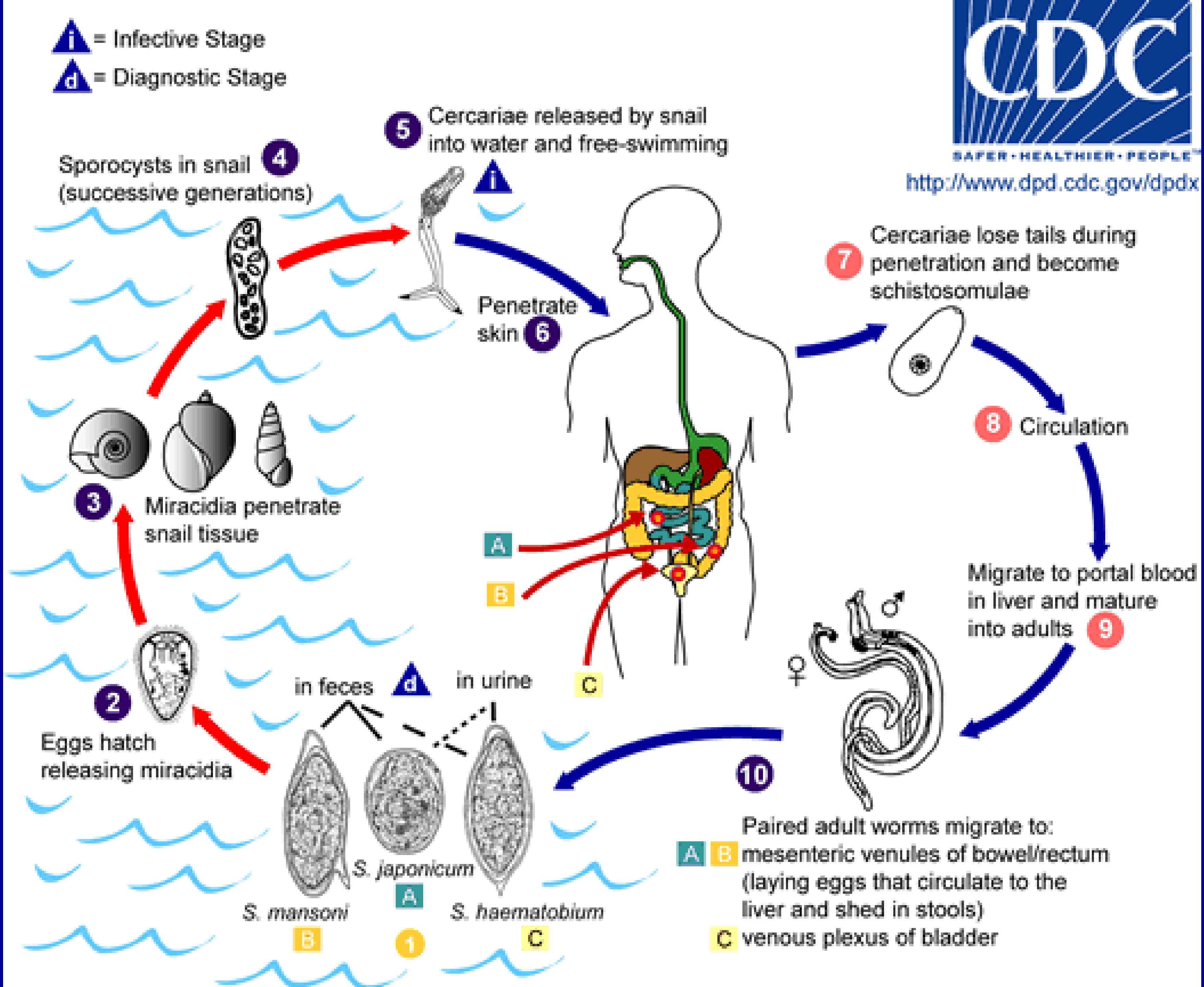




= Infective Stage
= Diagnostic Stage
CDC
SAFER · HEALTHIER · PEOPLE
http://www.dpd.cdc.gov/dpdx
5 Cercariae released by snail into water and free-swimming
4 Sporocysts in snail (successive generations)
Penetrate skin 6
7 Cercariae lose tails during penetration and become schistosomulae
8 Circulation
Migrate to portal blood in liver and mature into adults 9
3 Miracidia penetrate snail tissue
2 Eggs hatch releasing miracidia
in feces
in urine
A
B
C
10
Paired adult worms migrate to:
A B mesenteric venules of bowel/rectum (laying eggs that circulate to the liver and shed in stools)
C venous plexus of bladder
S. japonicum
A
S. mansoni
B
1
S. haematobium
C

BEWARE

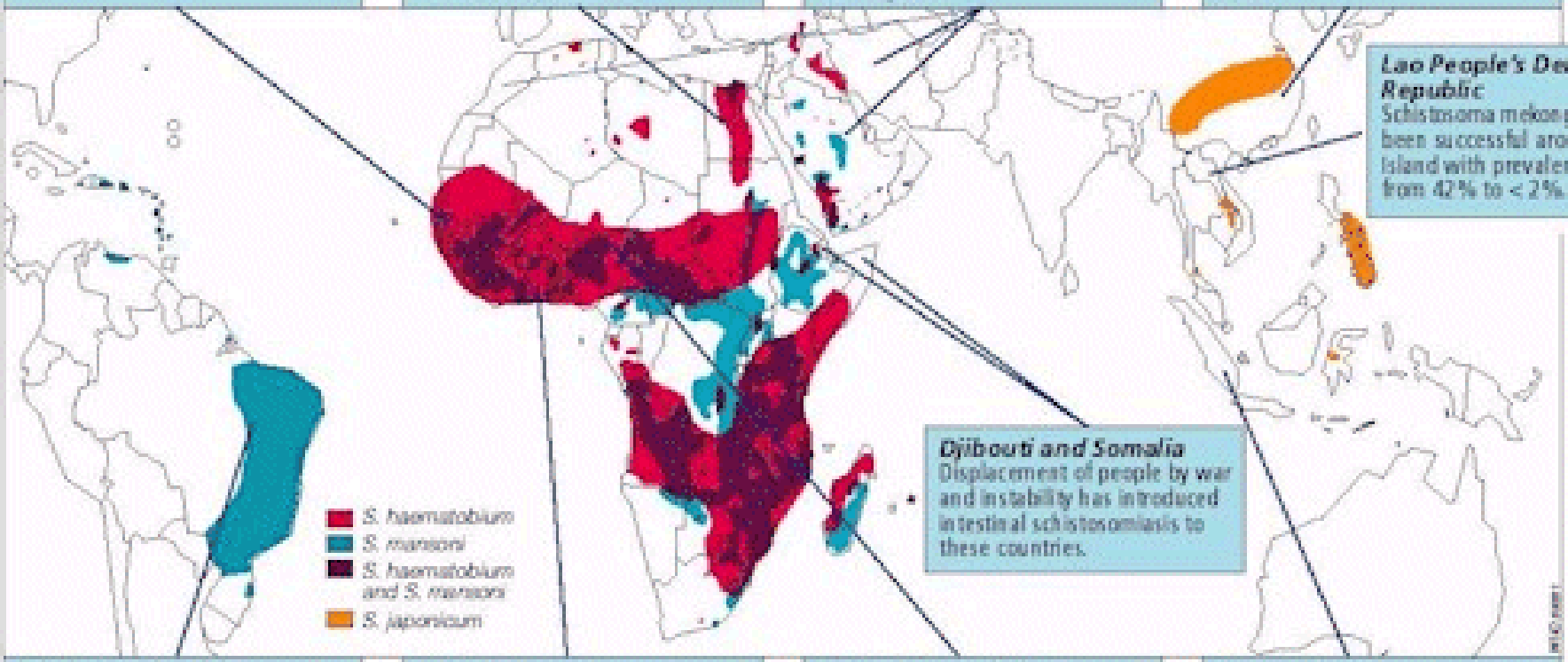
BILHARZIA

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



Senegal
An epidemic of schistomiasis along the senegal river basin caused by water-resource development schemes continues unabated.

Egypt
Praziquantel chemotherapy coupled to a vigourous media campaign has resulted in a significant decrease in the morbidity and prevalence of schistomiasis infection.

Iran, Morocco, and Saudi Arabia
Schistosomiasis control has been successful in those areas with elimination of the infection contemplated.

China
Schistosoma continues to be a major public health problem in the lake and marshy regions despite successful control in other endemic areas.

Lao People's Democratic Republic
Schistosoma mekongi control has been successful around Khong Island with prevalence reduced from 42% to < 2%.

Djibouti and Somalia
Displacement of people by war and instability has introduced intestinal schistosomiasis to these countries.

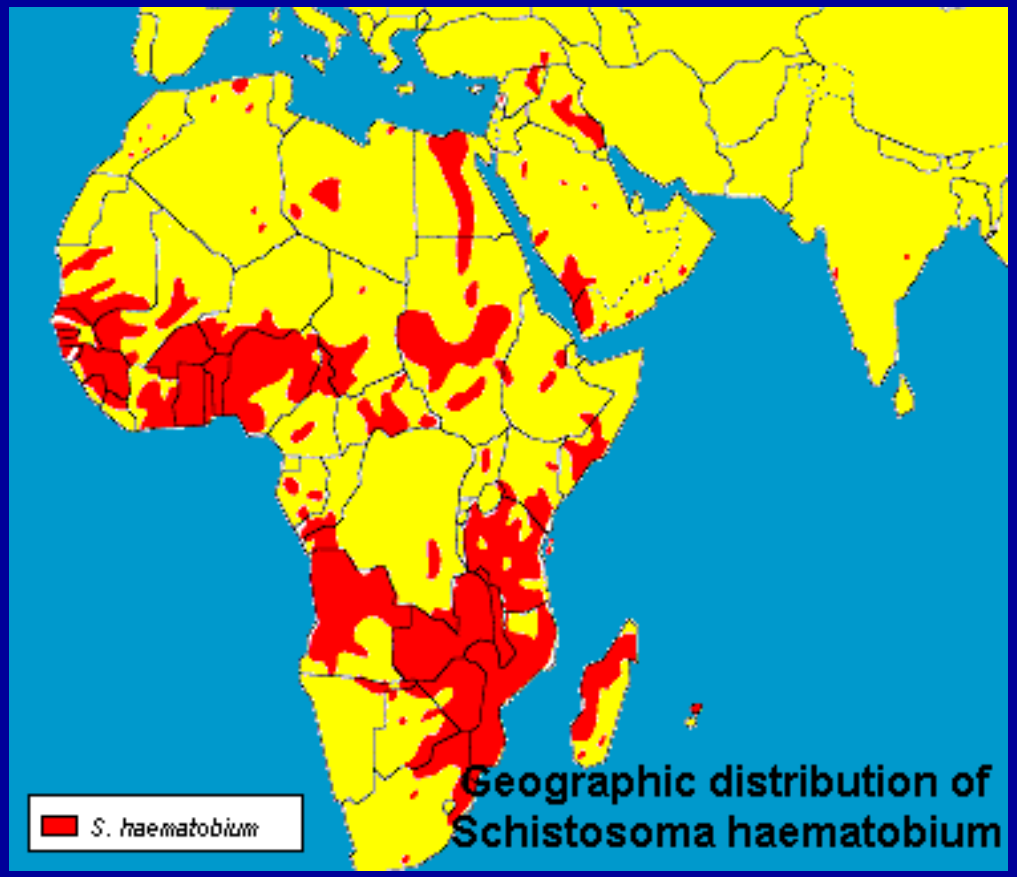
North-east Brazil
Urban schistosomiasis now present in and around many major cities

Ghana
Intestinal schistosomiasis has increased due to the construction of the Akosombo Dam and other much smaller dams.

sub-Saharan Africa
More than 85% of the estimated 200 million people globally with schistosomiasis and the majority of patients with severe disease live on this continent.

Indonesia
Schistosomiasis has been controlled in the Lindu region of Sulawesi such that the prevalence of infection is lower than 2%.

Hormones

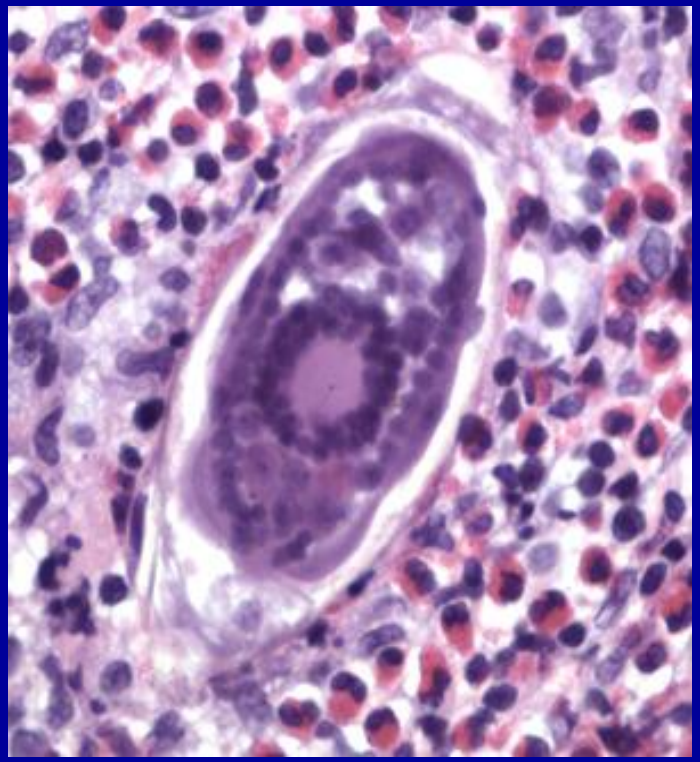

S. haematobium
Geographic distribution of
Schistosoma haematobium


Africa's
Major Rivers
Nile River
Senegal River
Niger River
Niger River
Volta River
Benue River
Blue Nile
White Nile
Congo River
Lake Albert
Lake Victoria
Lake Tanganyika
Lake Nyasa
Zambezi River
Limpopo River
Orange River
Kilometers

- 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 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).

Schistosomiasis and host hormones

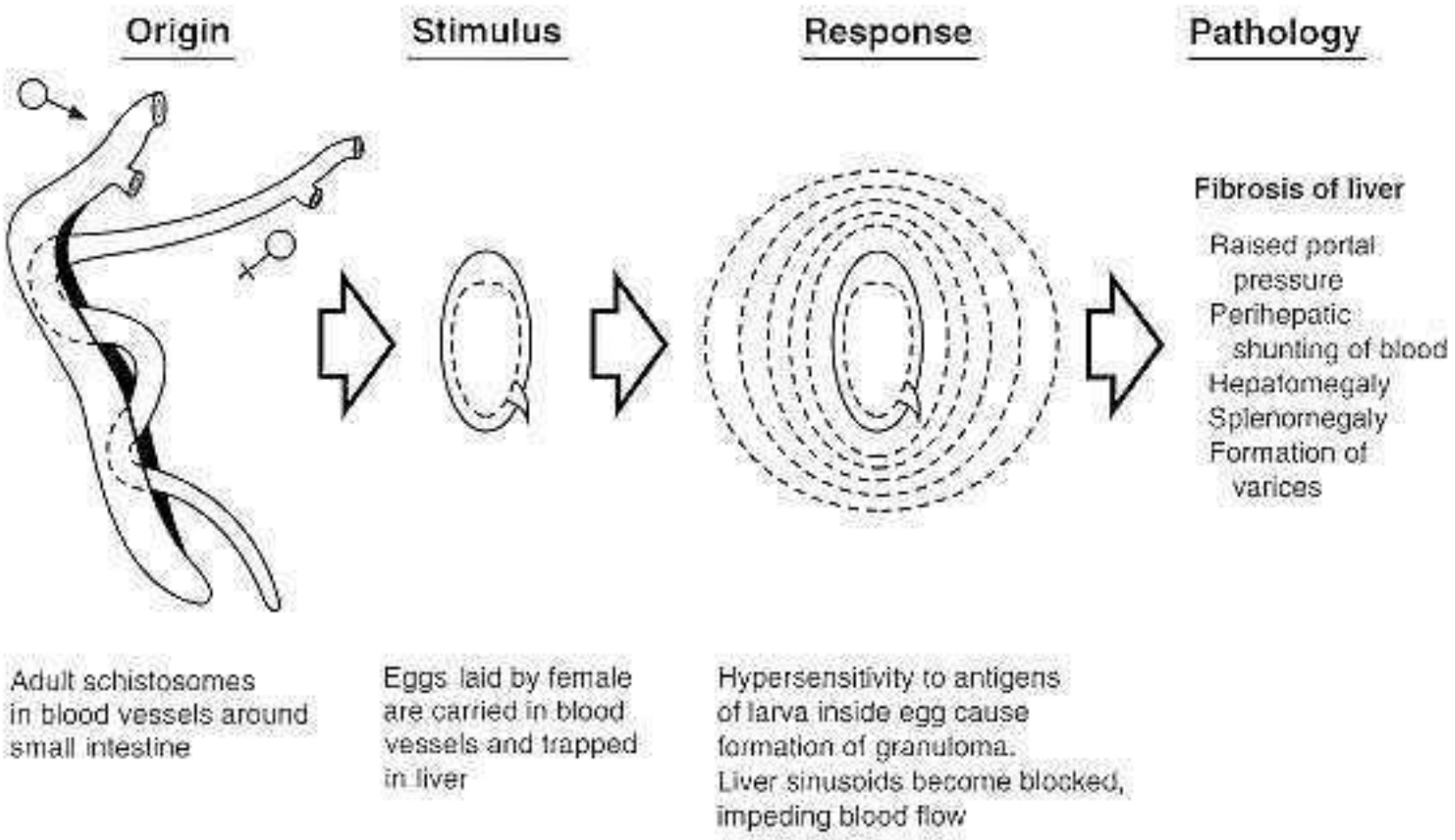
Recent experimental evidence suggests that schistosomes can not only evade immune responses actively but also exploit the hormonal microenvironment within the host to favor their establishment, growth and reproduction (Escobedo et al, Trends in Parasitology, 2005).

Aims

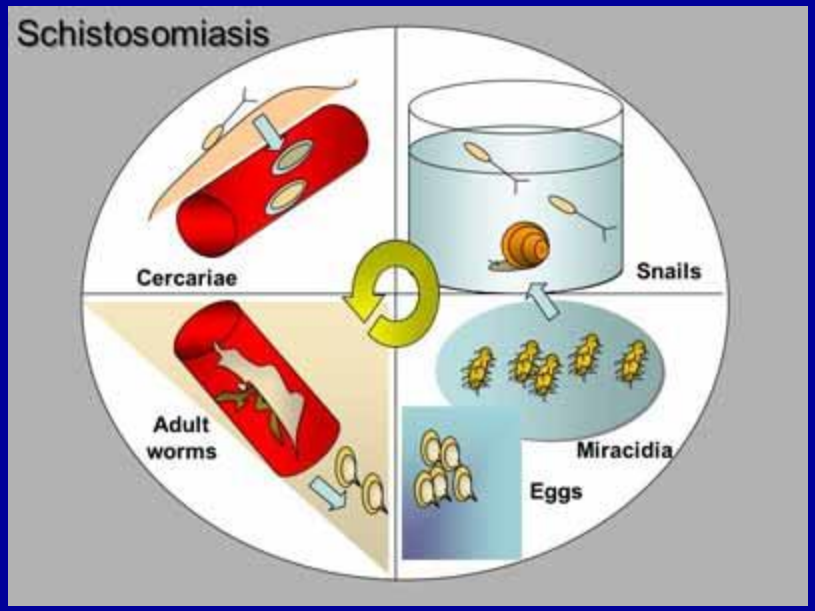
- A) To study the mechanisms underlying the association between S. haematobium and SCC of the bladder
- B) To understand how host endocrine system can favor the establishment of schistosomes

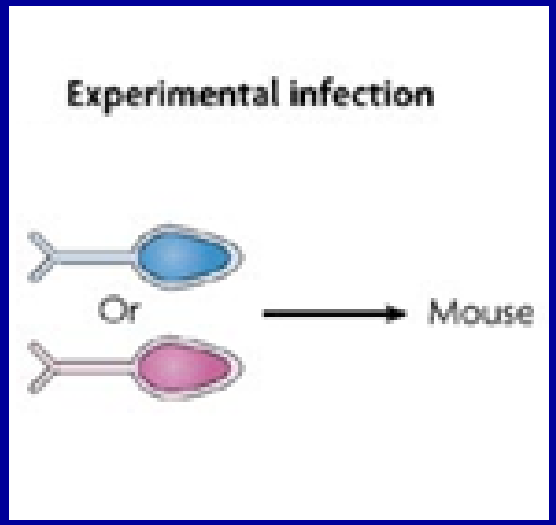
S. haematobium and bladder cancer

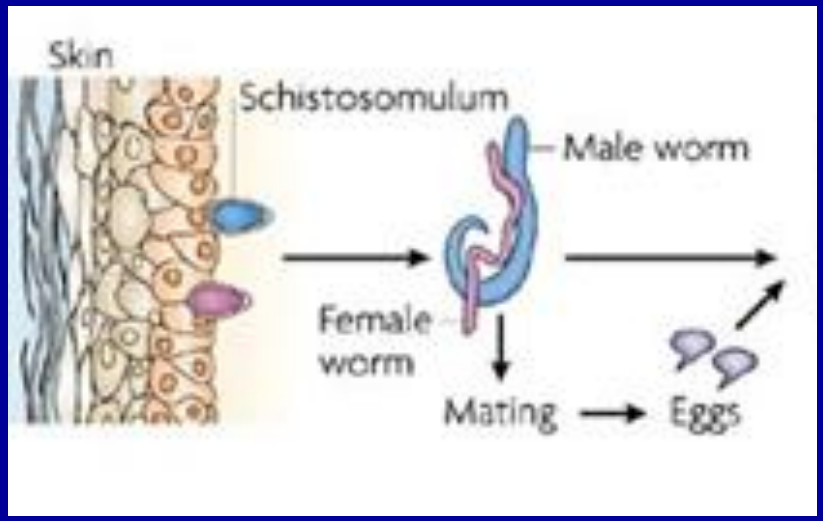
Schistosoma haematobium immature worms induce granulomatous-like immune reaction and hepatic fibrosis similar to parasite eggs



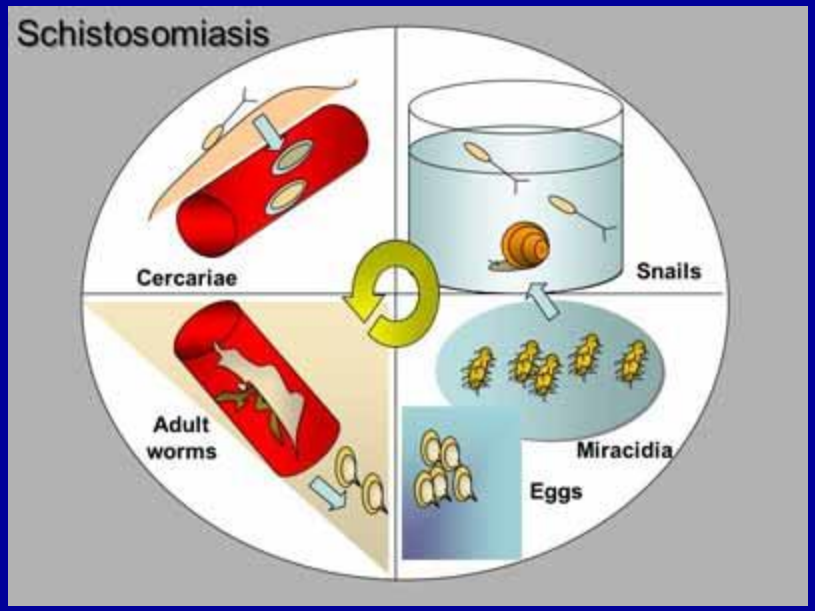
Methodological strategy

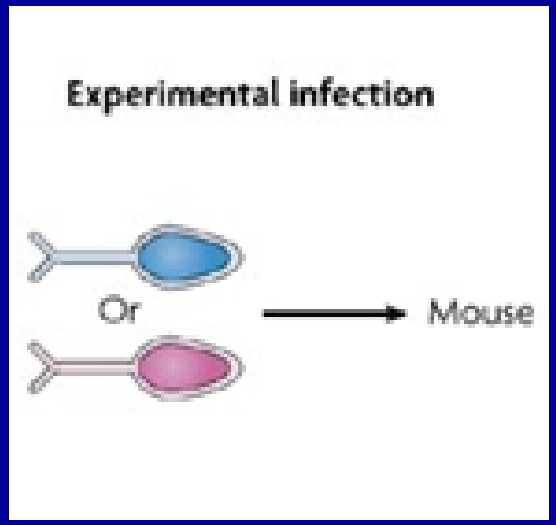


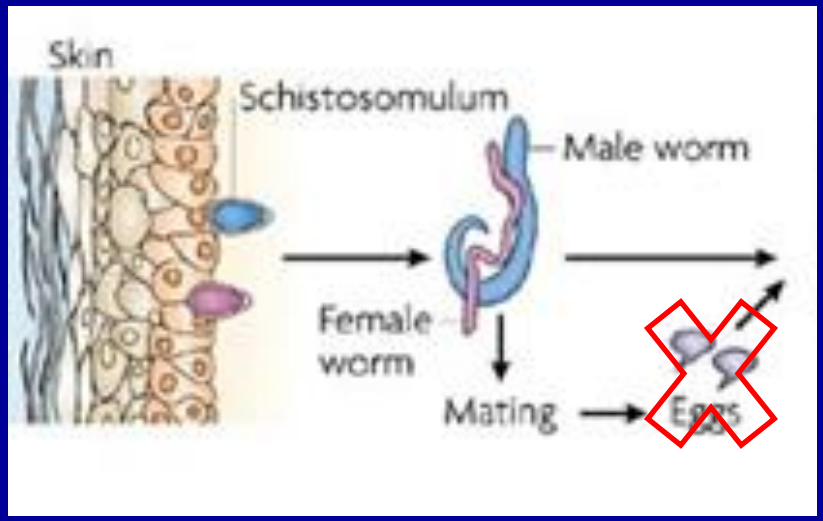




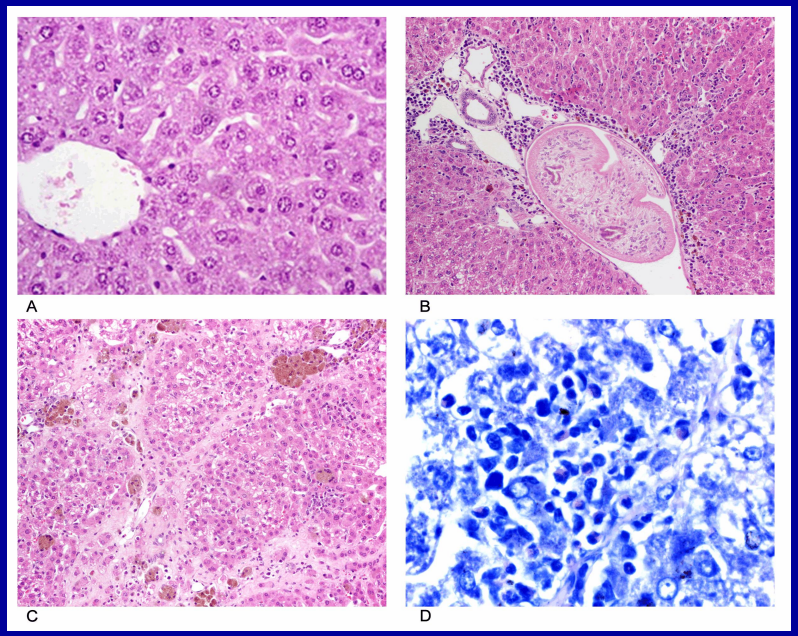
Methodological strategy

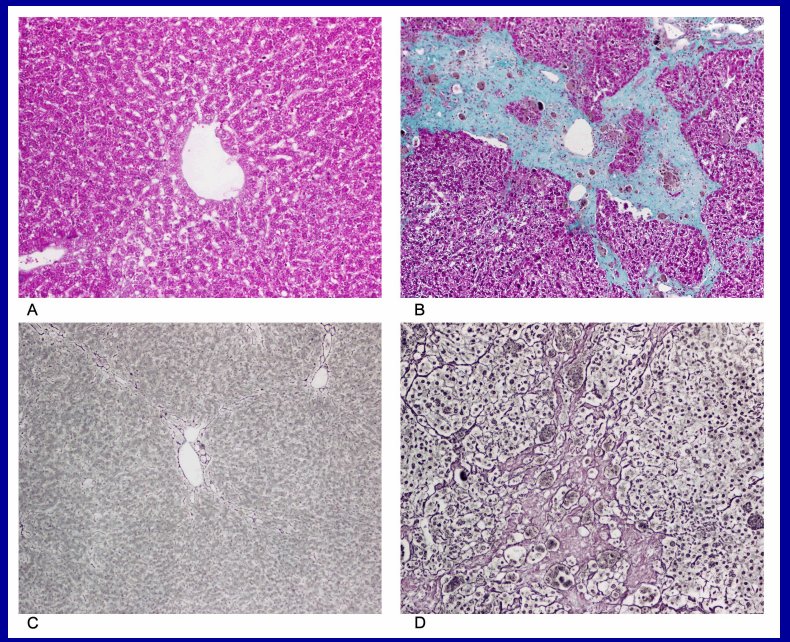






Schistosoma haematobium immature worms induce granulomatous-like immune reaction and hepatic fibrosis similar to parasite eggs

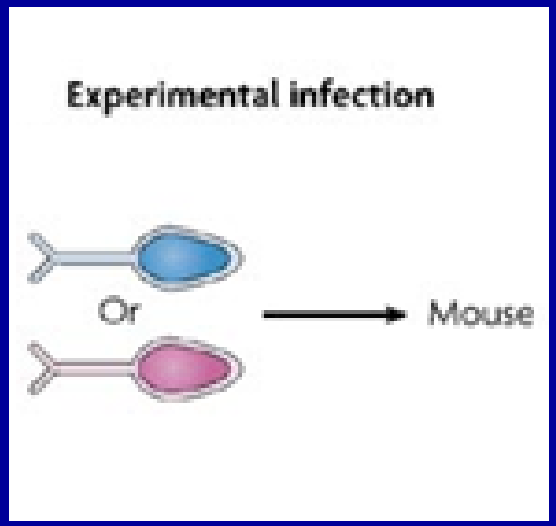


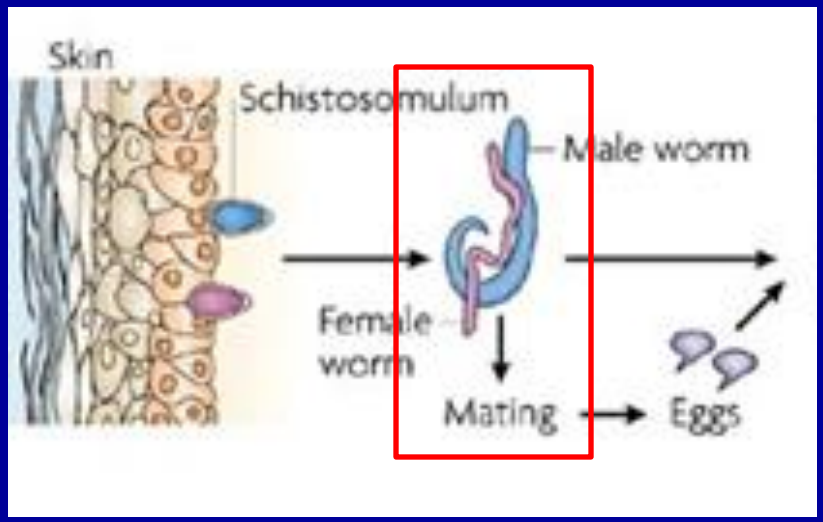


Botelho *et al.* Virulence 2010

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

Methodological Strategy

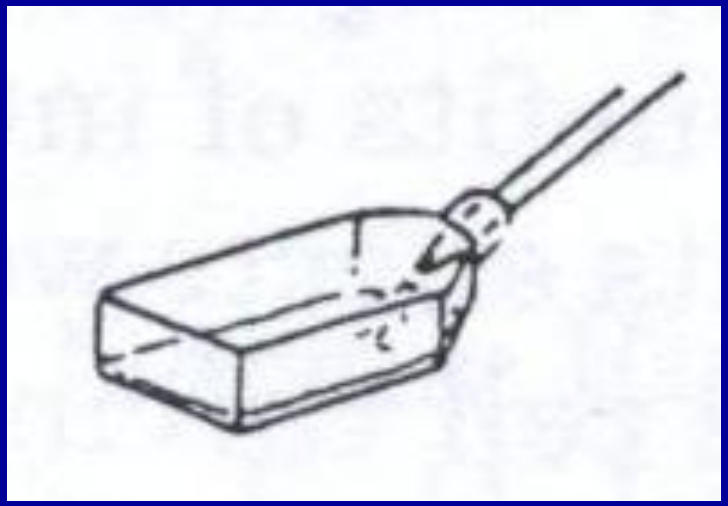




S. haematobium
total antigen (Sh)

Methodological Strategy

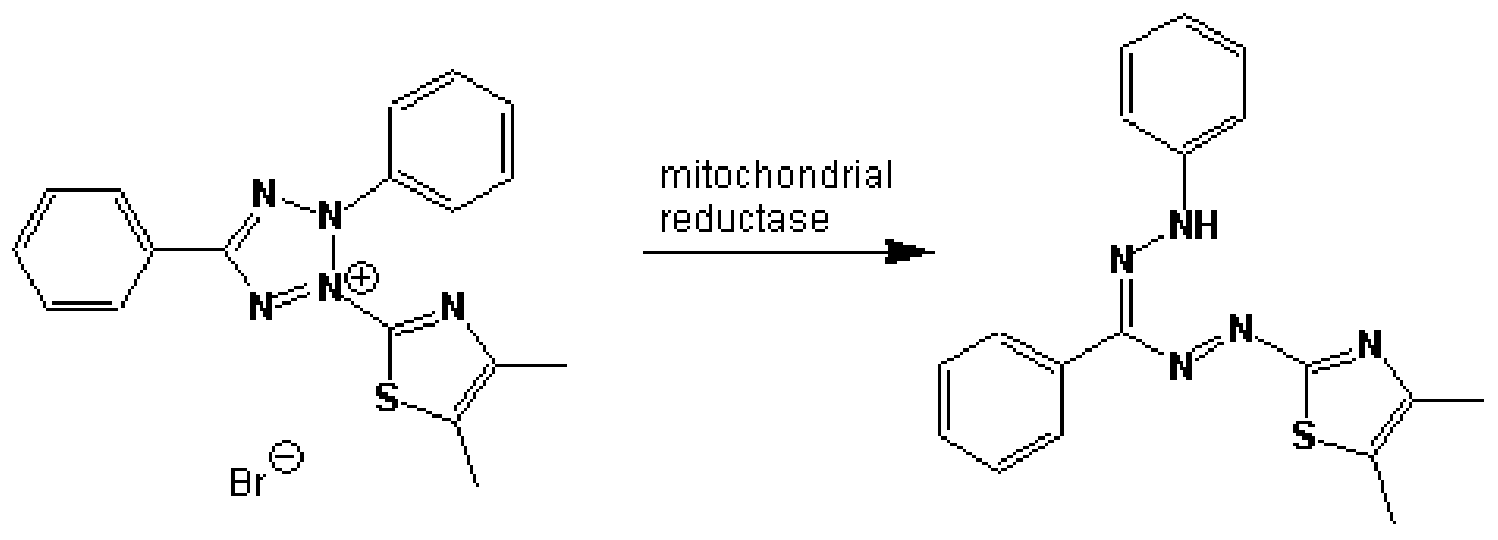
Chinese Hamster Ovary (CHO) cells



Sh-treatment 48 h

Methodological Strategy

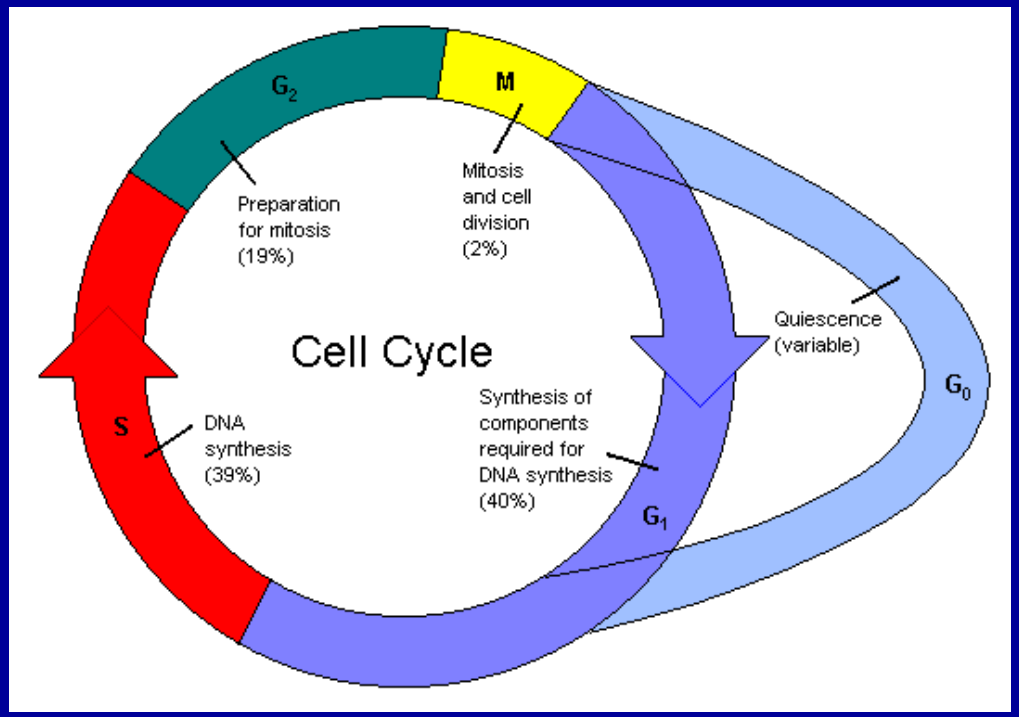
Proliferation MTT assay

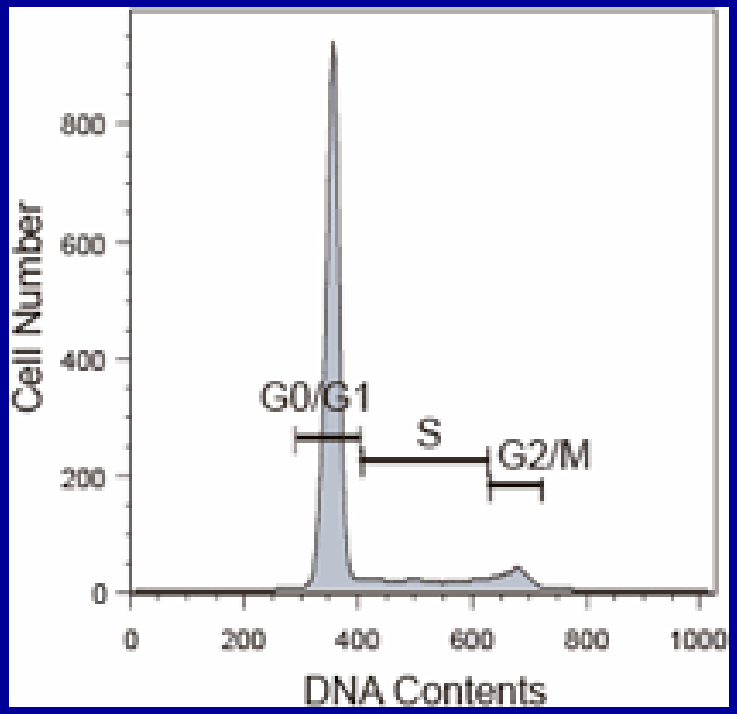


Yellow **MTT** (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells.

Methodological Strategy

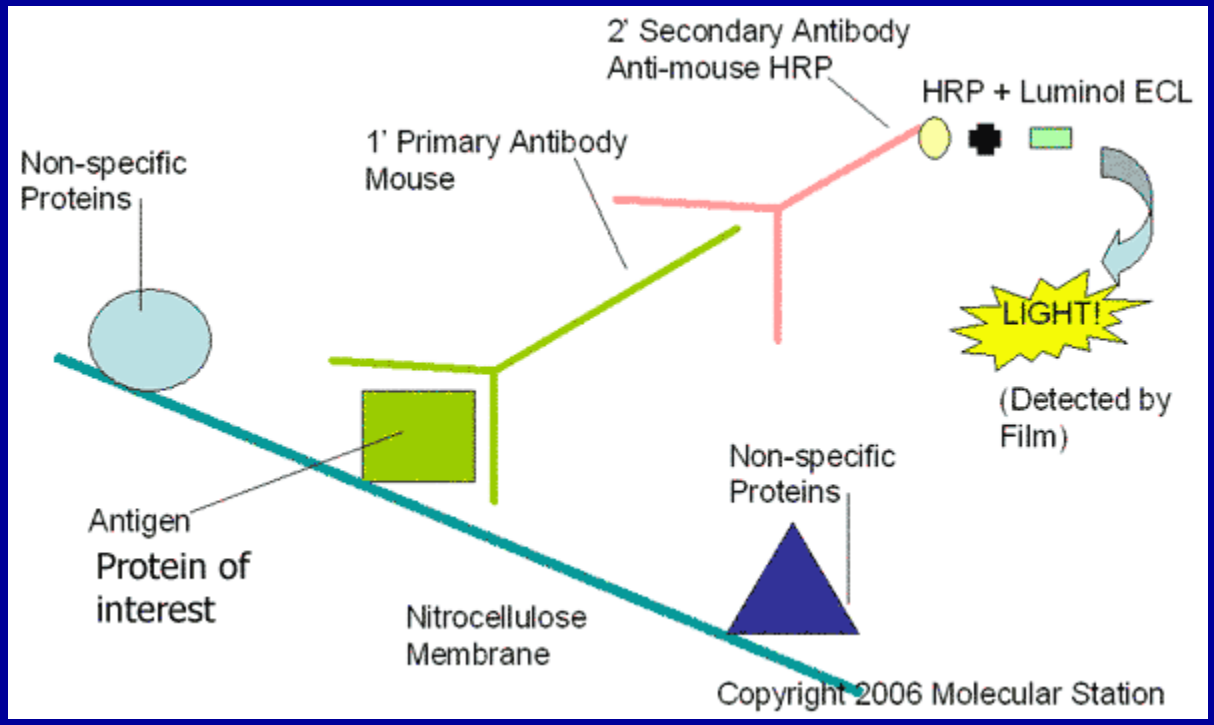
DNA content Flow cytometry





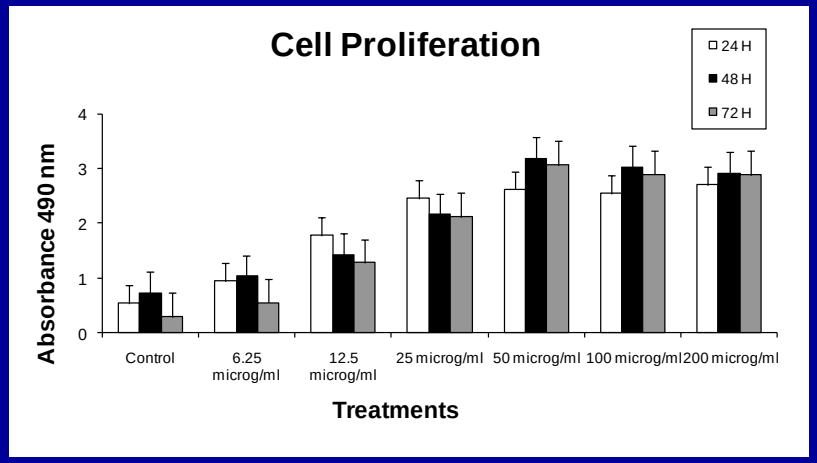
Methodological Strategy

Protein expression Western blot

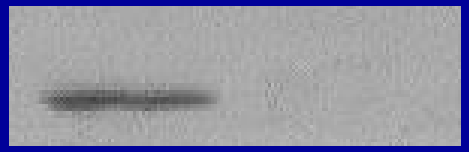


The **western blot** is used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins. The proteins are then transferred to a membrane where they are detected using antibodies specific to the target protein.

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

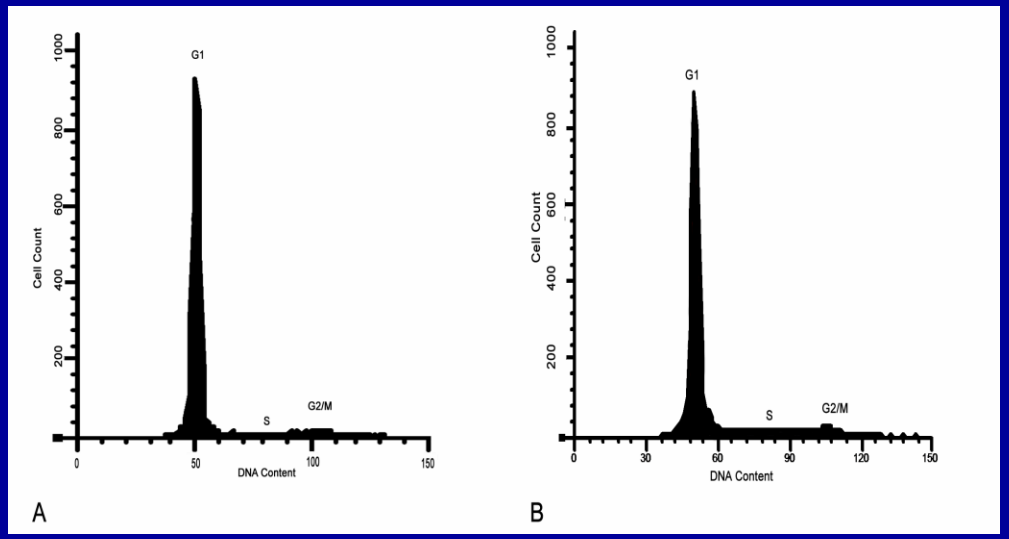


beta-Actin



p27

Control Sh-treated

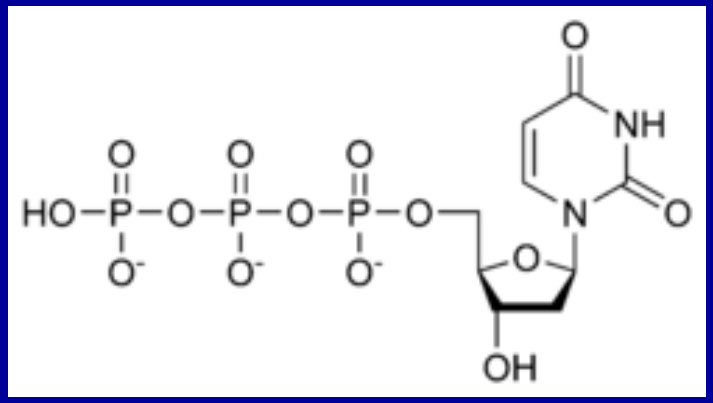


Botelho *et al.* Int J Parsitol 2009

Methodological Strategy

Apoptosis

TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling)

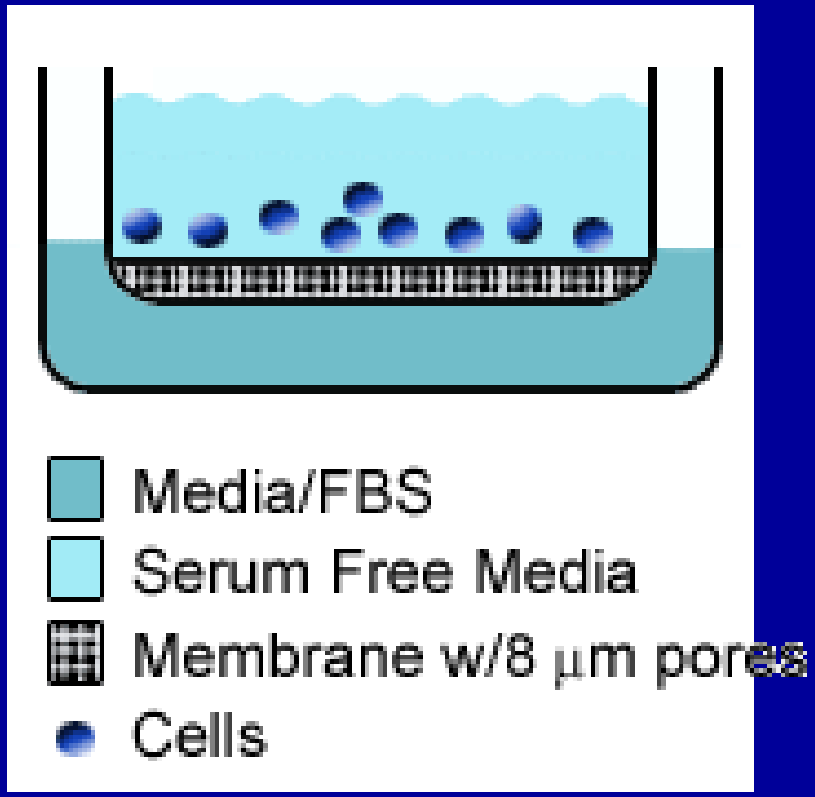


The assay relies on the presence of nicks in the DNA which can be identified by terminal deoxynucleotidyl transferase, an enzyme that will catalyze the addition of dUTPs that are secondarily labeled with a marker. It may also label cells that have suffered severe DNA damage.

Methodological Strategy

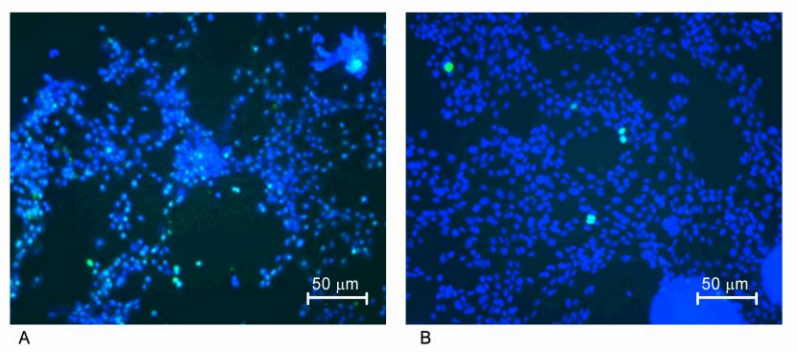
Invasion

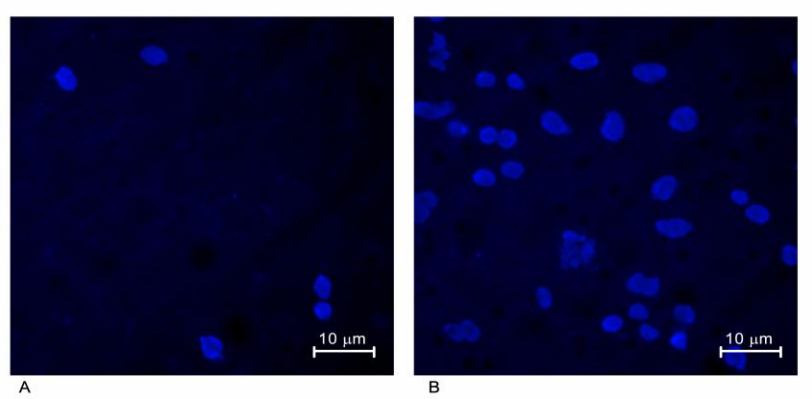
Boyden chamber

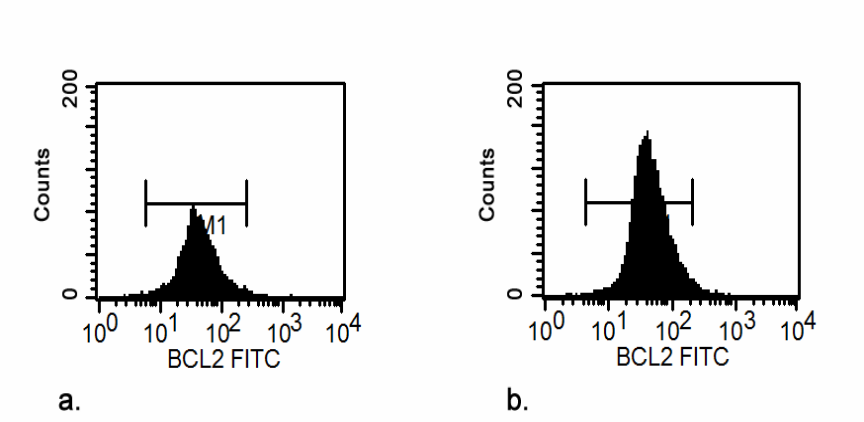


1. Cell suspension is placed in upper chamber and incubated for 24-72 hours at 37 C.
2. Invasive cells pass through the membrane and cling to the bottom of the polycarbonate membrane that is tissue culture-treated to enhance cell attachment.
3. Non-invading cells cannot pass through the membrane and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

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

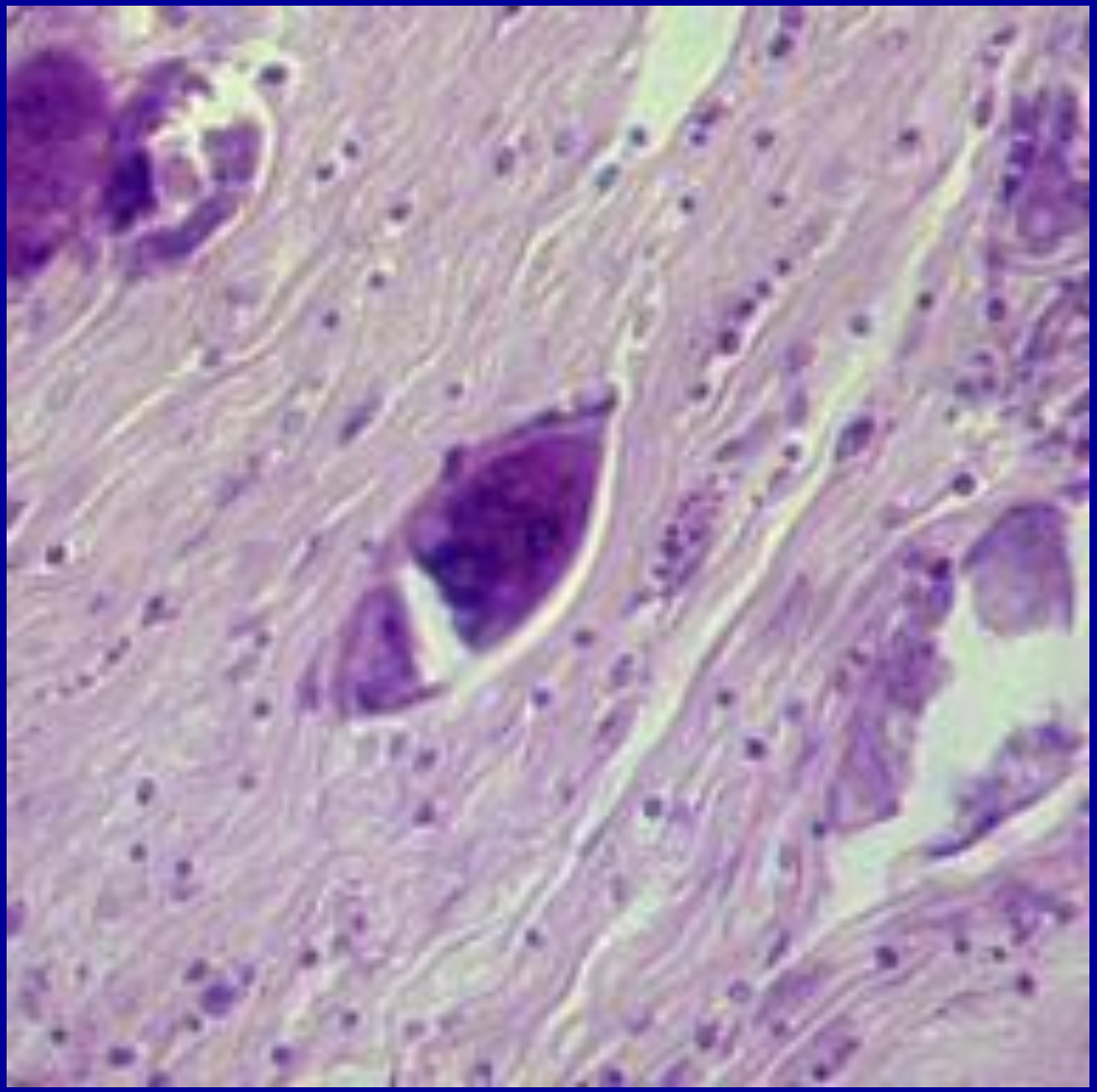






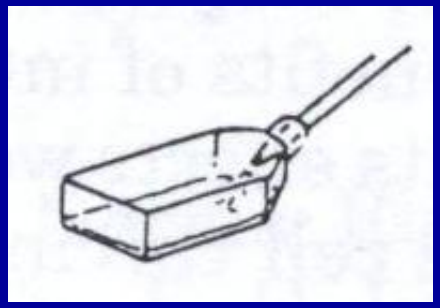
Botelho *et al.* Int J Parsitol 2009

S. haematobium total antigen induces tumorigenesis



Methodological Strategy

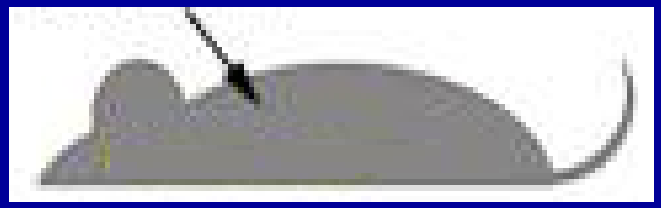
Chinese Hamster Ovary (CHO) cells

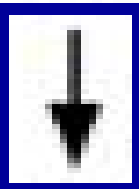


Sh-treatment 48 hours

Nude mice

Control CHO cells

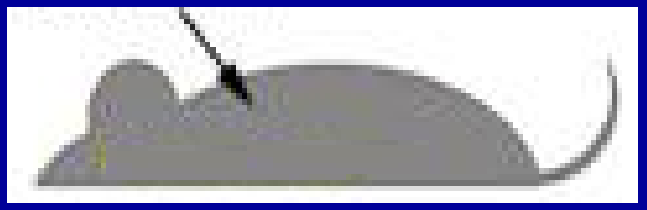


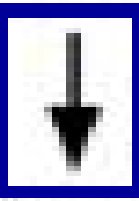


No Tumour

2 weeks

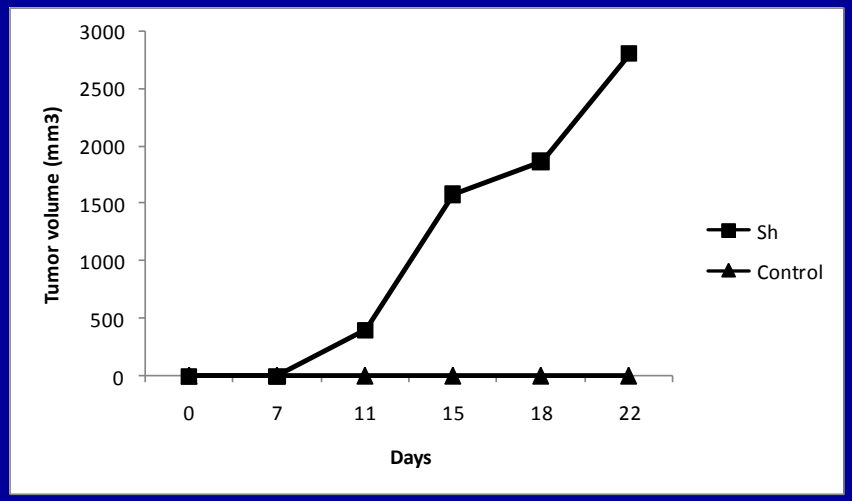
Sh-treated CHO cells

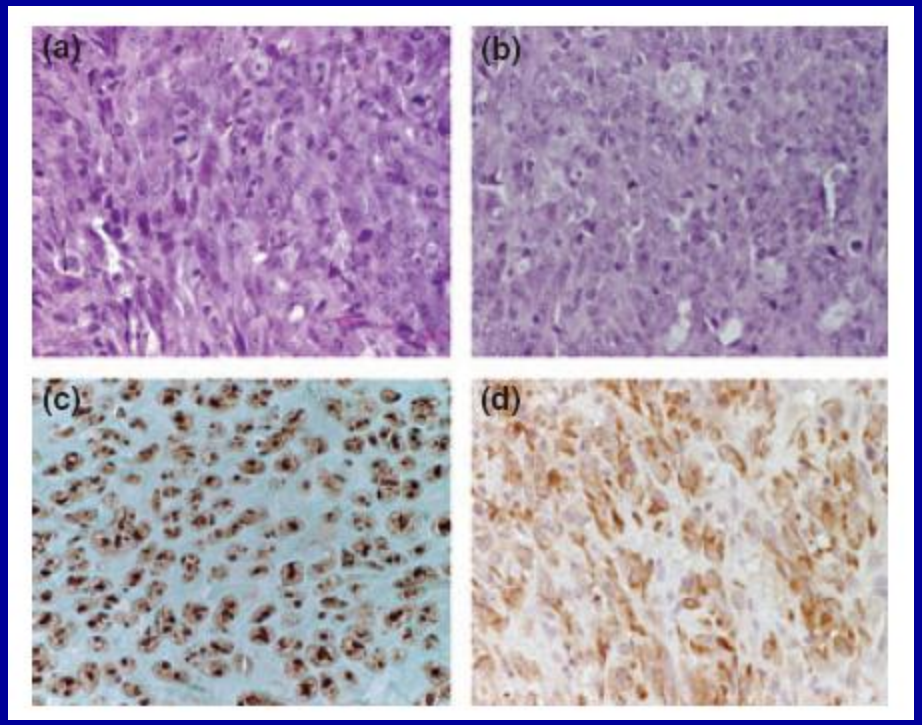




Tumour-Bearing Nude Mouse

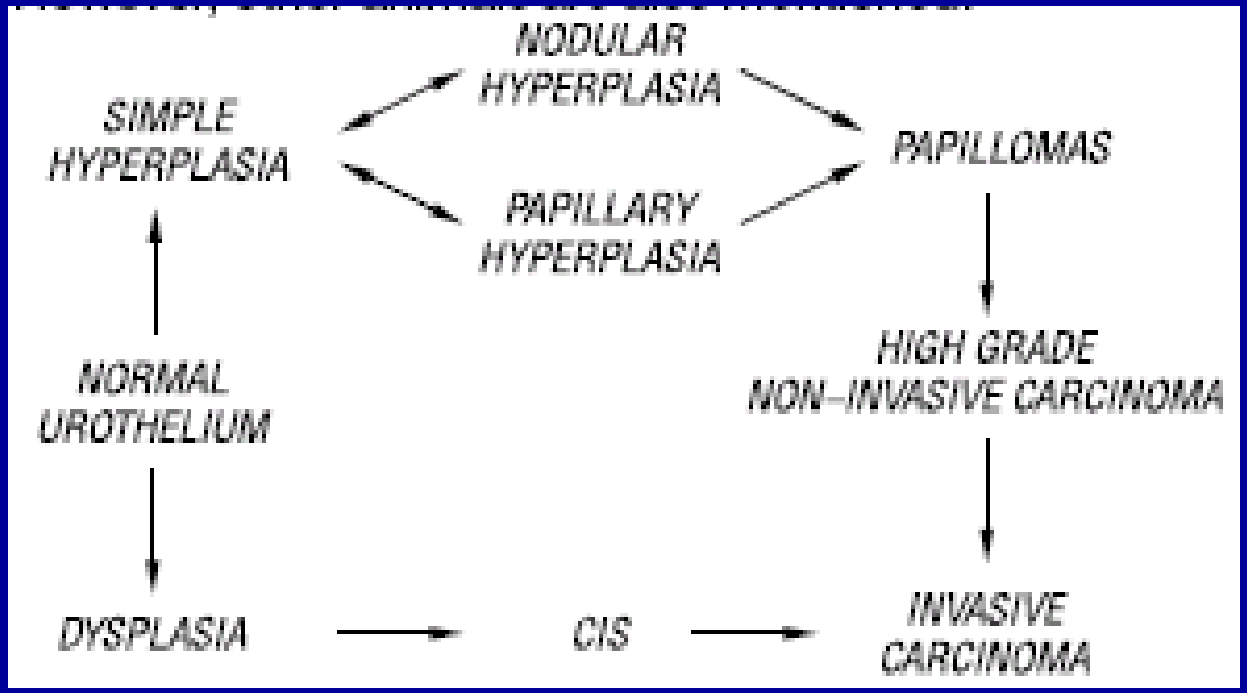
S. haematobium total antigen induces tumorigenesis





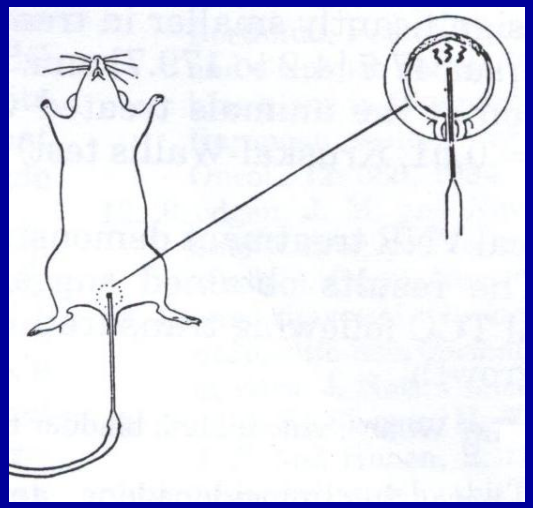
Botelho *et al.* Int J Exp Path 2009

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

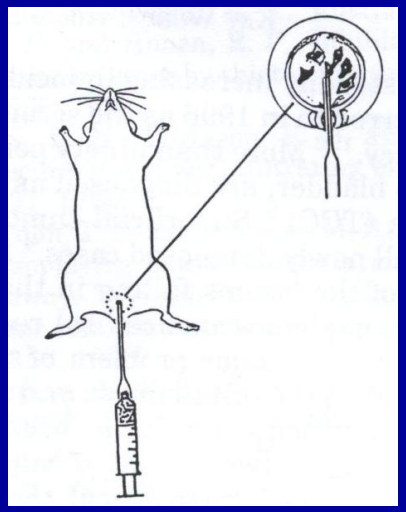


Pathogenic pathways of rat and mice urinary bladder carcinogenesis.

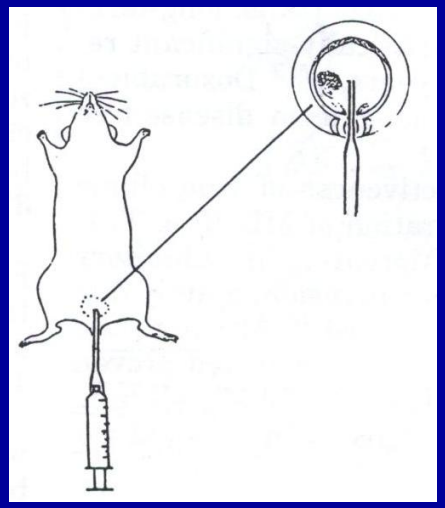
Methodological Strategy



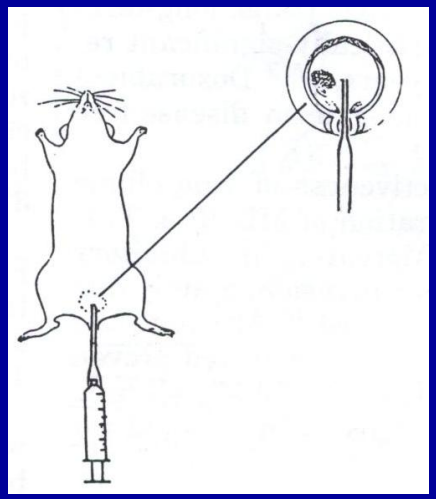
Transurethral Catheterization



Urothelial Denudation



Sh-treatment /PBS 1 hour

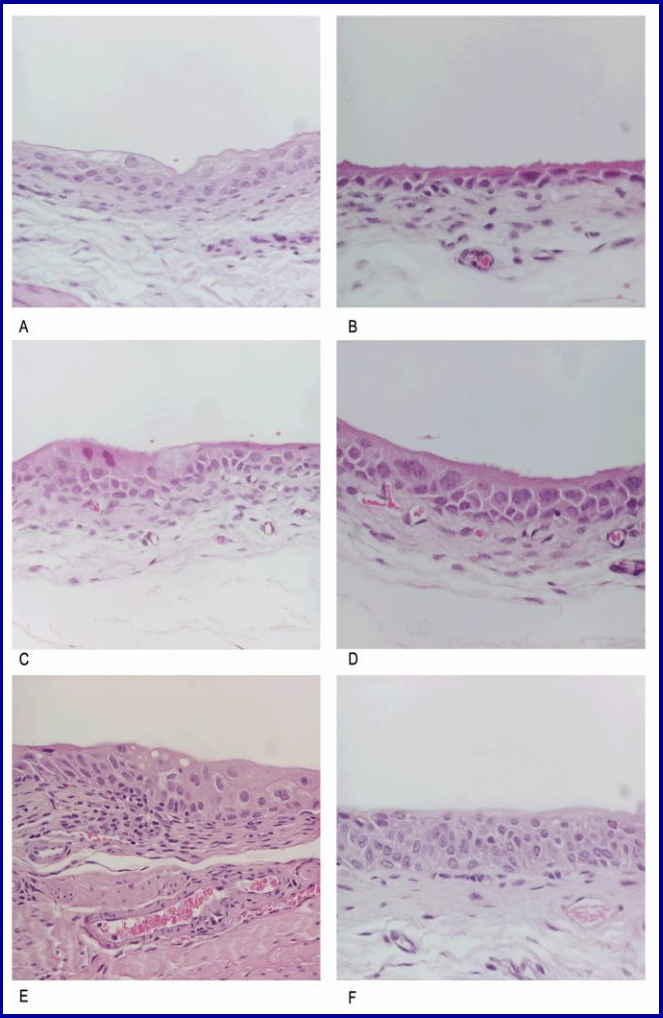


In situ fixation 10% PPA

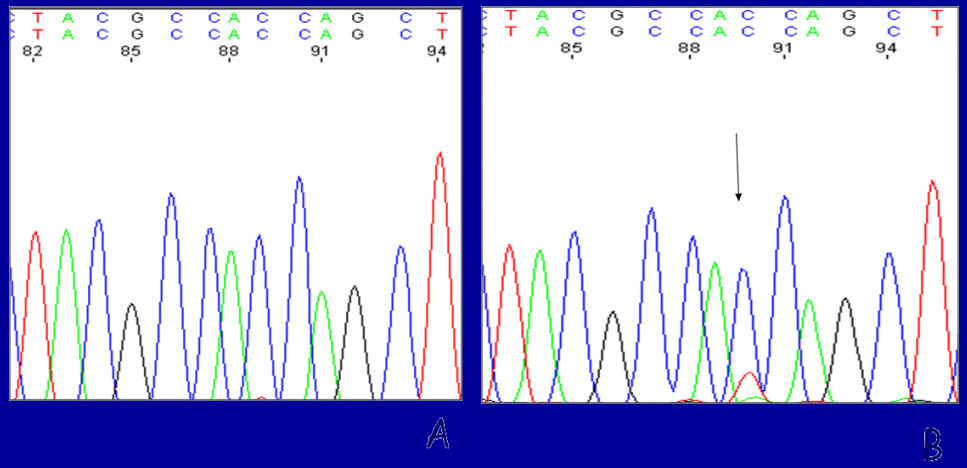
20 weeks
40 weeks

Methodological Strategy

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



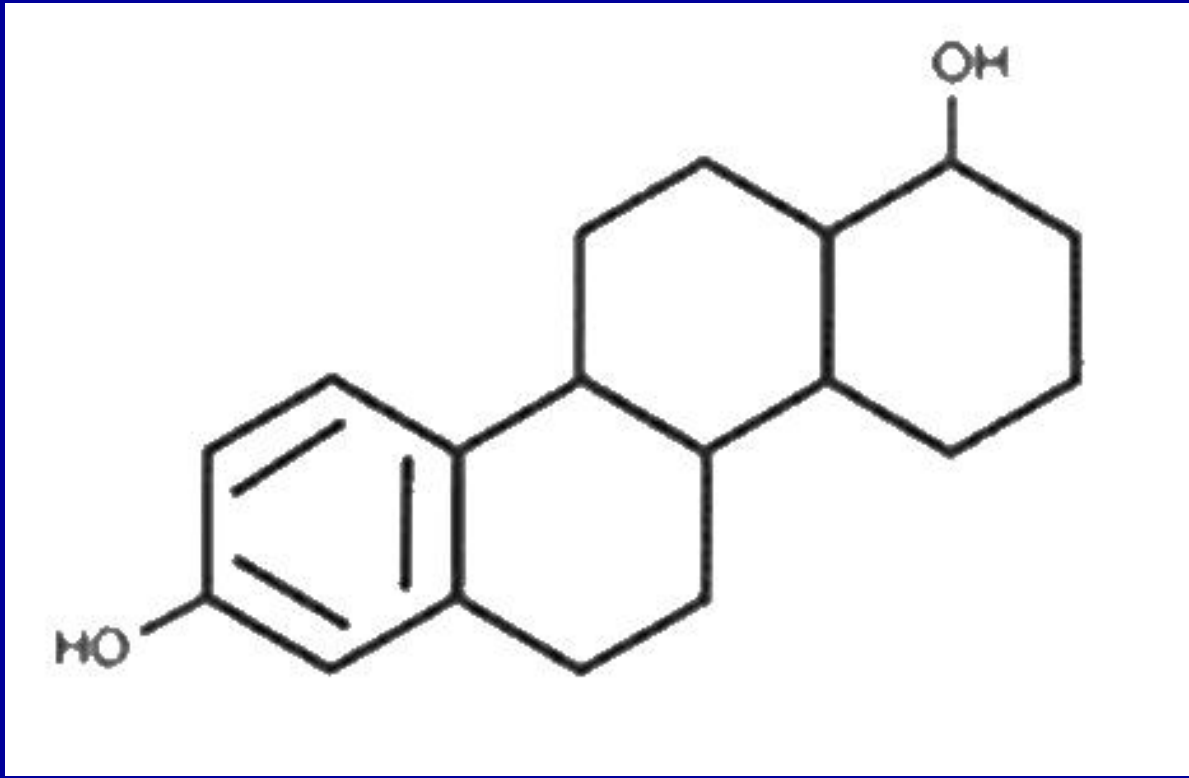
Botelho *et al.* Urol Oncol 2009



Botelho *et al.* (Submitted)

Schistosomiasis and host hormones

Shistosoma haematobium produces an estradiol-related molecule



Estradiol (E2)

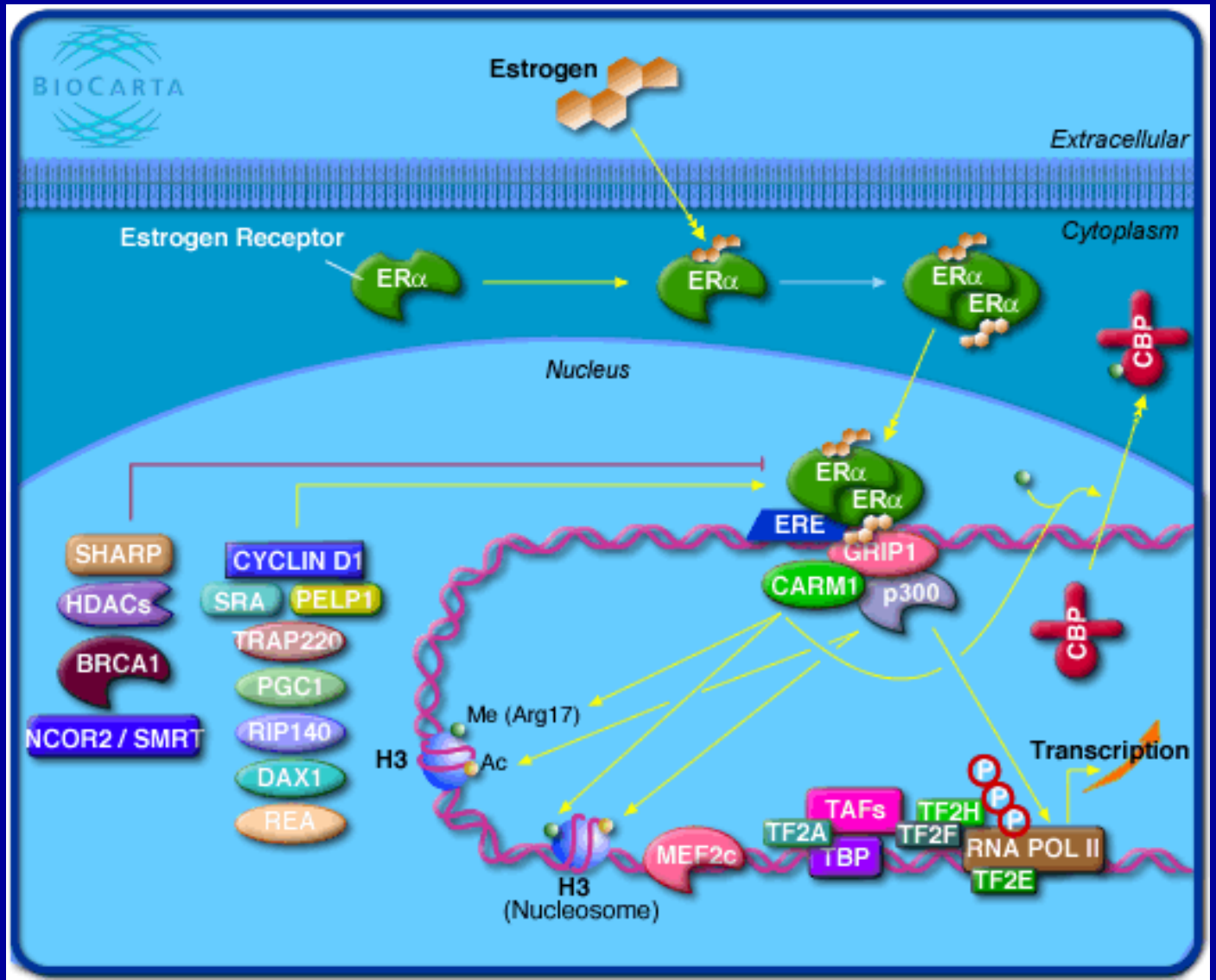
Shistosoma haematobium produces an estradiol-related molecule

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
S. haematobium	14,84±0,14
S. mansoni	12,63±0,27
F. haepatica	<10
H_2Od	<10
10 nM E2	1632,99±2,55

Botelho *et al.* Exp Parasitol 2009

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

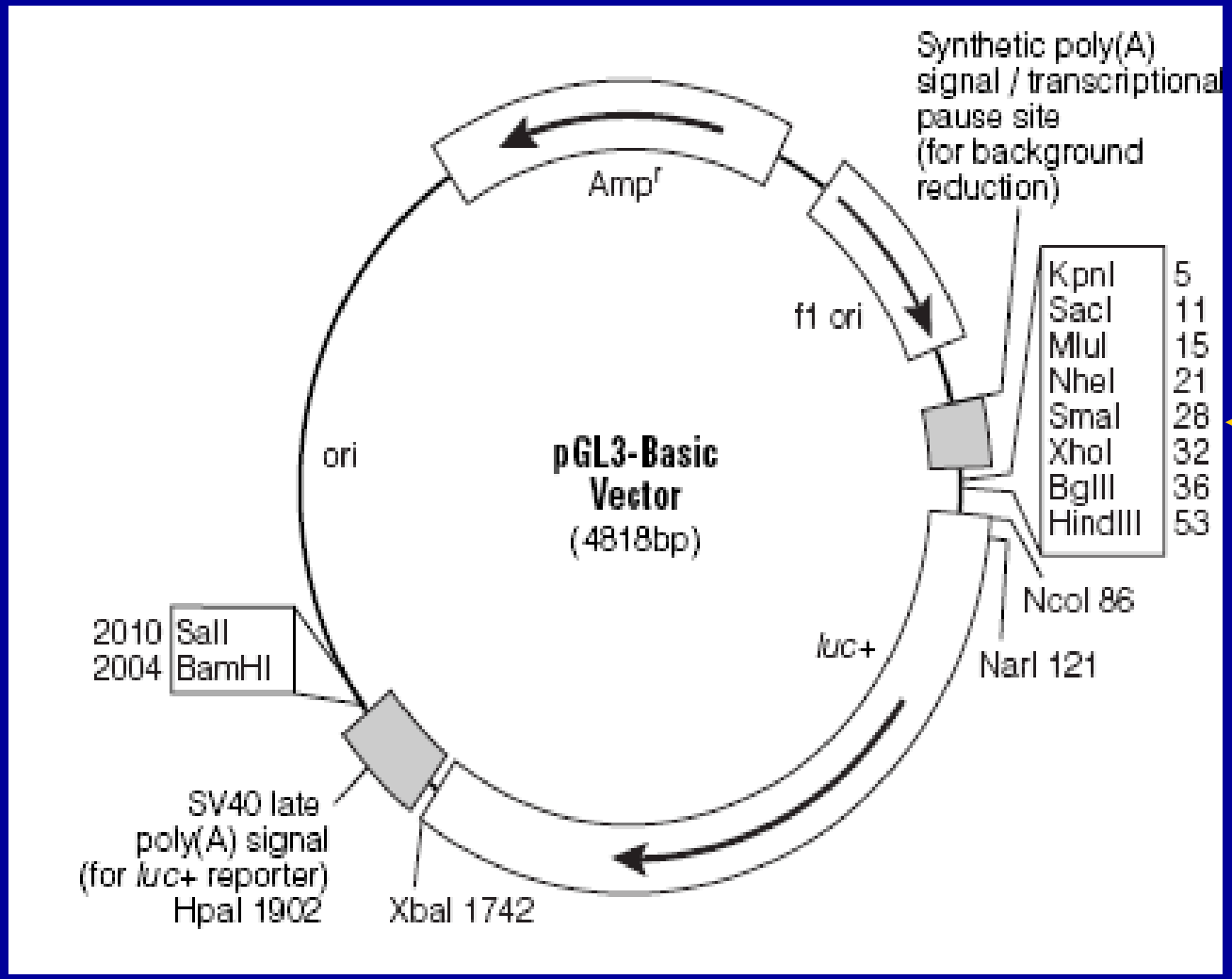


Methodological Strategy

Gene expression Real-Time PCR

Methodological Strategy

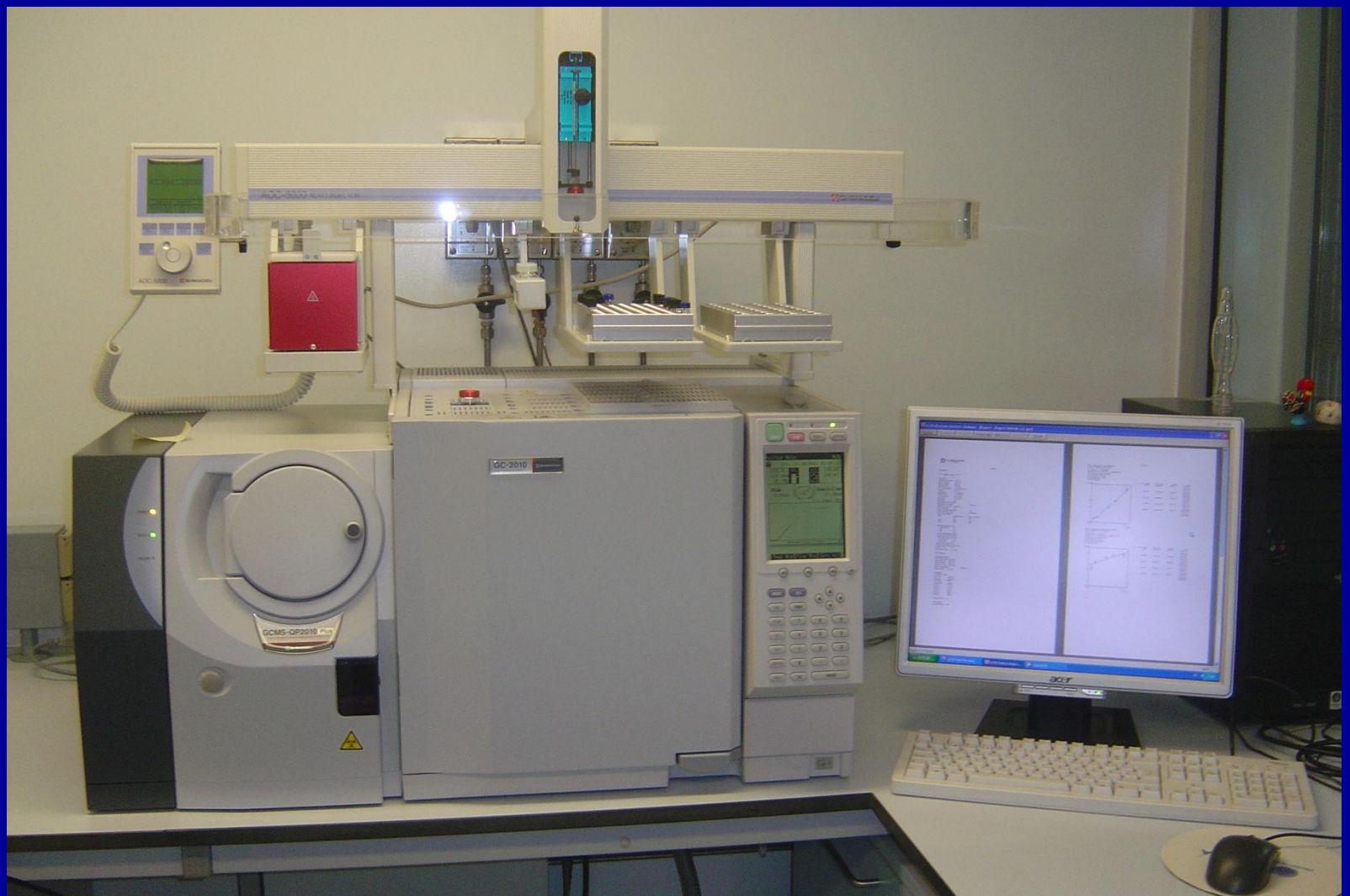
Transfection pERE-Luc



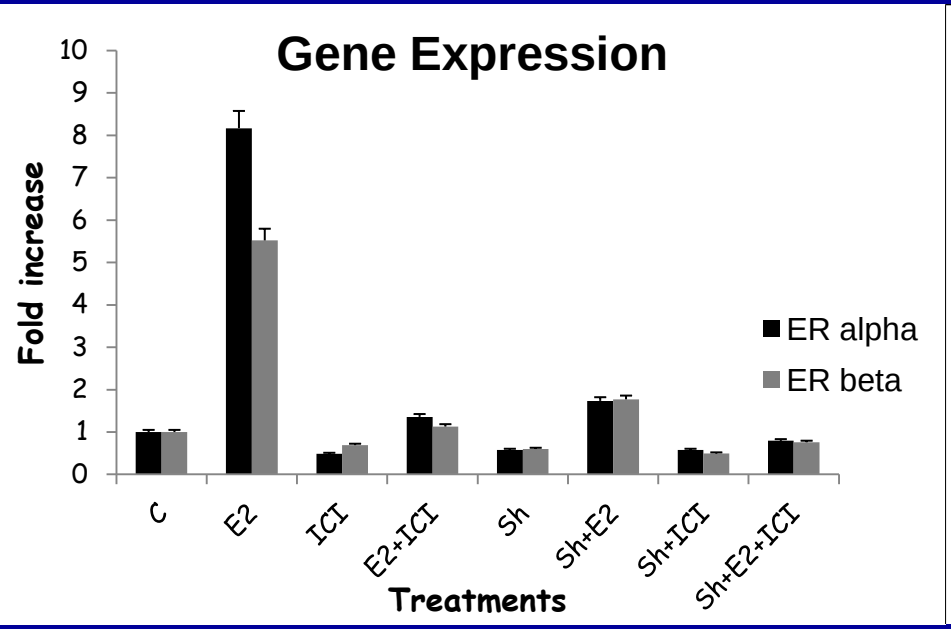
Estrogen Responsive Element (ERE)

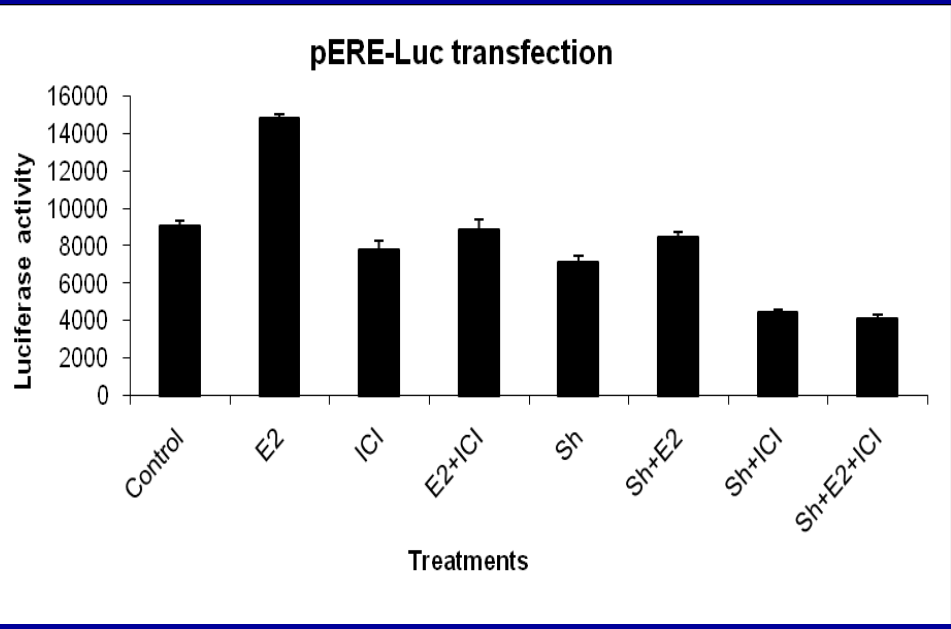
Methodological Strategy

Mass spectrometry LC-ESI-MS



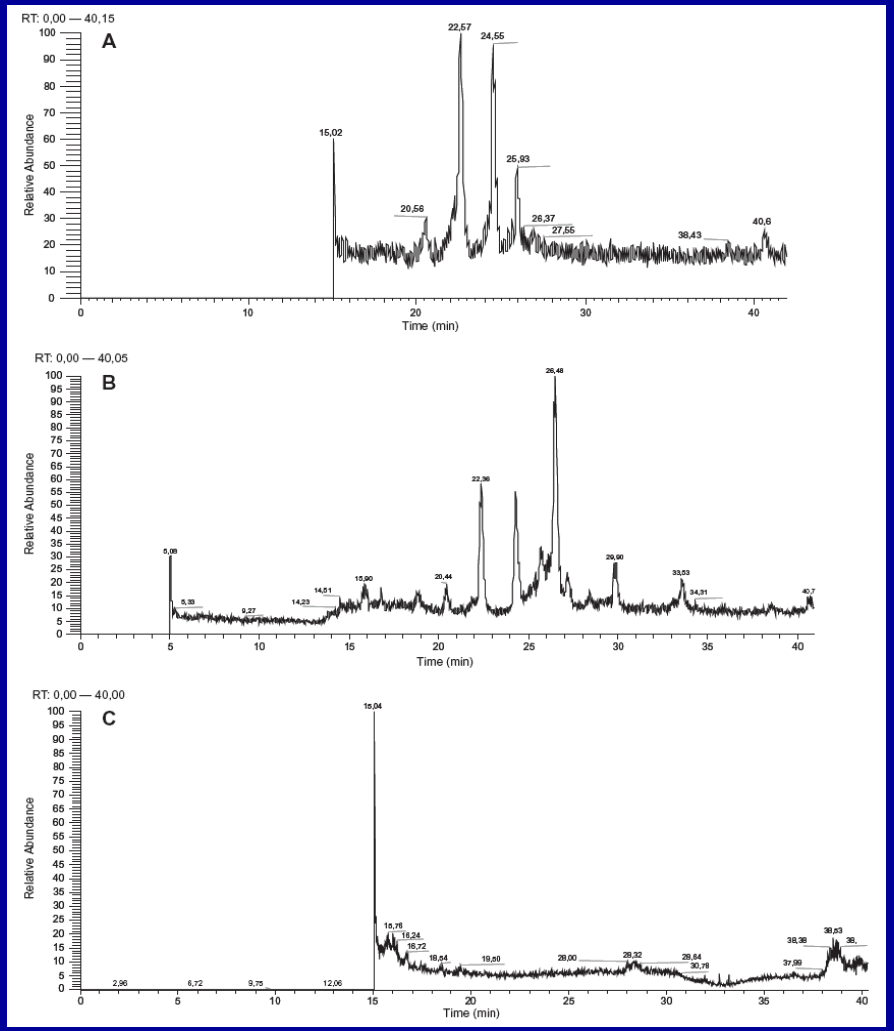
S. haematobium produces estrogenic molecules that are able to down-regulate ER alpha and ER beta and repress ER transcriptional activity

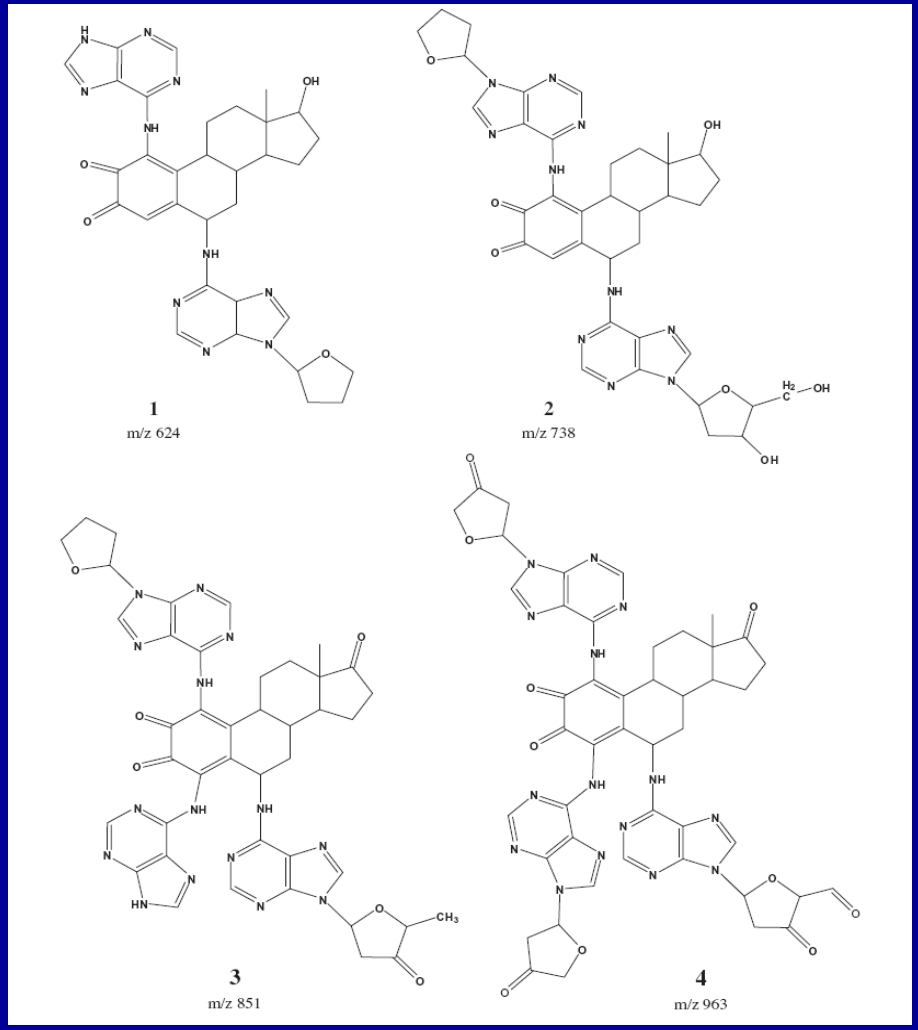




Botelho *et al.* Exp Parasitol 2010

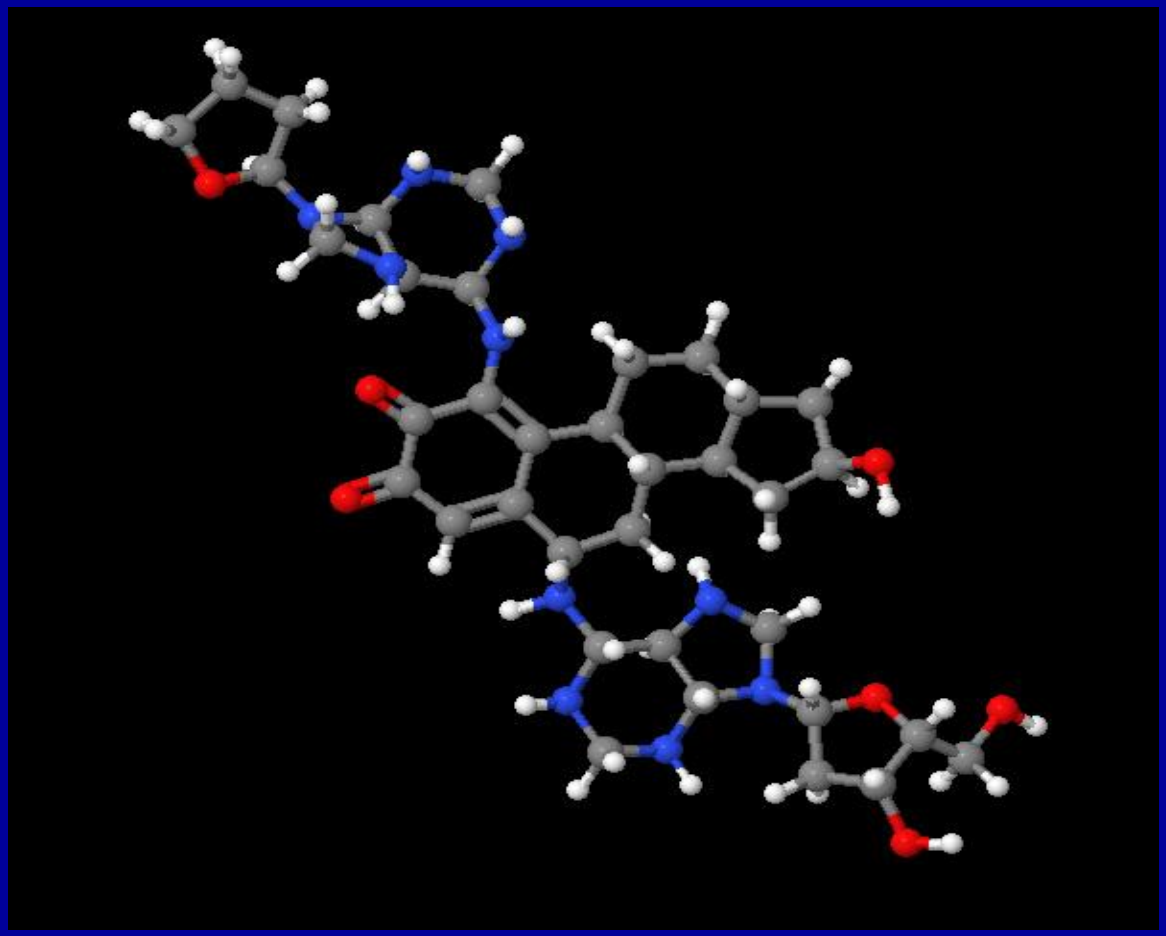
S. haematobium produces estrogenic molecules that are able to down-regulate ER alpha and ER beta and repress ER transcriptional activity





Botelho *et al.* Exp Parasitol 2010

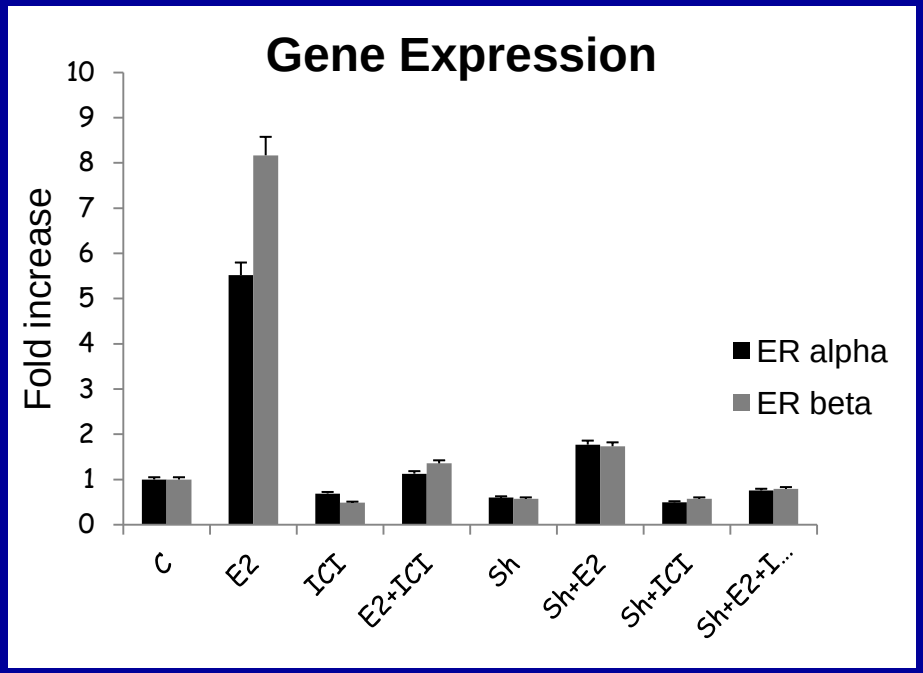
S. haematobium produces estrogenic molecules that are able to down-regulate ER alpha and ER beta and repress ER transcriptional activity

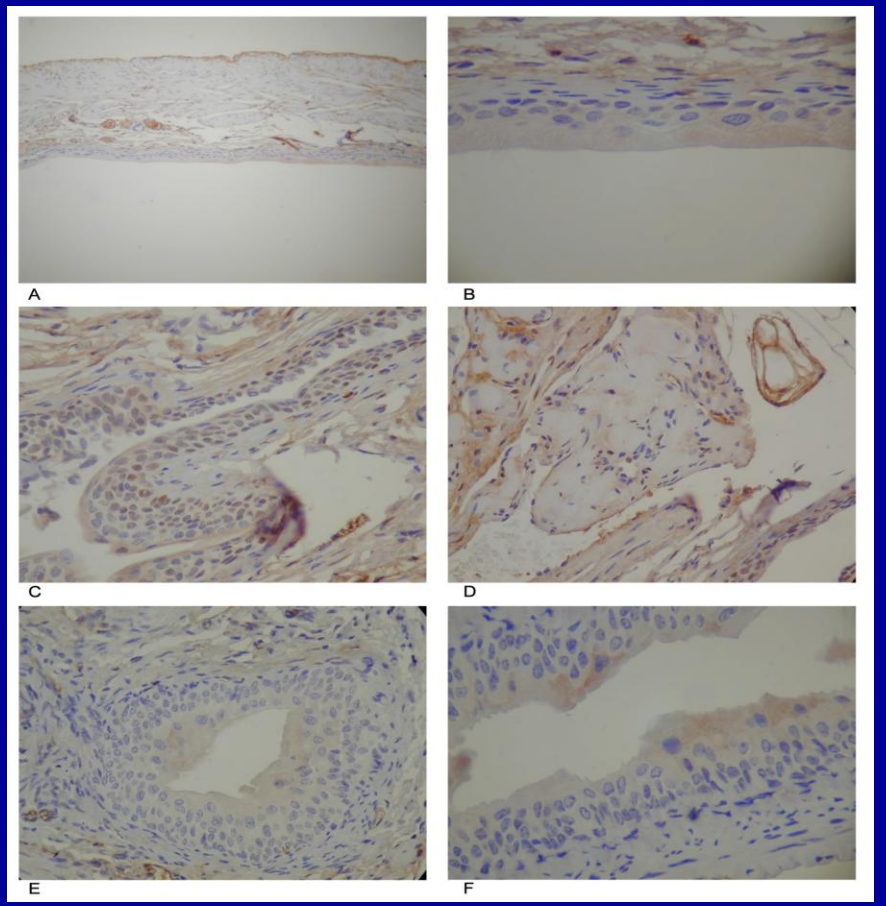


Botelho *et al.* Exp Parasitol 2010

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





Botelho *et al.* Oncol Rep 2011

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.

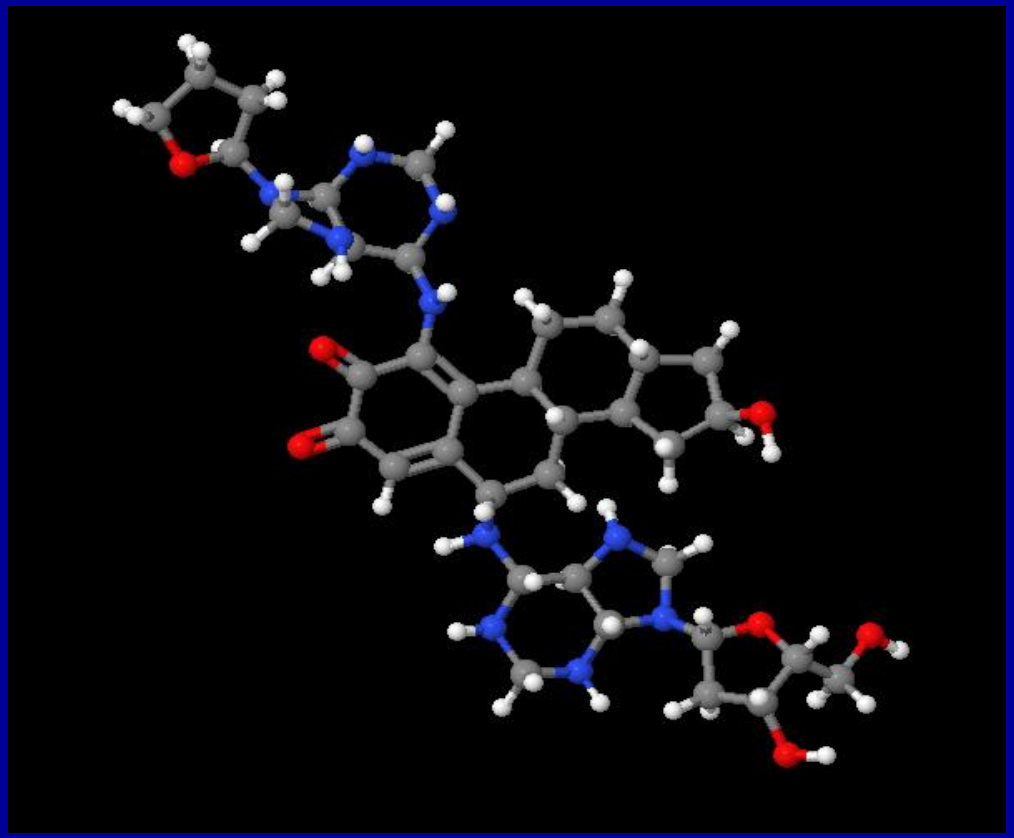
Conclusions
S. haematobium and hormones

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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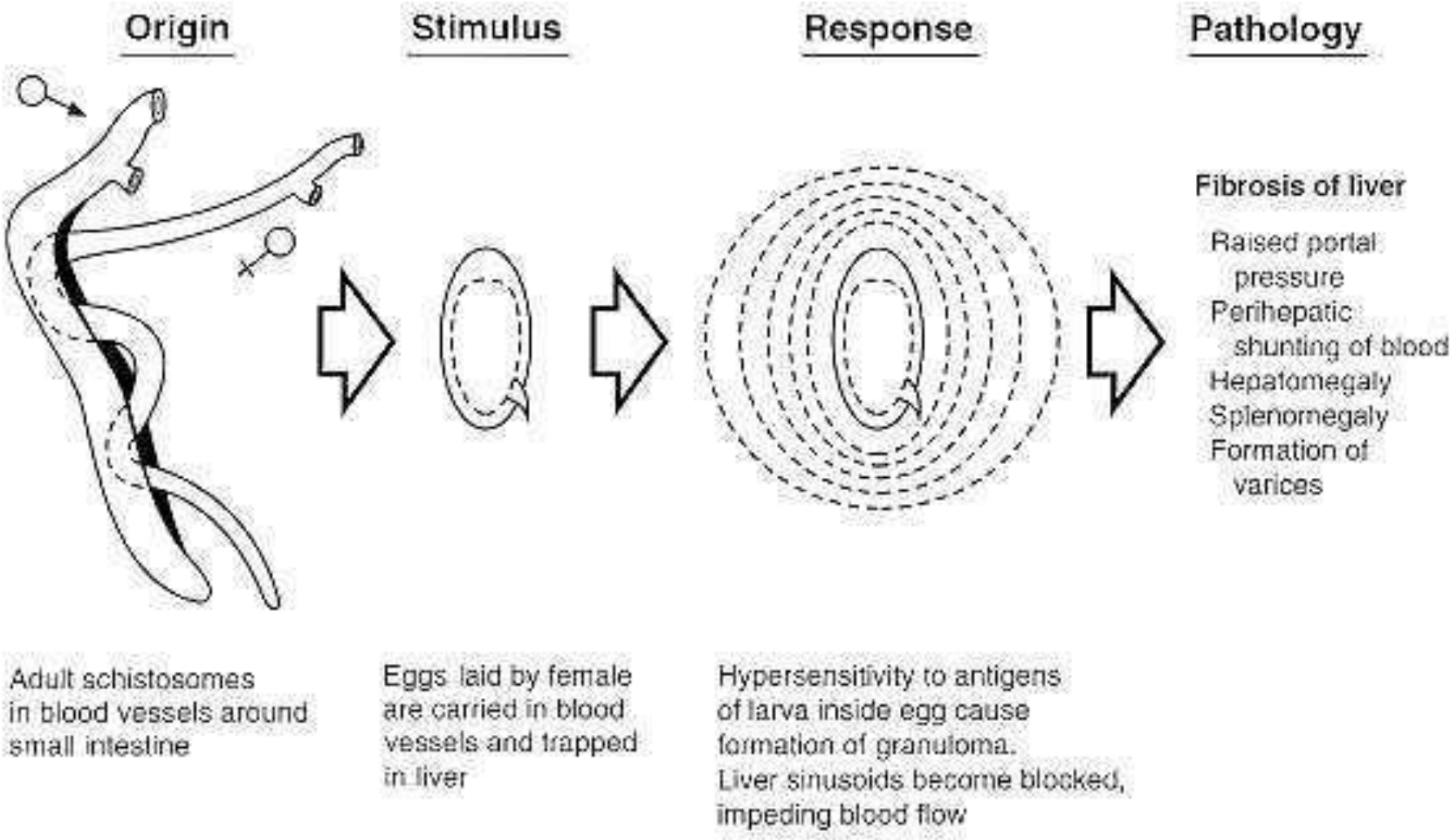
Madalena Crespo
Carlos Palmeira

Thank you for your attention

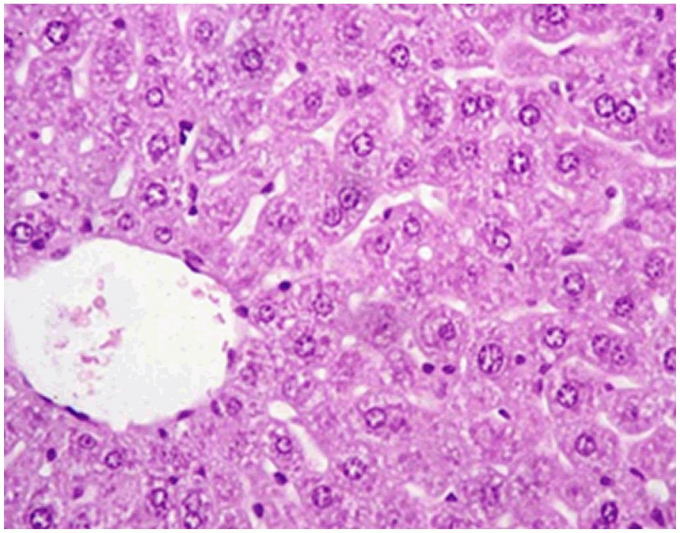

Origin
Stimulus
Response
Pathology
Fibrosis of liver
Raised portal pressure
Perihepatic shunting of blood
Hepatomegaly
Splenomegaly
Formation of varices
Adult schistosomes in blood vessels around small intestine
Eggs laid by female are carried in blood vessels and trapped in liver
Hypersensitivity to antigens of larva inside egg cause formation of granuloma.
Liver sinusoids become blocked, impeding blood flow

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.



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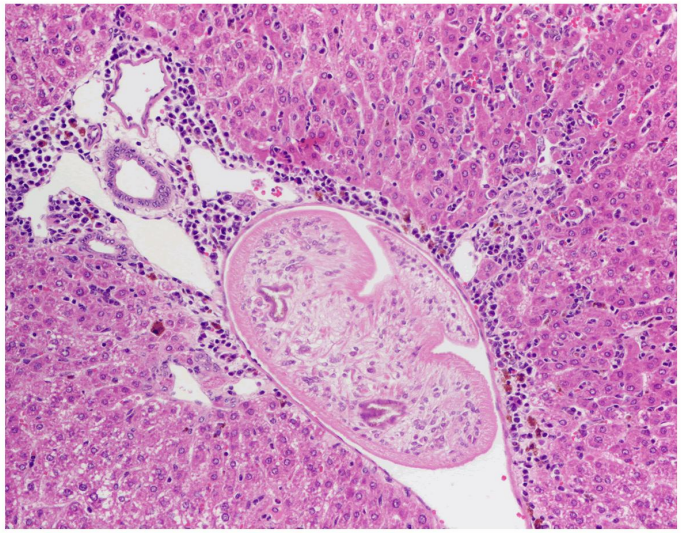

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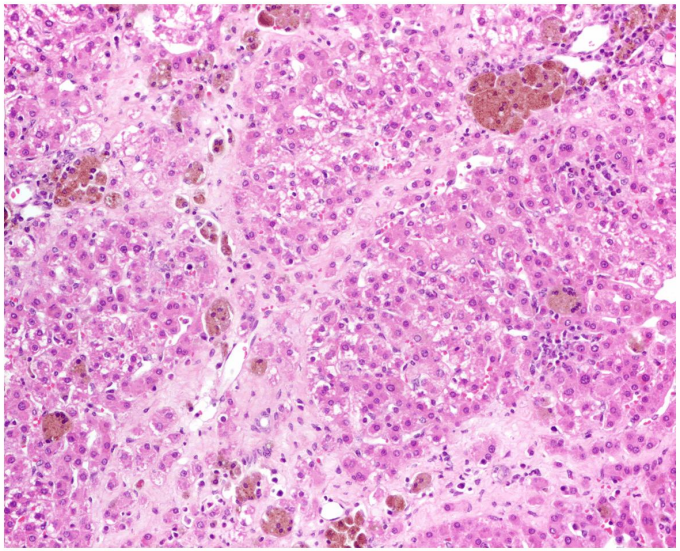

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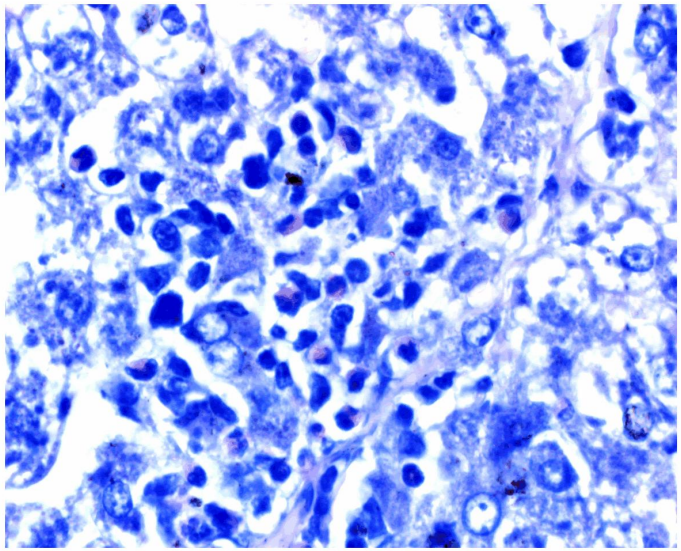
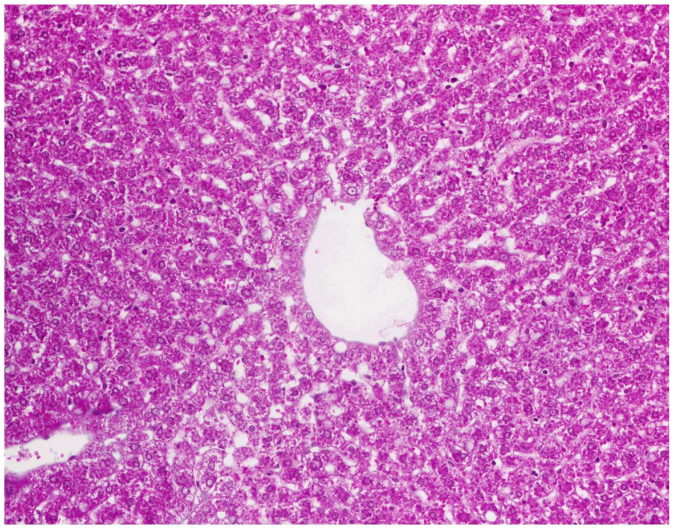
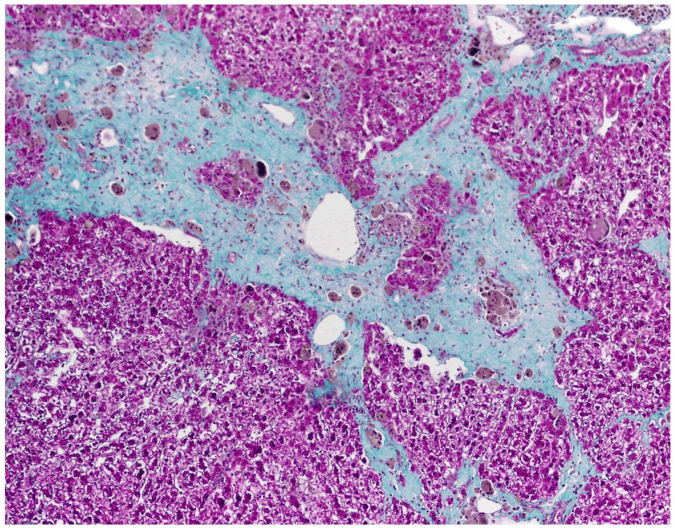


D

Histological findings of livers of infected animals.



A



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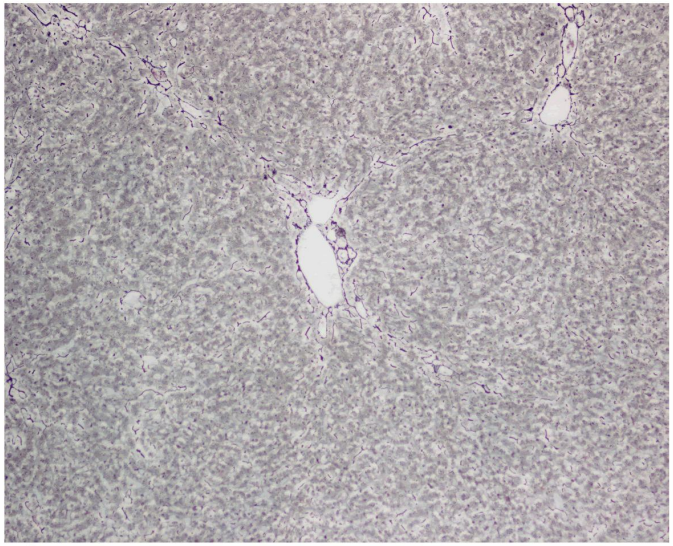

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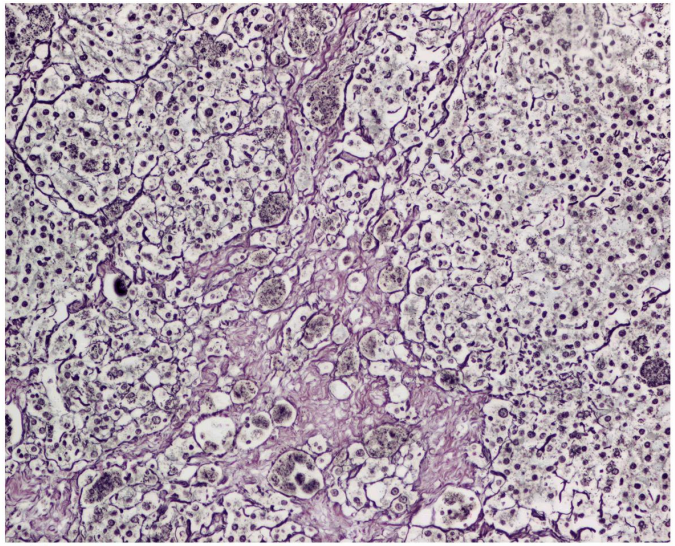


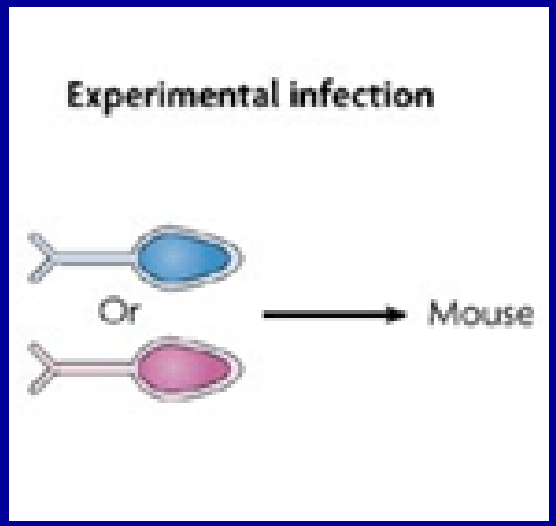
D

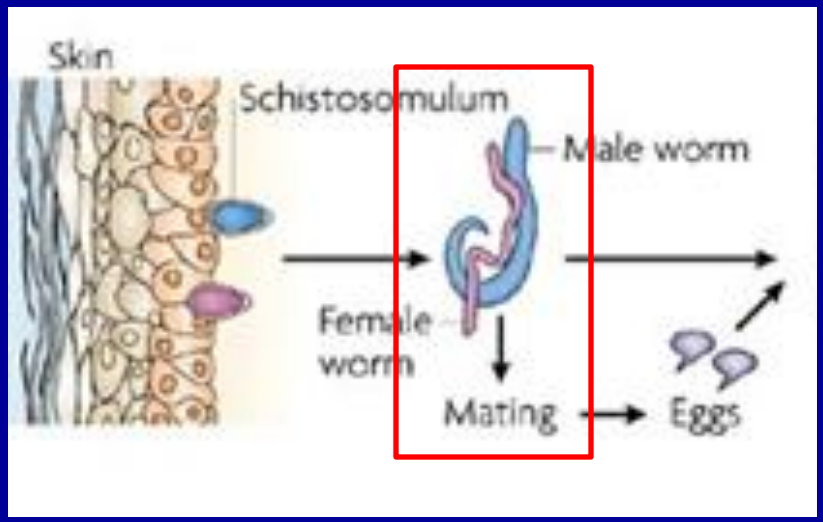
Schistosoma haematobium total antigen induces increased proliferation, migration and invasion, and decreases apoptosis of normal epithelial cells

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

(International Journal for Parasitology, 2009)

Methodological Strategy

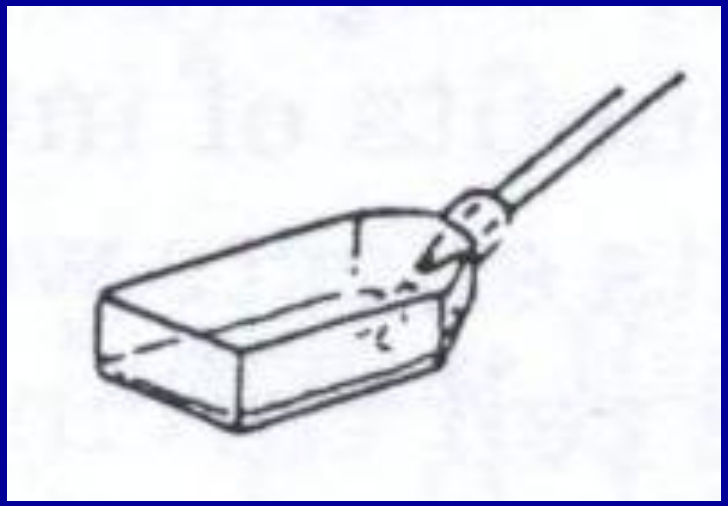




S. haematobium
total antigen (Sh)

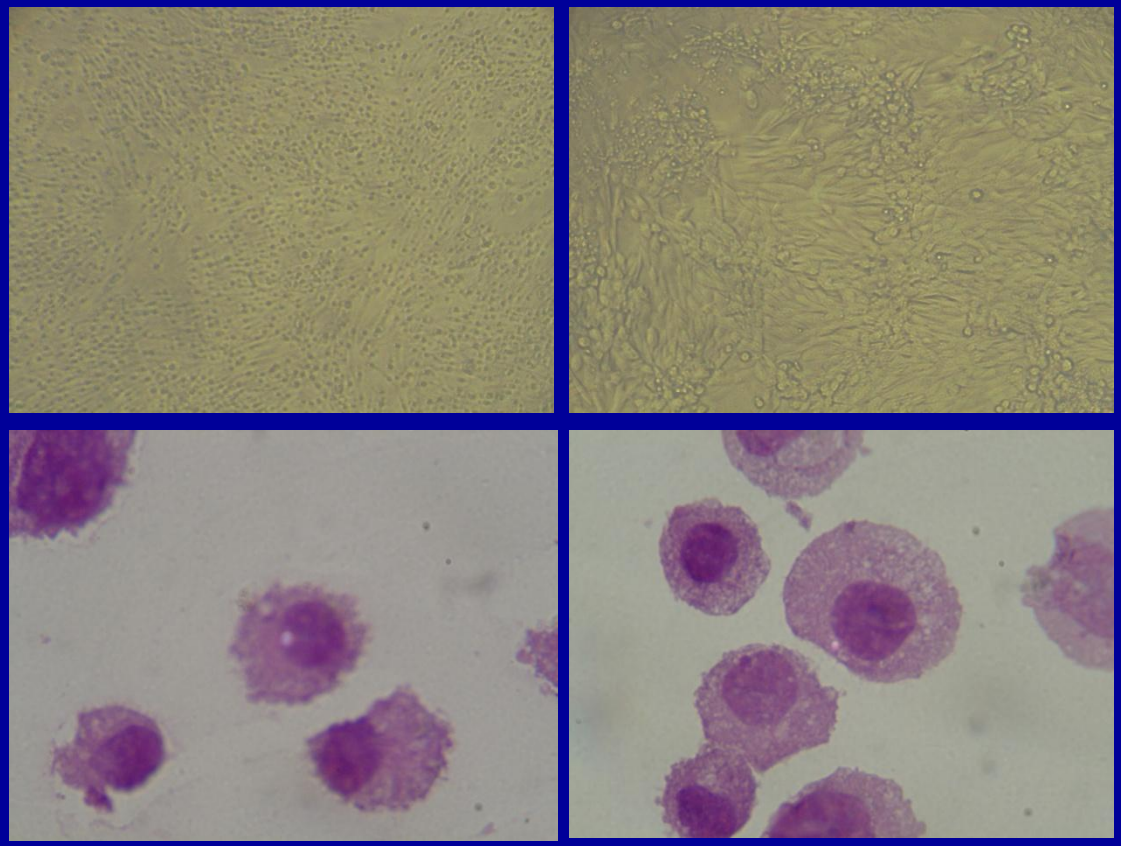
Methodological Strategy

Chinese Hamster Ovary (CHO) cells



Sh-treatment 48 h

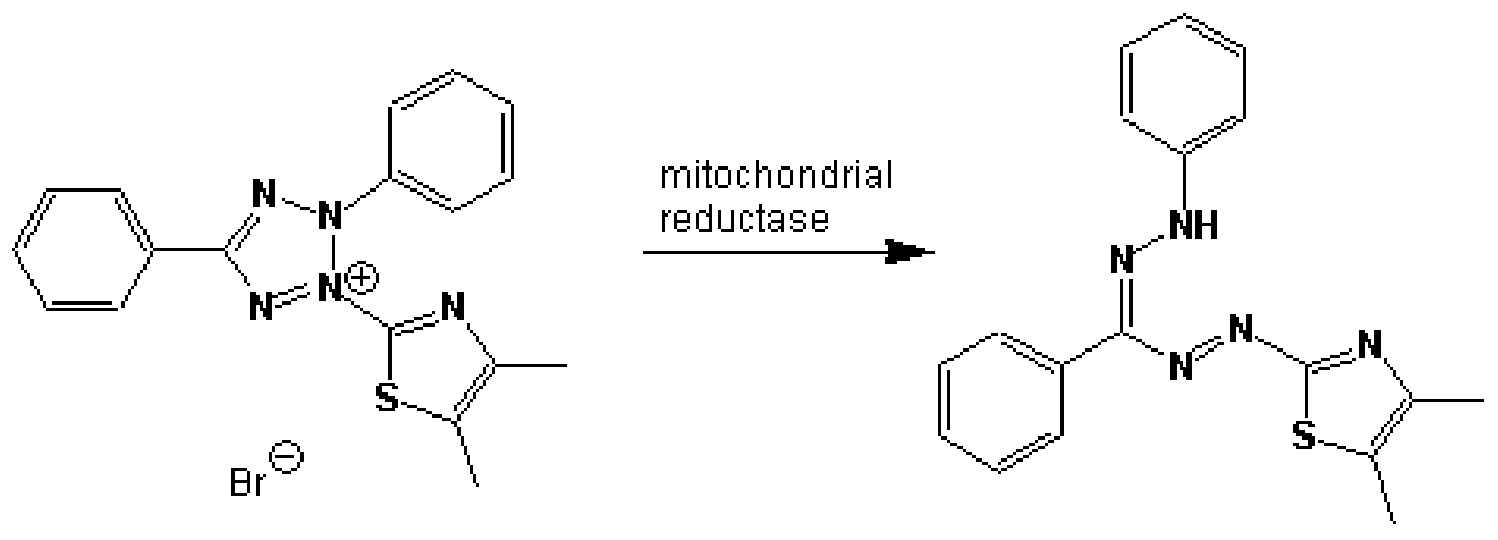
S. haematobium total antigen induces alterations in the morphology of treated cells



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

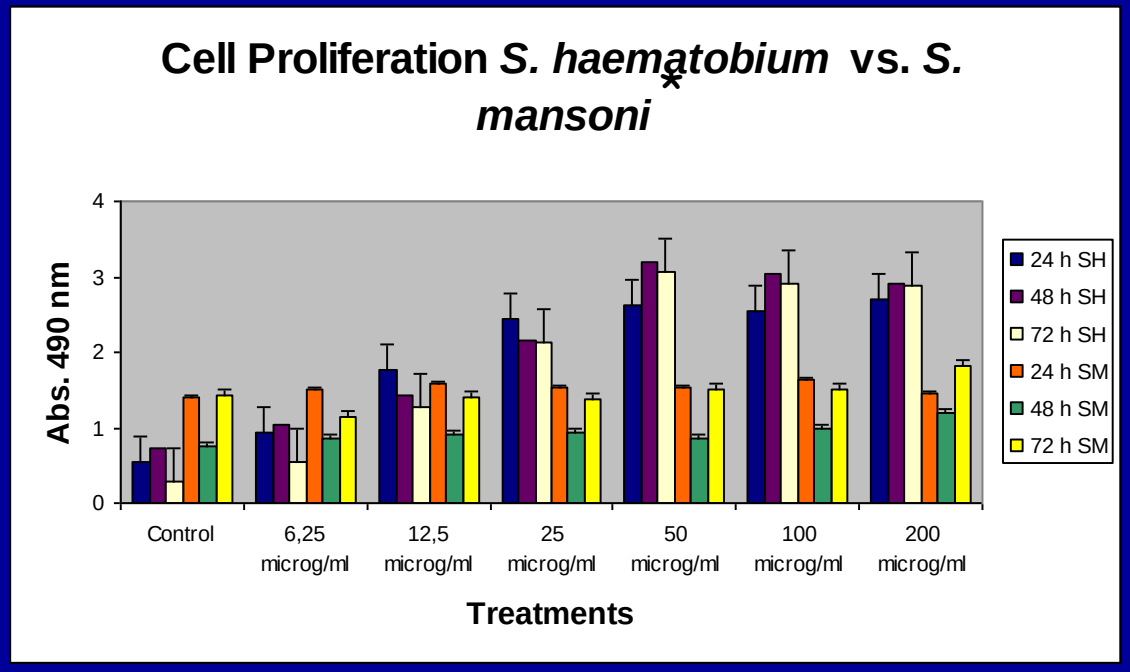
Methodological Strategy

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.[1

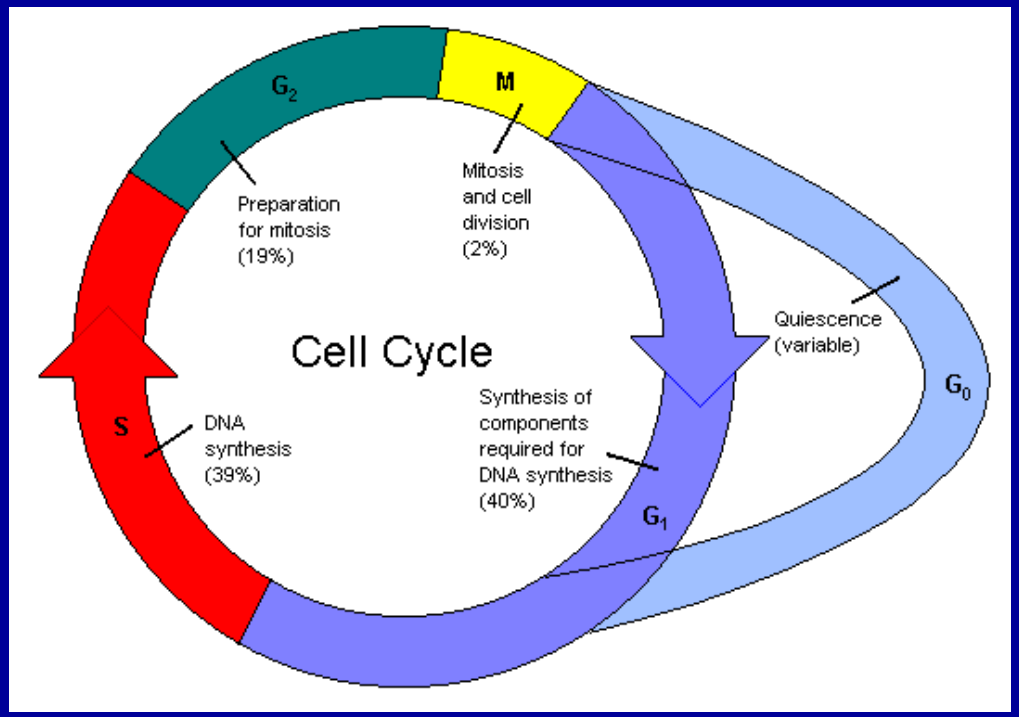
S. haematobium total antigen, but not *S. mansoni* total antigen, increased the proliferation of epithelial cells in vitro

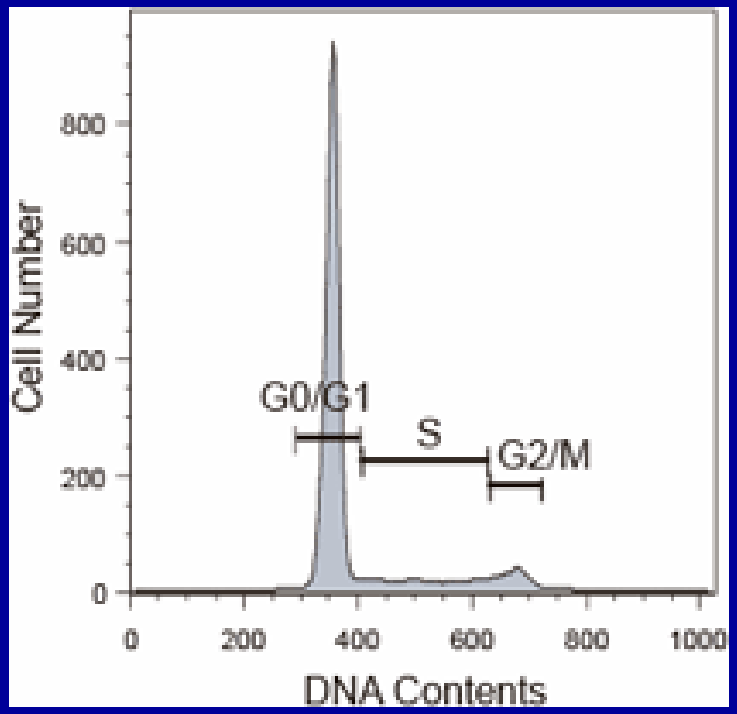


Comparison of proliferation between Sh and Sm treated cells (*p<0,01; Sh vs. Sm).

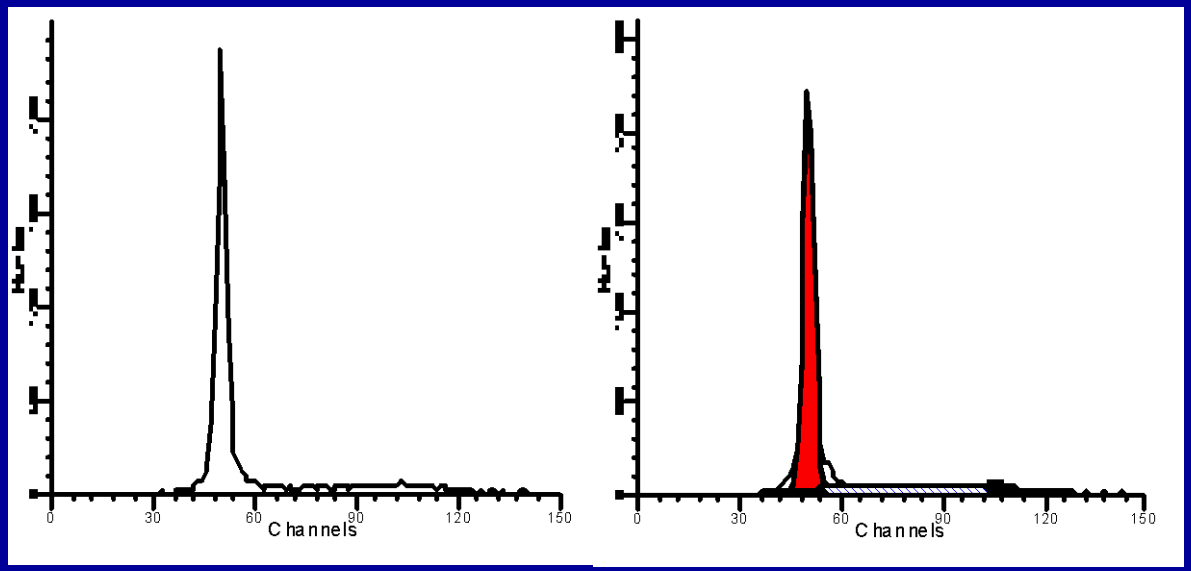
Methodological Strategy

DNA content Flow cytometry





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

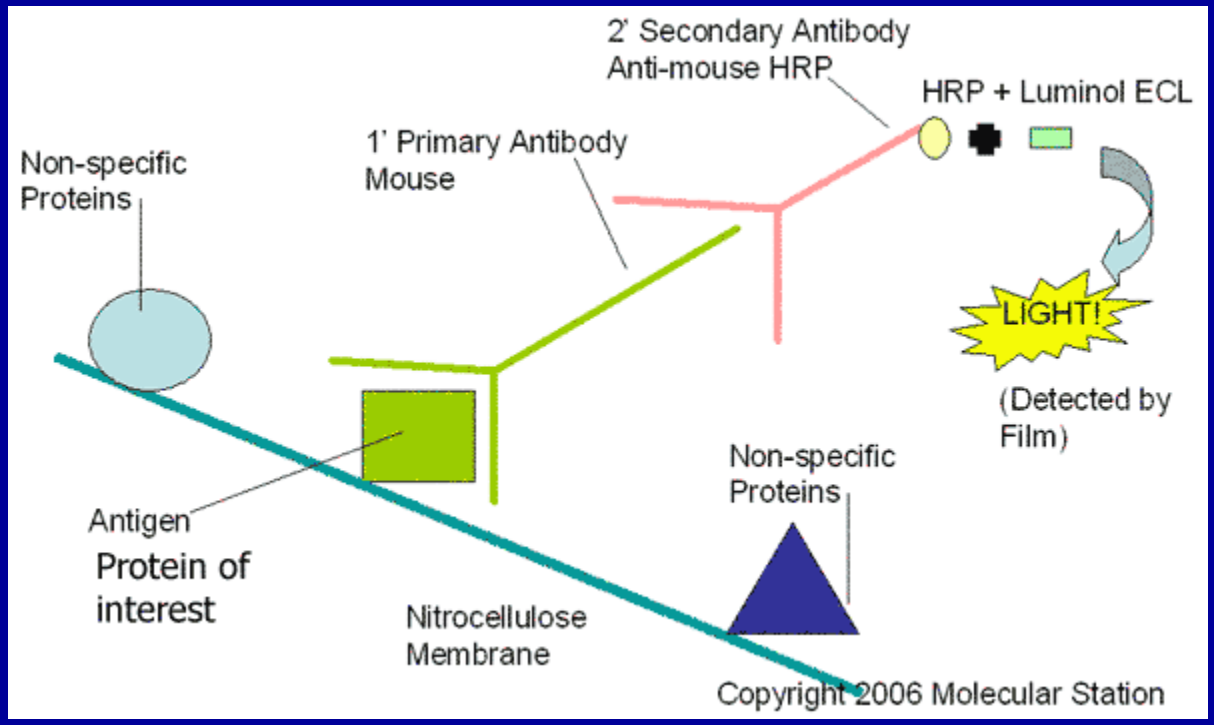


Control Sh-treated

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



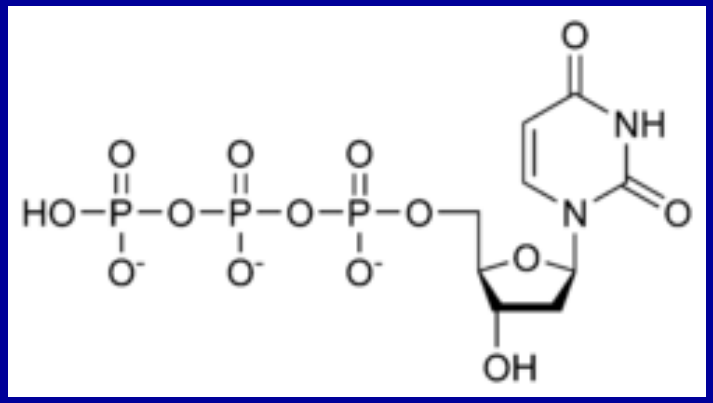
The **western blot** is used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins. The proteins are then transferred to a membrane where they are detected using antibodies specific to the target protein.

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

Methodological Strategy

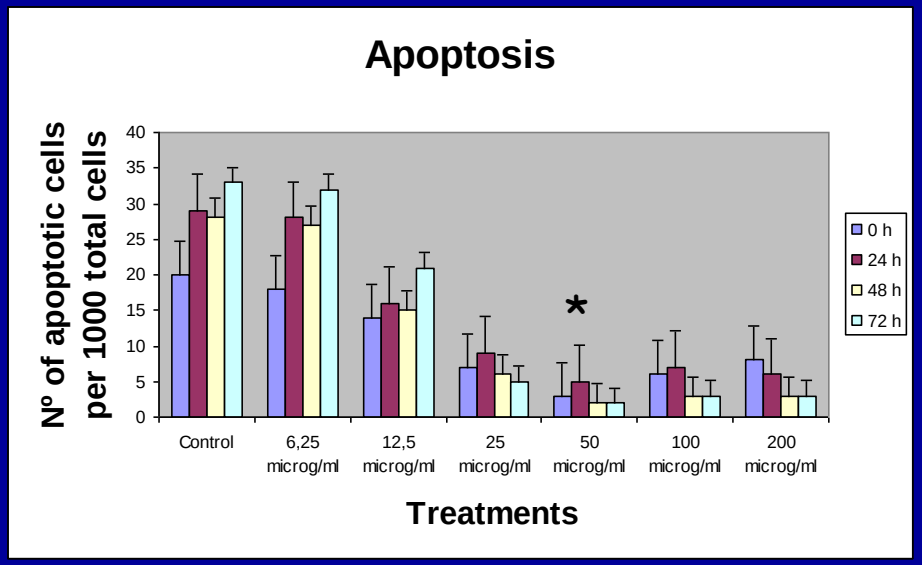
Apoptosis

TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling)

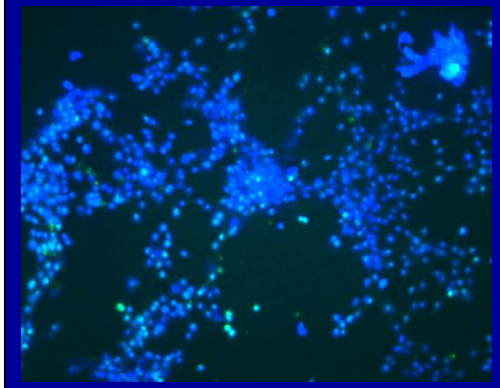


The assay relies on the presence of nicks in the DNA which can be identified by terminal deoxynucleotidyl transferase, an enzyme that will catalyze the addition of dUTPs that are secondarily labeled with a marker. It may also label cells that have suffered severe DNA damage.

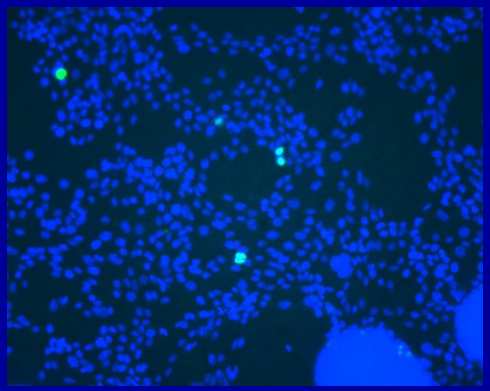
S. haematobium total antigen decreased the apoptosis of epithelial cells *in vitro*



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

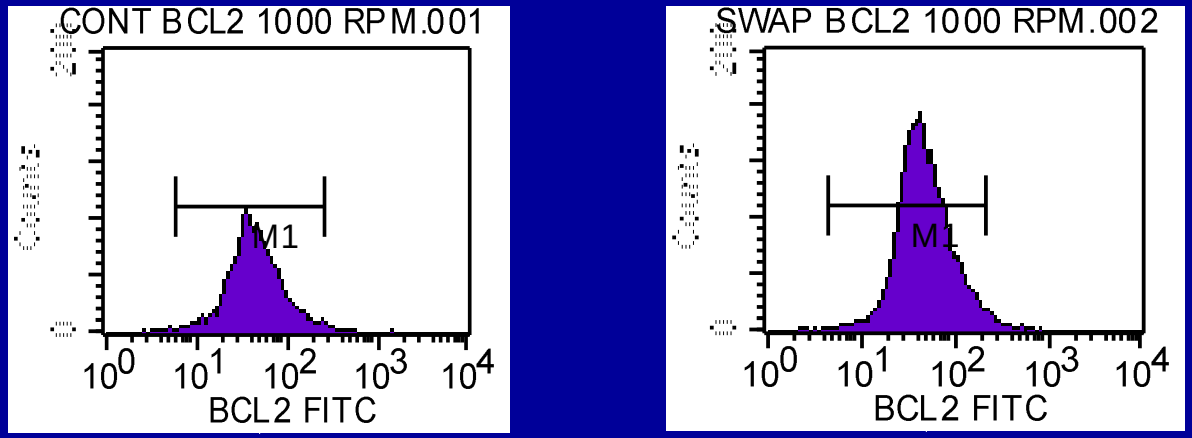


Control



Sh-treated

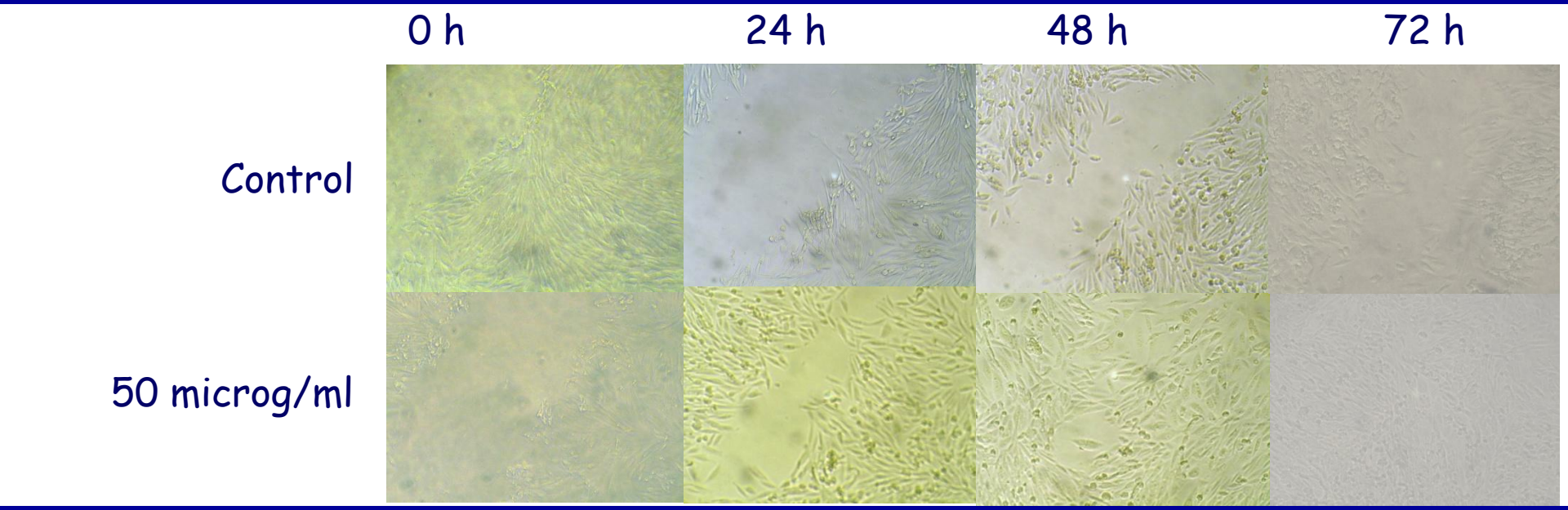
Up-regulation of the anti-apoptotic protein Bcl-2, *in vitro*, by *S. haematobium* total antigen



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

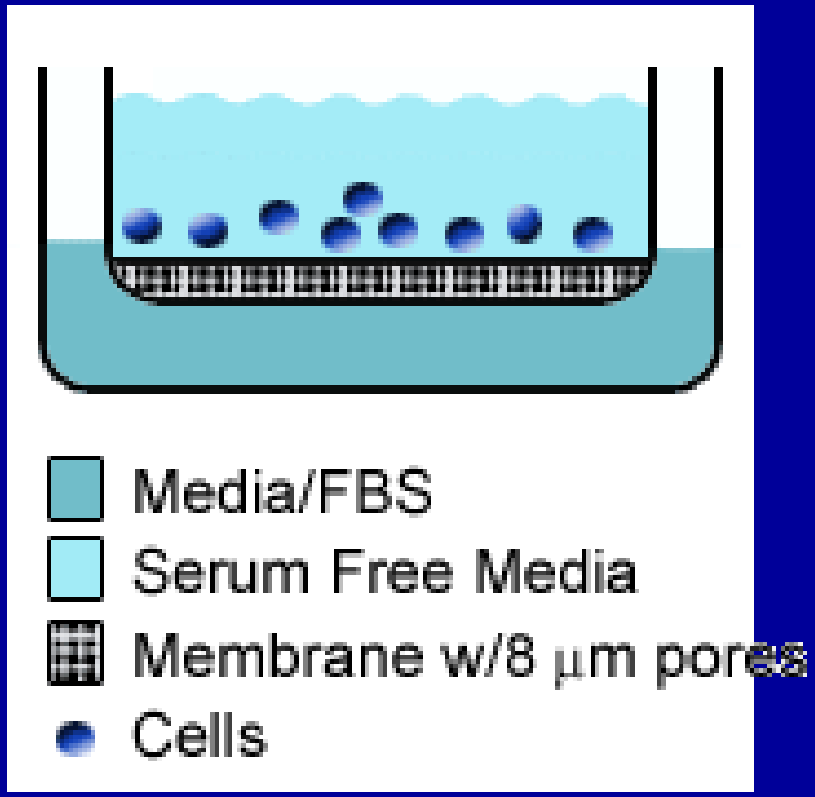


Wound closure assay

Methodological Strategy

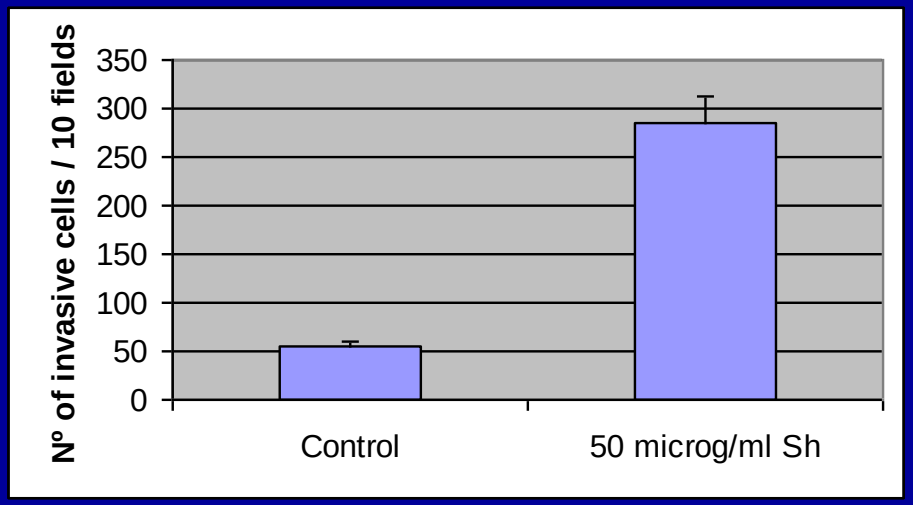
Invasion

Boyden chamber

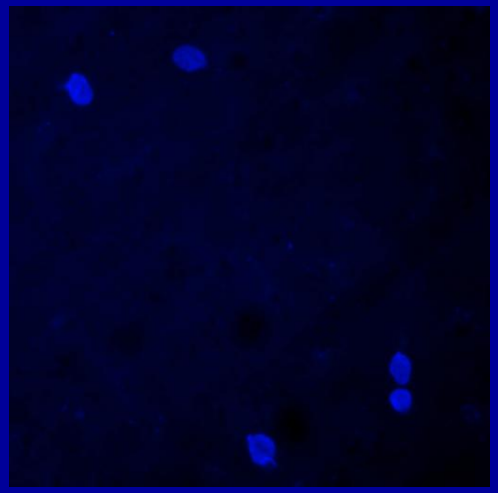


1. Cell suspension is placed in upper chamber and incubated for 24-72 hours at 37 C.
2. Invasive cells pass through the membrane and cling to the bottom of the polycarbonate membrane that is tissue culture-treated to enhance cell attachment.
3. Non-invading cells cannot pass through the membrane and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

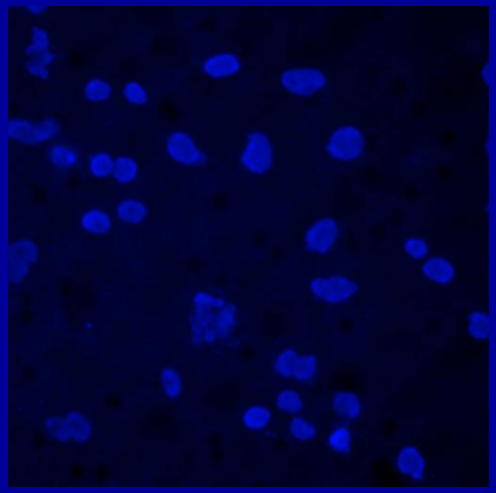
Induced invasion of epithelial cells *in vitro* by *S. haematobium* total antigen



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



Control



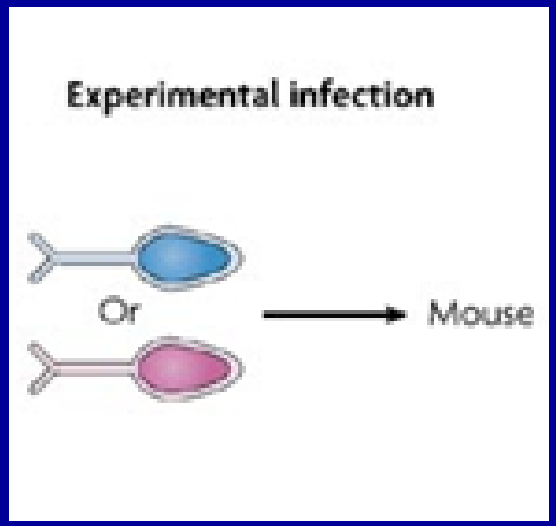
Sh-treated

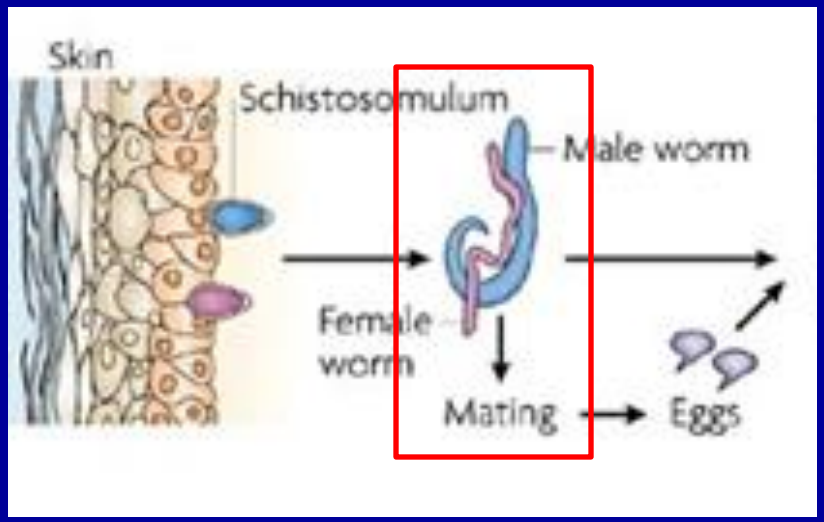
Tumorigenic effect of *Schistosoma haematobium* total antigen in normal mammalian cells

Botelho M, Oliveira PA, Gomes J, Gartner F, Lopes C, Correia da Costa JM, Machado JC

(Int. J. Exp. Path., 2009)

Methodological Strategy

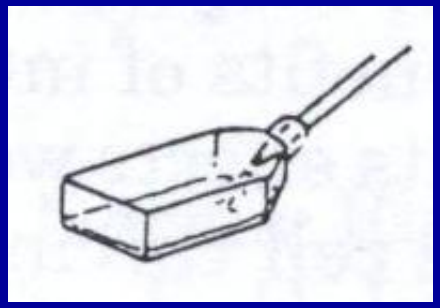




S. haematobium
total antigen (Sh)

Methodological Strategy

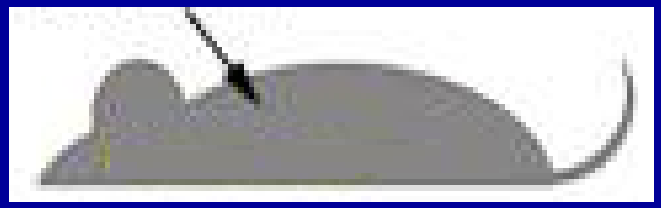
Chinese Hamster Ovary (CHO) cells

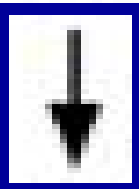


Sh-treatment 48 hours

Nude mice

Control CHO cells

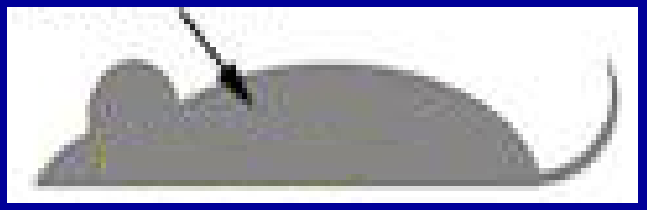


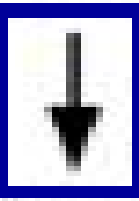


No Tumour

2 weeks

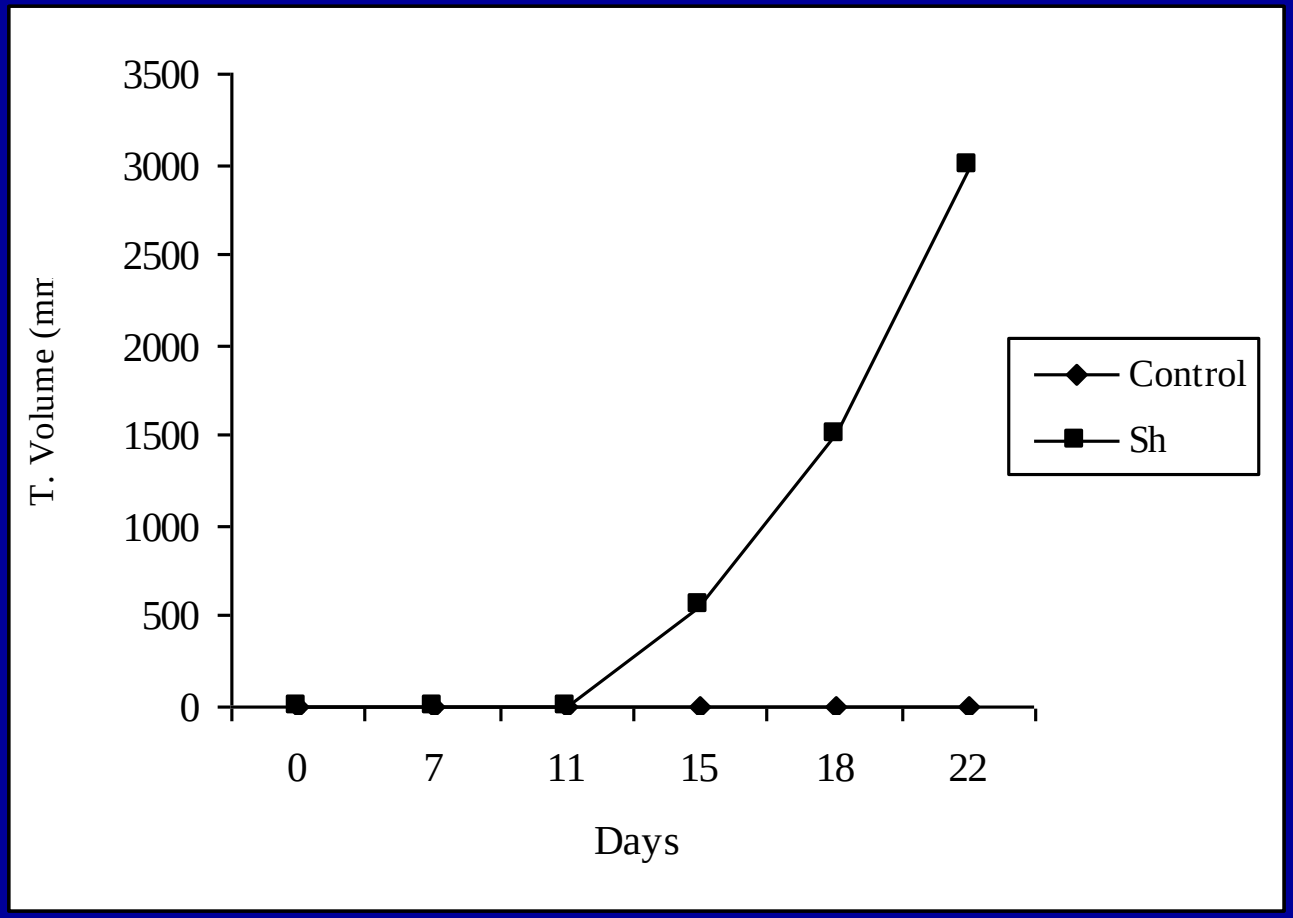
Sh-treated CHO cells





Tumour-Bearing Nude Mouse

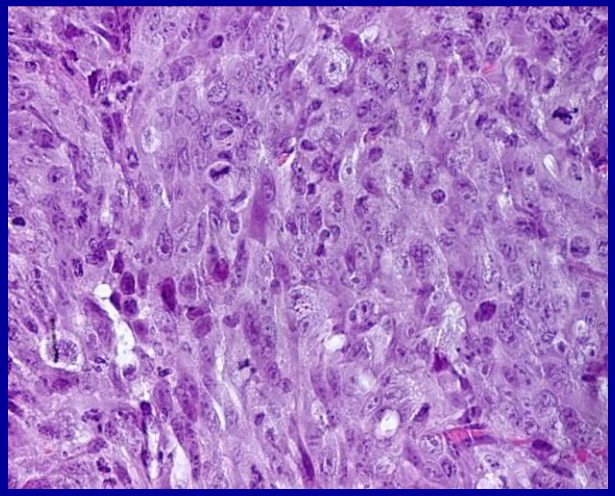
Sh allows the vigorous growth of CHO xenografts in nude mice



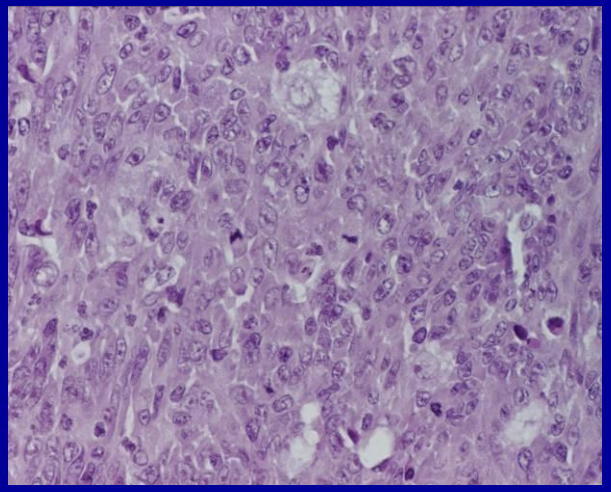
Immunohistochemistry and histochemical staining

		Immunohistochemistry		Histochemistry
Group	Tumour	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



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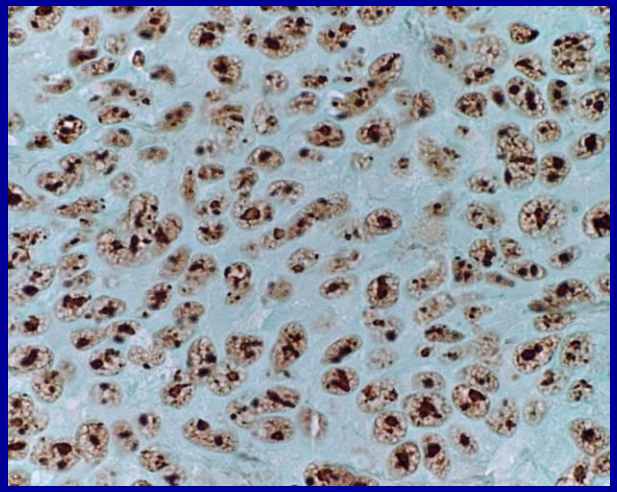
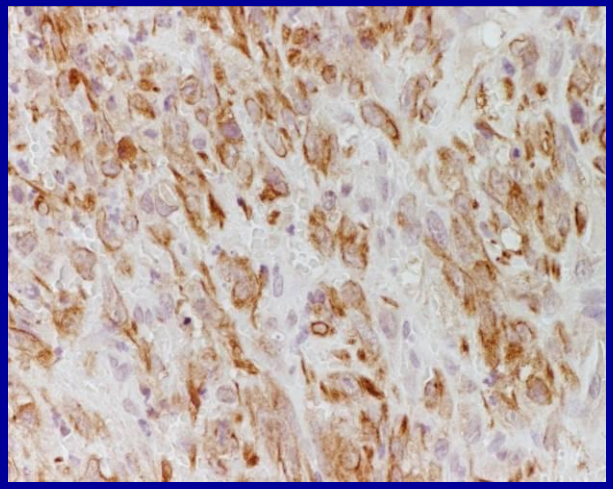


C



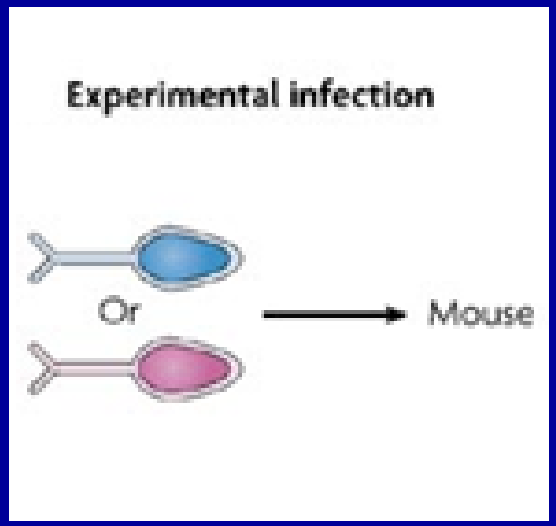
D

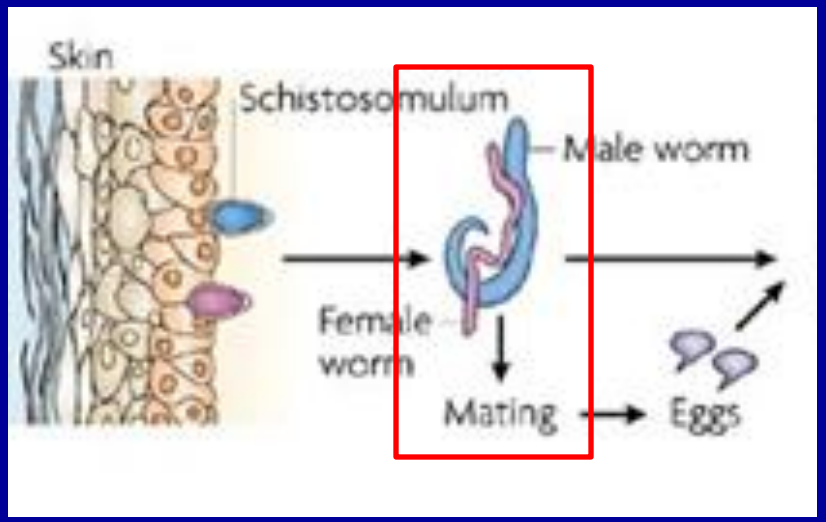
Induced dysplasia and inflammation of *Schistosoma haematobium* total antigen on normal urothelium of CD-1 mice

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

(Urologic Oncology: Seminars and Original Investigations, 2009)

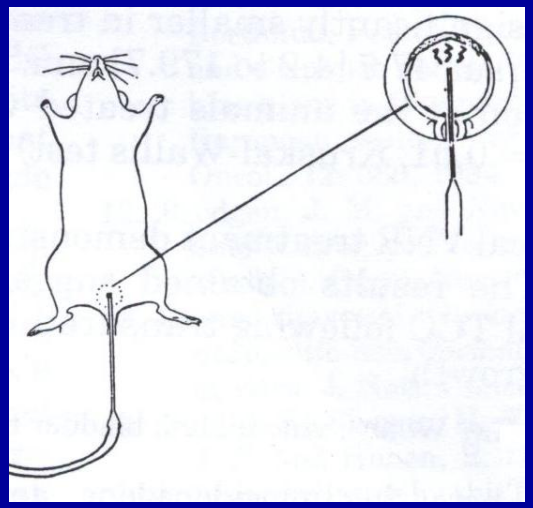
Methodological Strategy



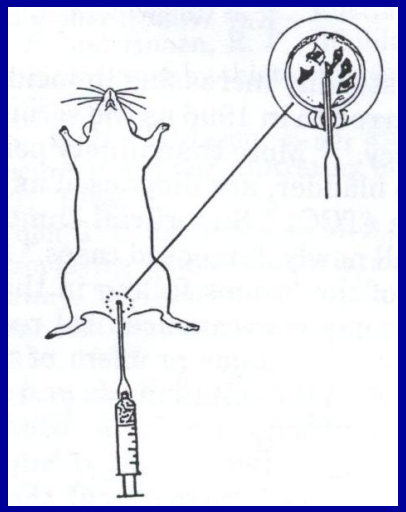


S. haematobium
total antigen (Sh)

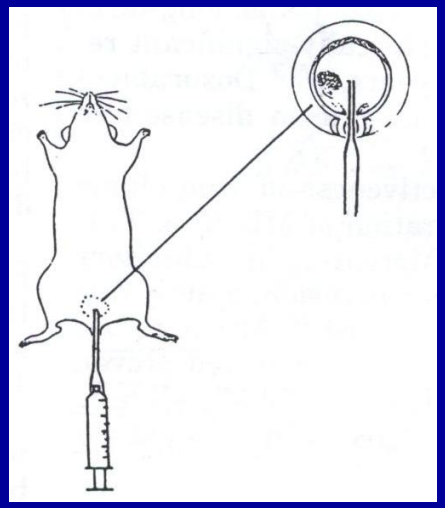
Methodological Strategy



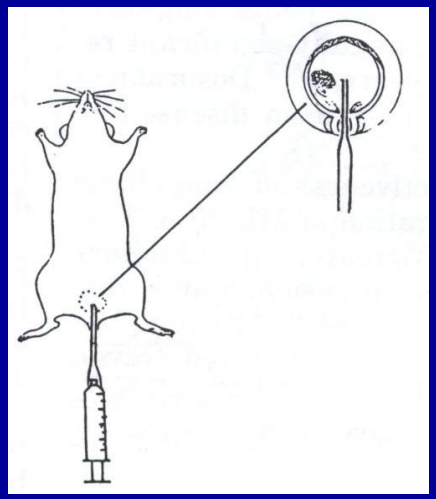
Transurethral Catheterization



Urothelial Denudation



Sh-treatment /PBS 1 hour



In situ fixation 10% PPA

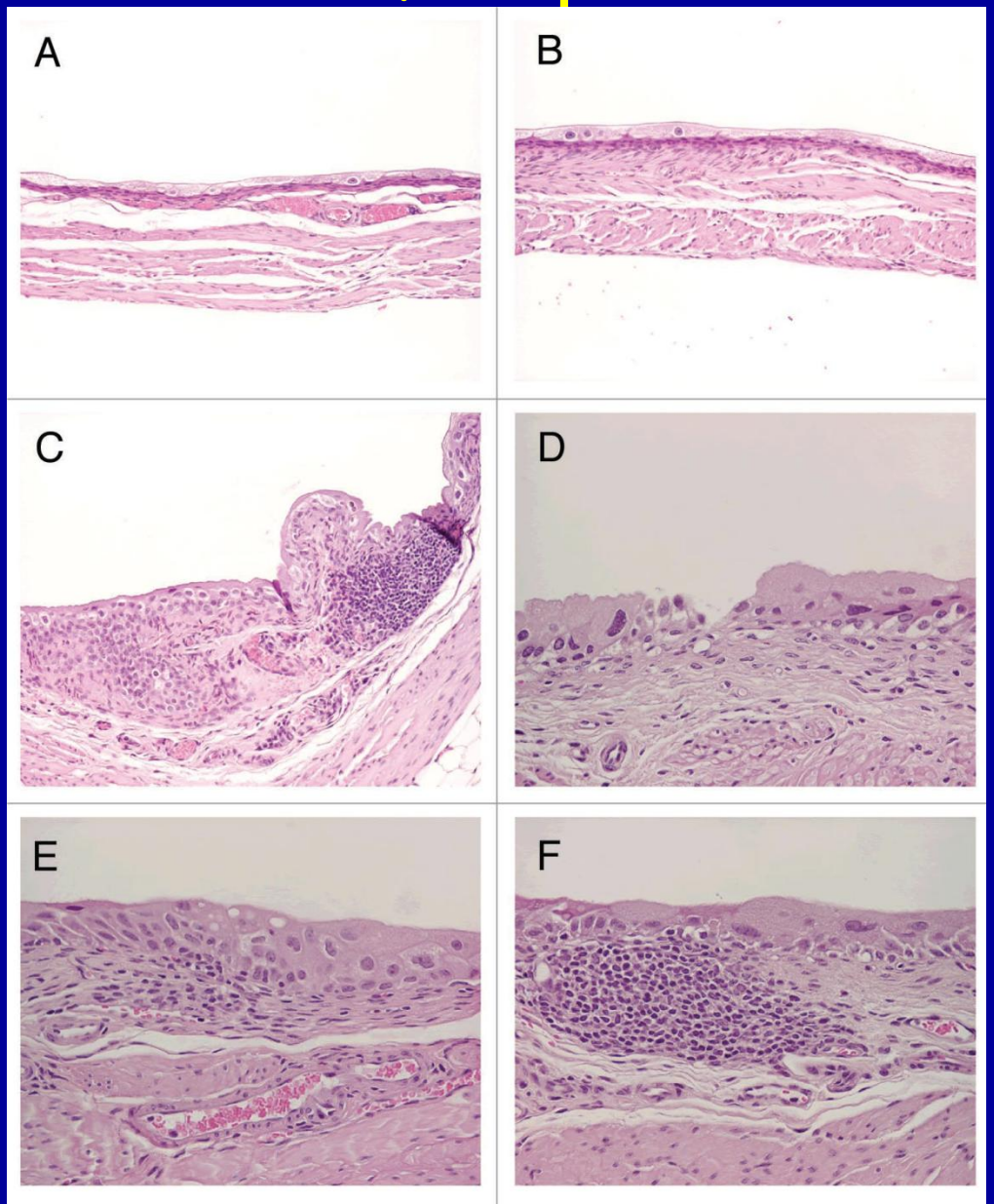
20 weeks
40 weeks

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

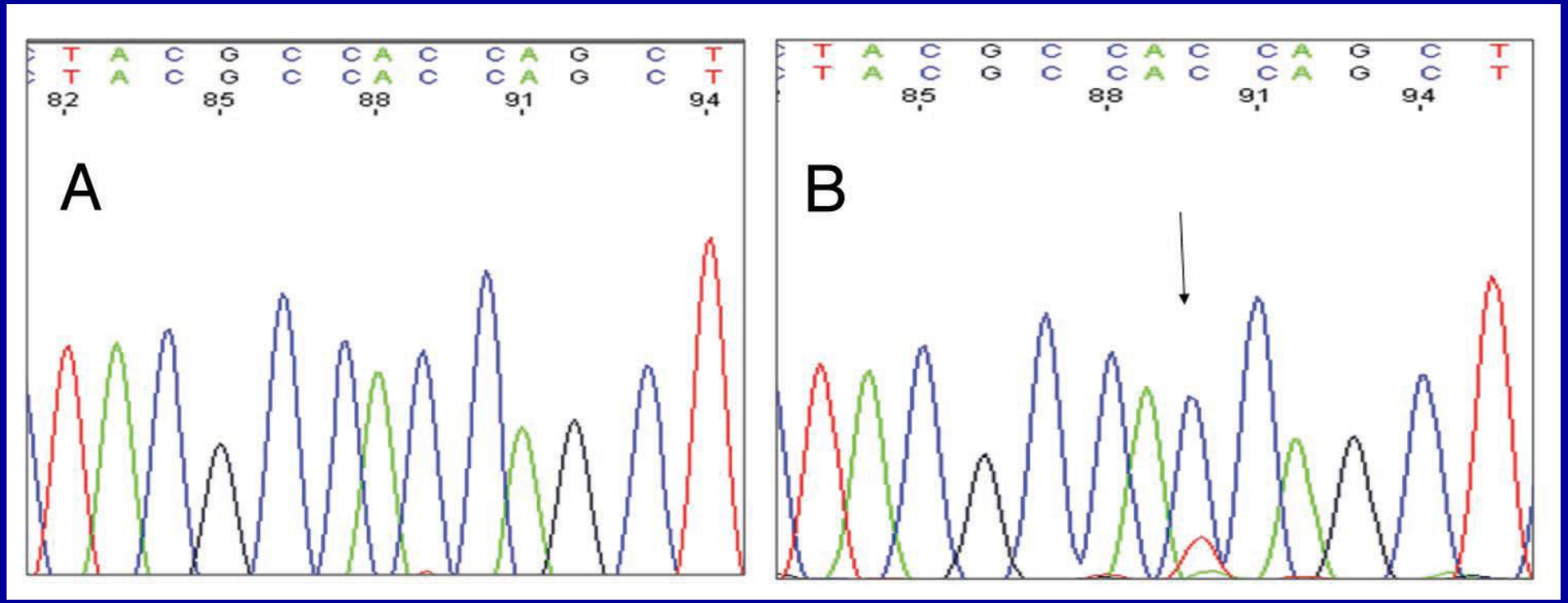


Carcinogenic ability of *S. haematobium* through oncogenic mutation of KRAS gene

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

(Virulence, 2010)

KRAS exon 2 sequencing results

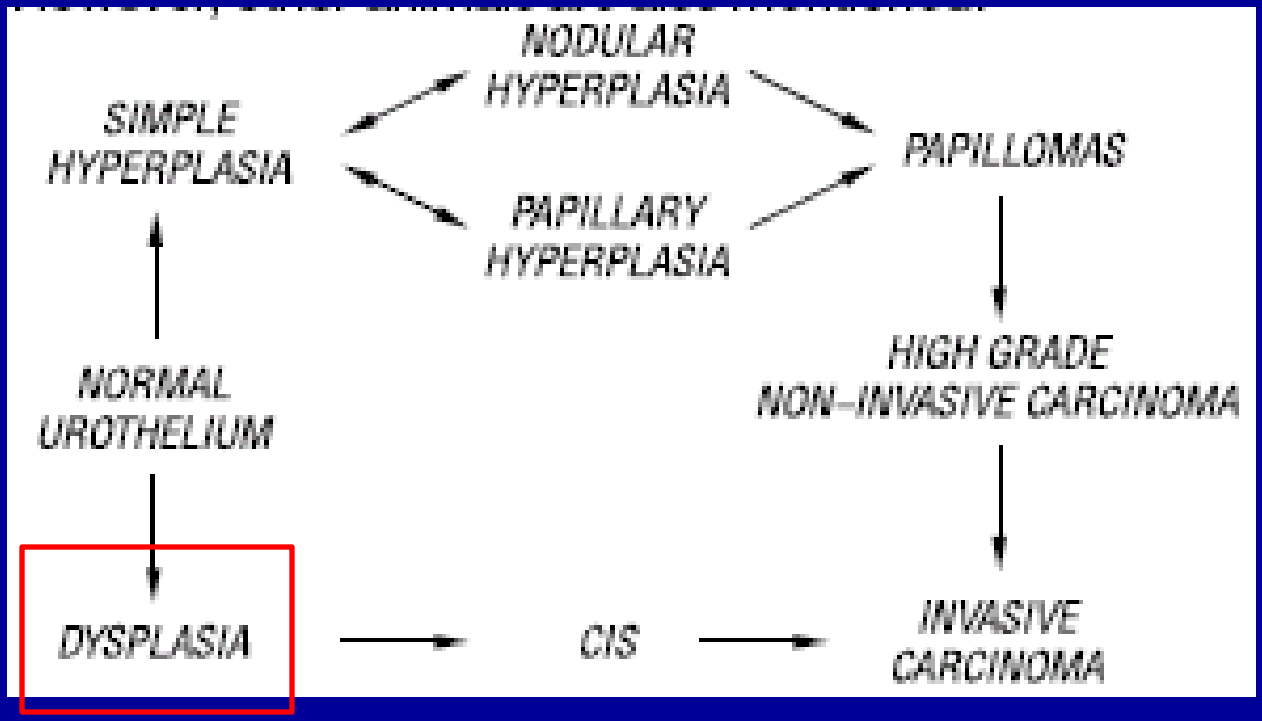


- Is there a role for *S. haematobium* in bladder cancer?

- 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

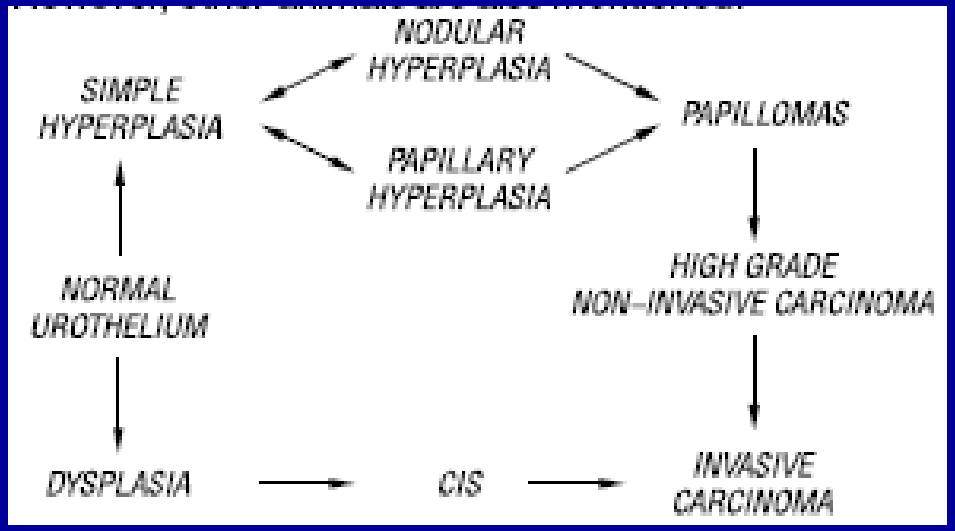
- CIBP-INSA Porto
  - **José Manuel Costa**
  - Paulo Vieira
  - Lurdes Delgado
- IPATIMUP
  - **José Carlos Machado**
  - Maria José Oliveira
  - António Carlos Ferreira
  - **Fatima Gartner**
  - Joana Gomes
- FMUP
  - Raquel Soares
- UTAD
  - Paula Oliveira
- ICBAS
  - Carlos Lopes
- IPO Porto
  -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



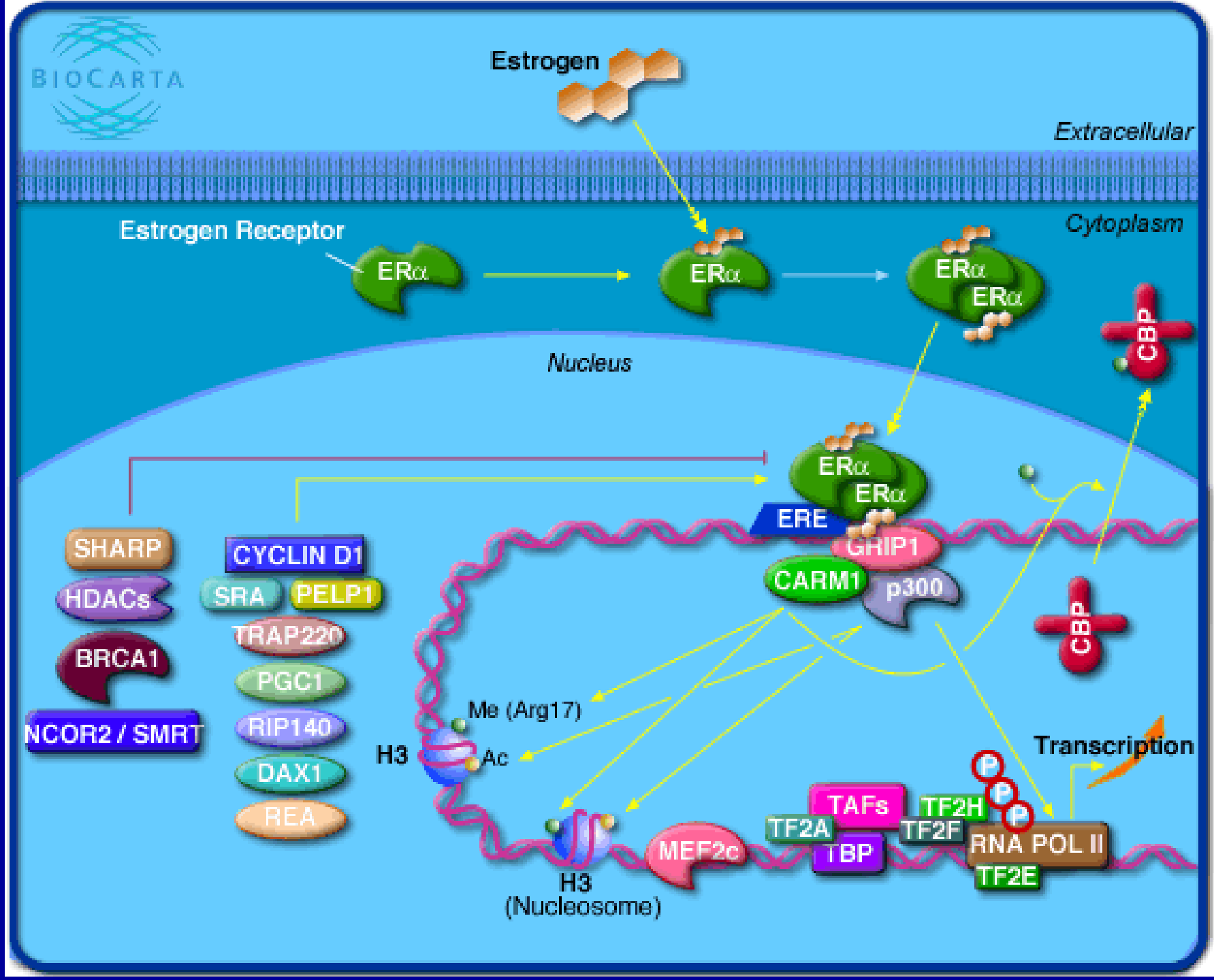
Pathogenic pathways of rat and mice urinary bladder carcinogenesis.

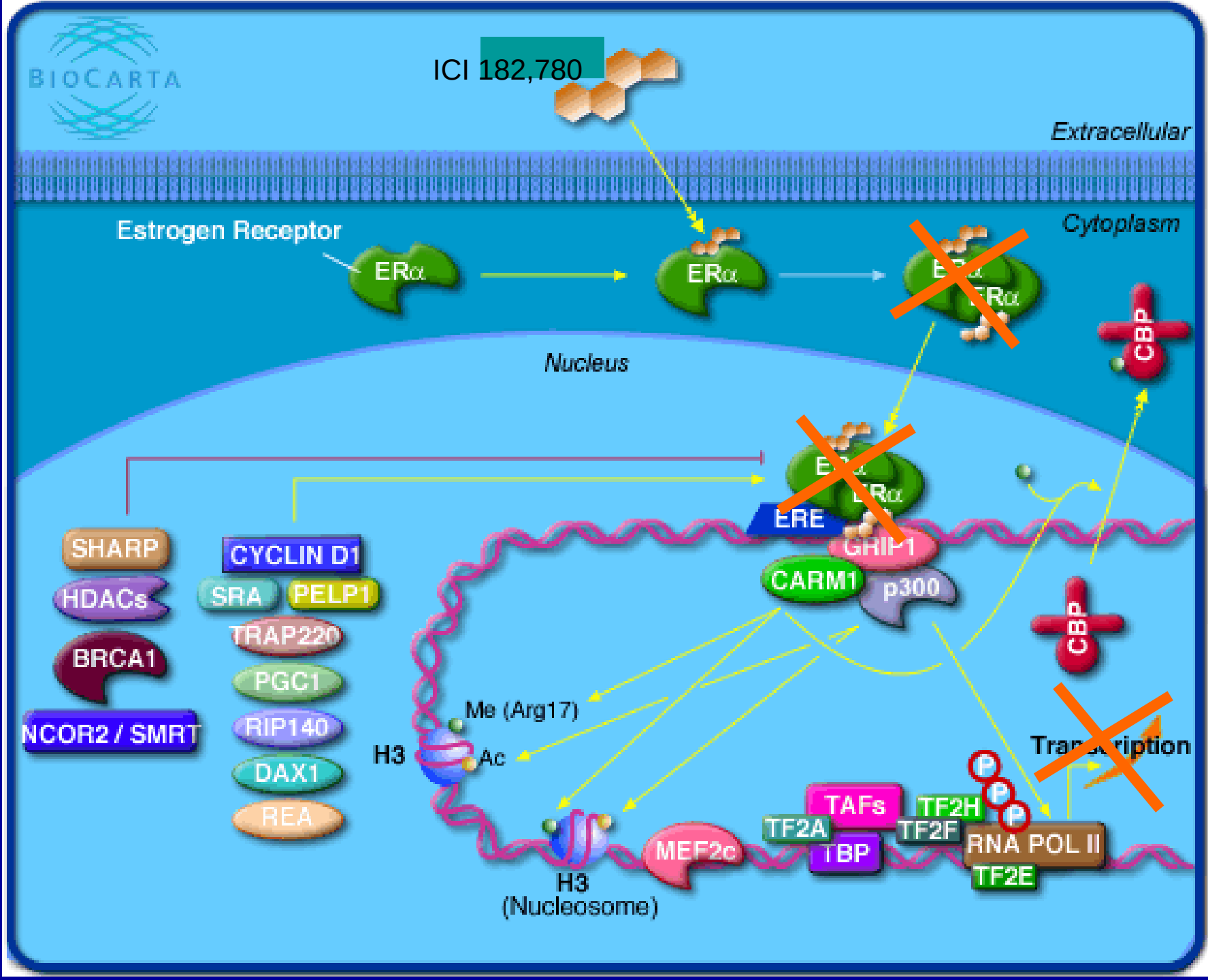
S. haematobium and host hormones

- 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).


BIOCARTA
Estrogen
Extracellular
Estrogen Receptor
ERα
Cytoplasm
Nucleus
CBP
ERE
GHIP1
CARM1
p300
SHARP
HDACs
BRCA1
NCOR2 / SMRT
CYCLIN D1
SRA
PELP1
TRAP220
PGC1
RIP140
DAX1
REA
Me (Arg17)
H3
Ac
H3
(Nucleosome)
MEF2c
TAFs
TF2A
TBP
TF2H
TF2F
RNA POL II
TF2E
Transcription


BIOCARTA
ICI 182,780
Extracellular
Estrogen Receptor
ERα
ERα
Cytoplasm
CBP
Nucleus
ERE
GHIP1
CARM1
p300
CBP
SHARP
HDACs
BRCA1
NCOR2 / SMRT
CYCLIN D1
SRA
PELP1
TRAP220
PGC1
RIP140
DAX1
REA
Me (Arg17)
H3
Ac
H3
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RNA POL II
TF2E
P
P
P
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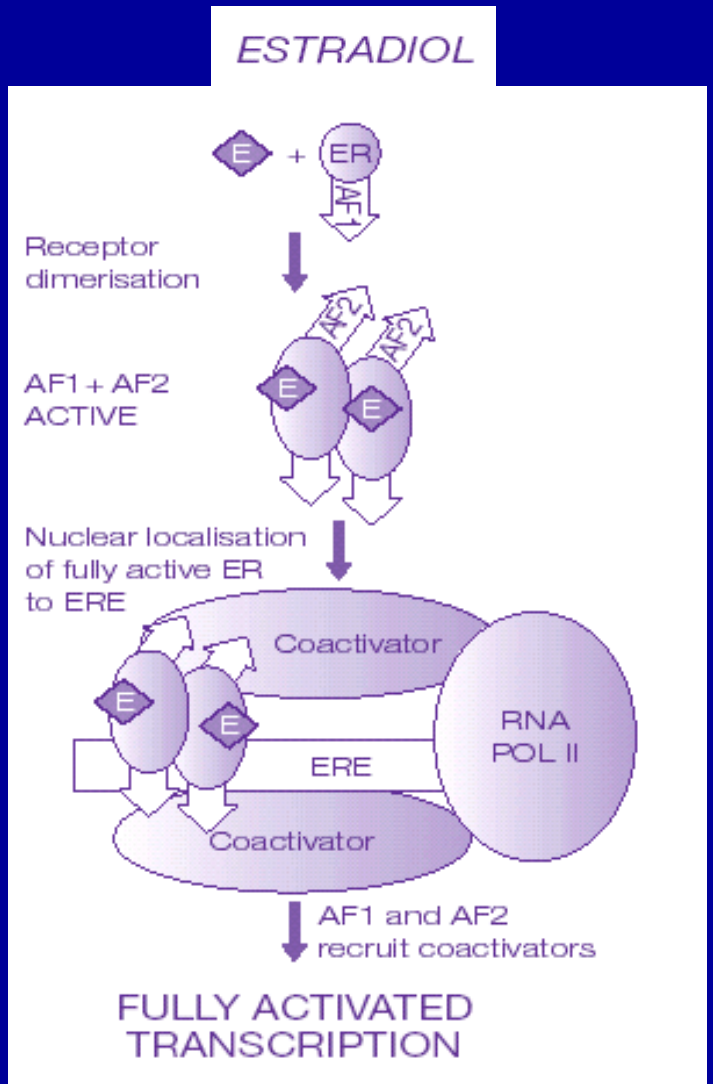
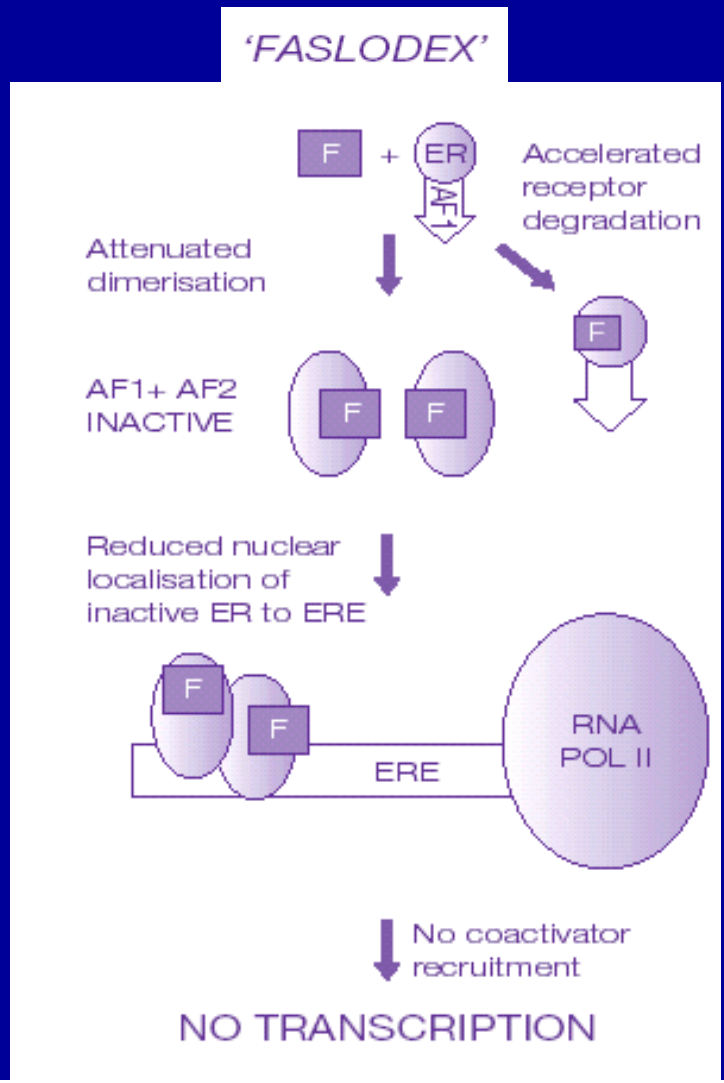




Key:

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

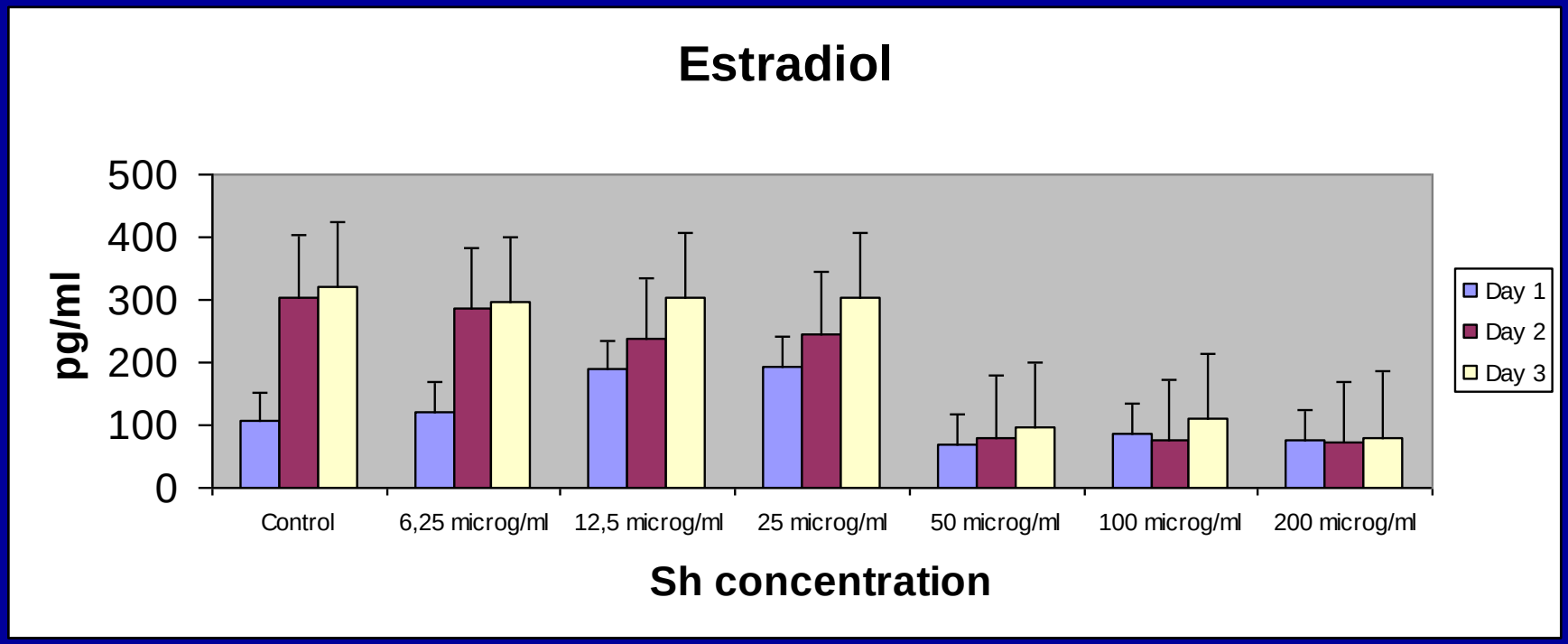
S. haematobium and *S. mansoni* express an estradiol analogue but not *Fasciola hepatica*

Antigenic preparations	E2 (pg/ml) SD
S. haematobium	14,84 0,14
S. mansoni	12,63 0,27
F. haepatica	<10
H_2Od	<10
10 nM E2	1632,99 2,55

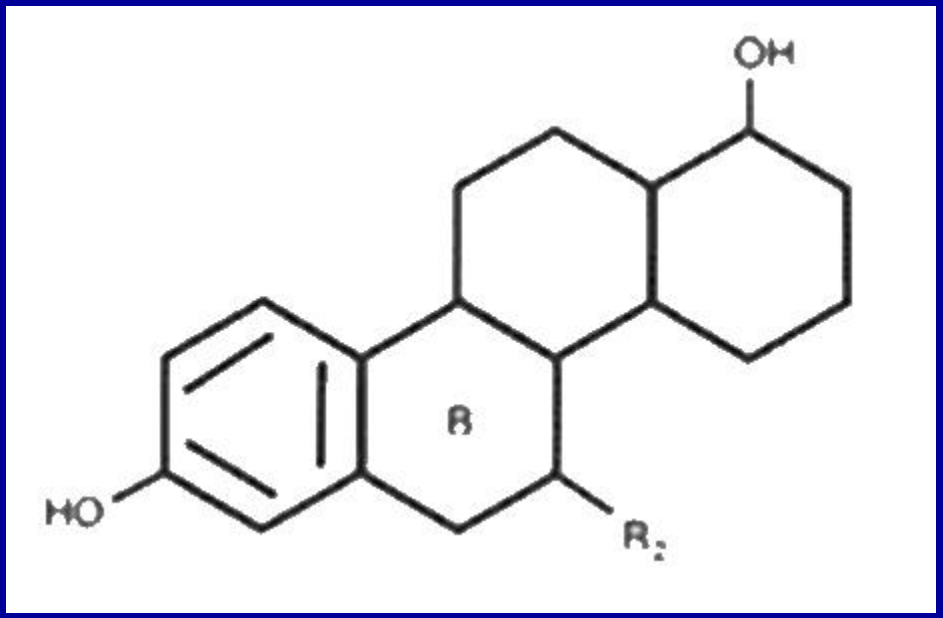
Hormones

Botelho M et al. Journal of Parasitology (In

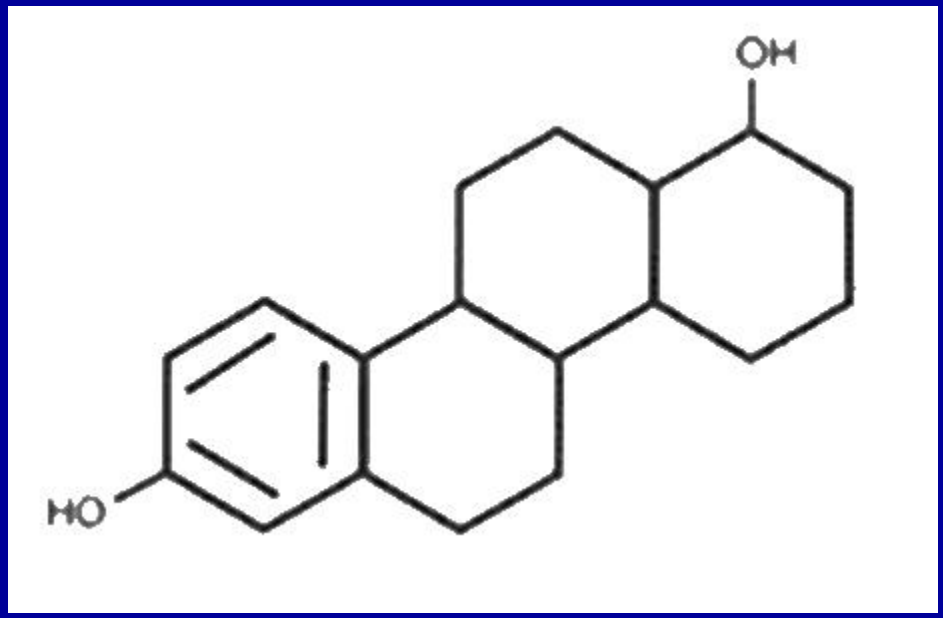
S. haematobium total antigen decreases estradiol production of CHO cells *in vitro*



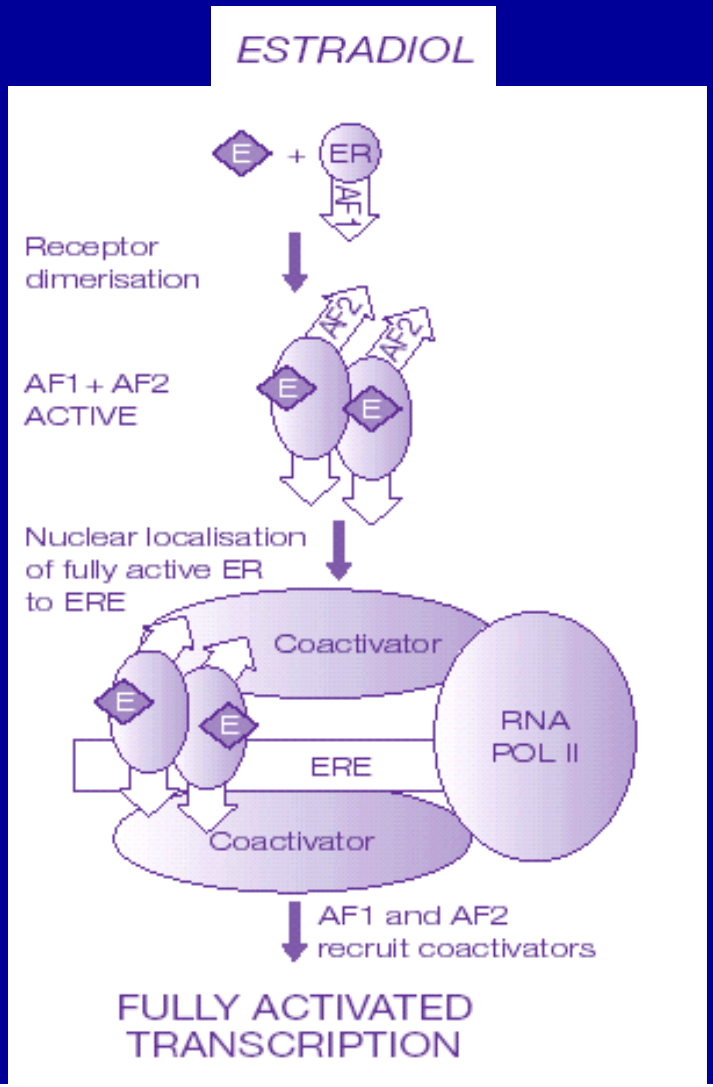
Estradiol production in culture. The experiments were done in triplicate ($p<0,01$; control vs. 50 microg/ml).

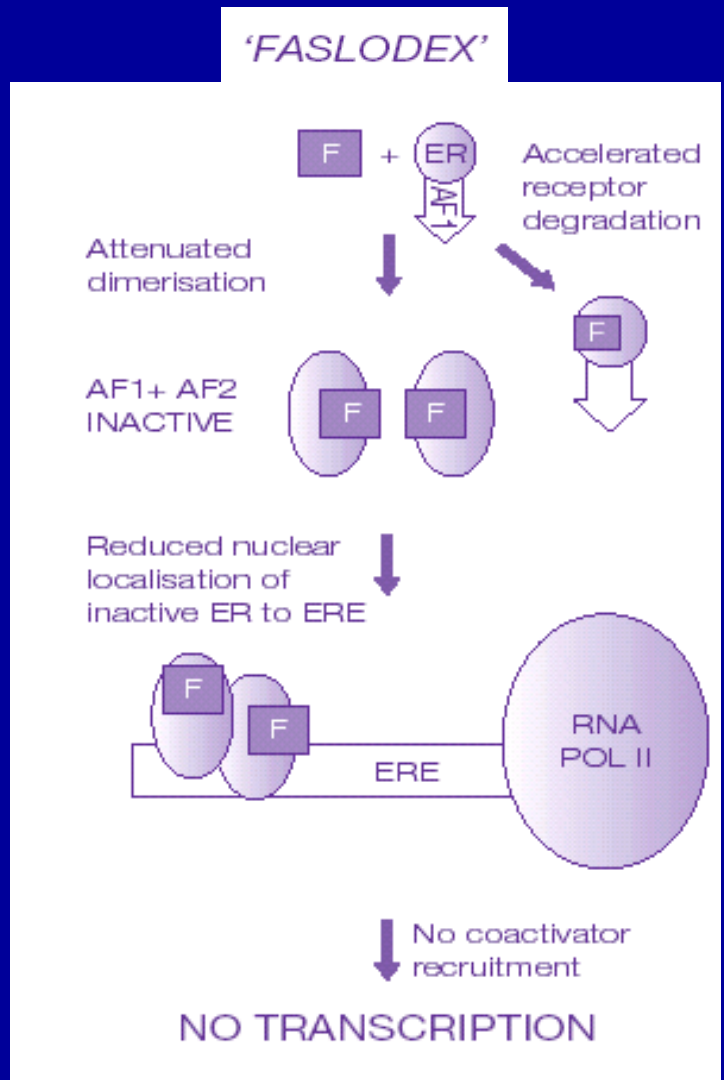


ICI 182,780



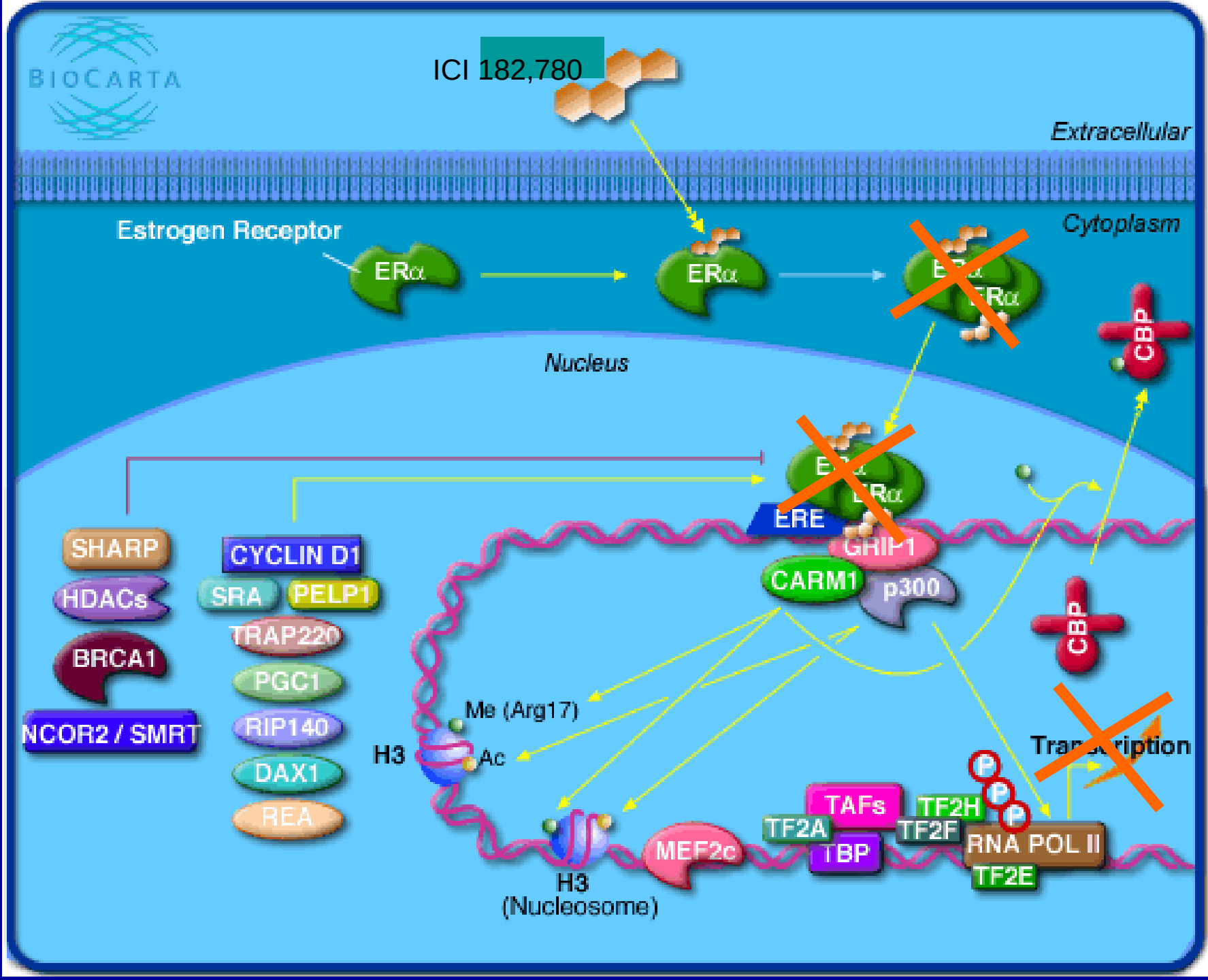
Estradiol (E2)





Key:

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


BIOCARTA
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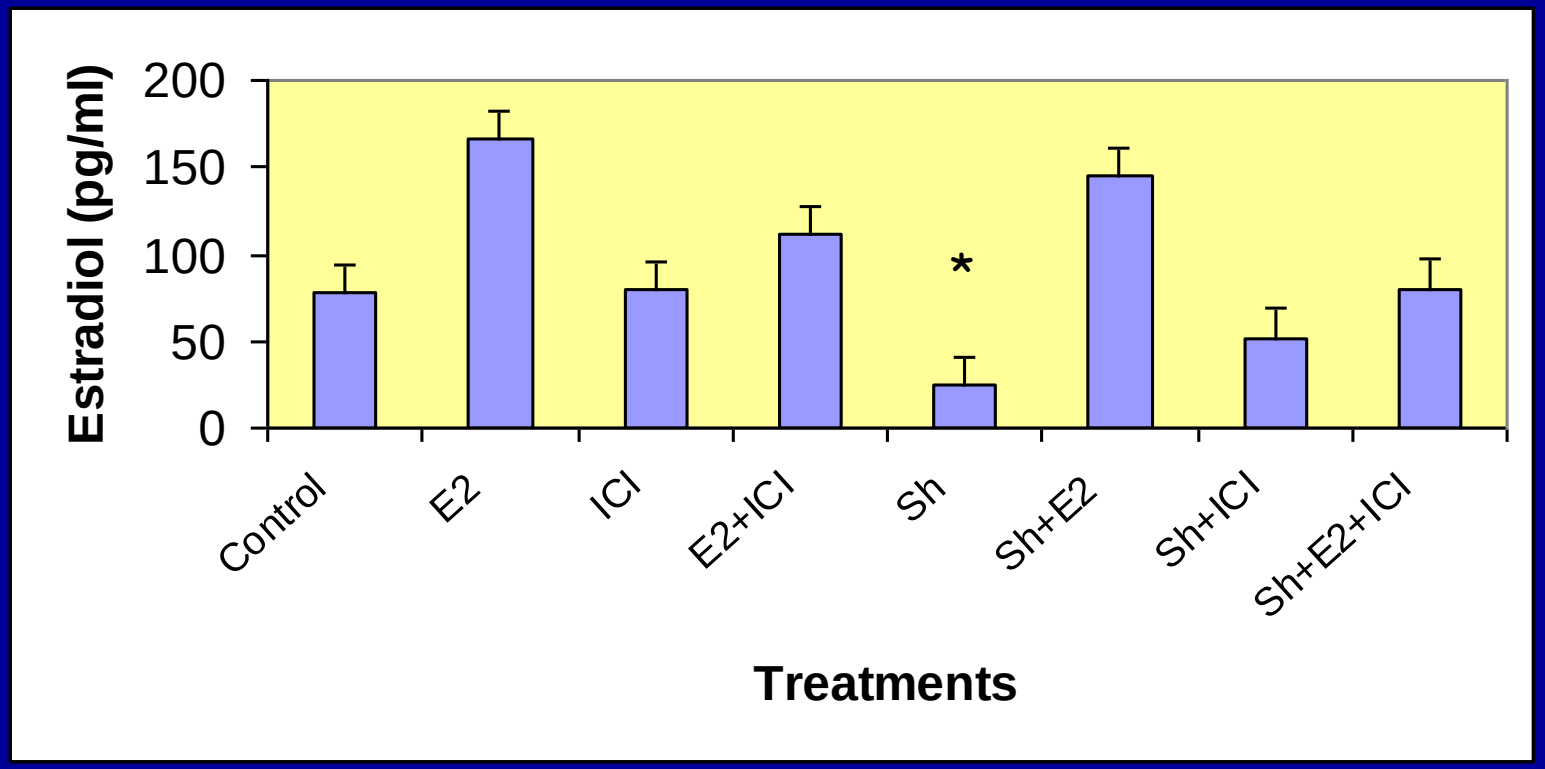
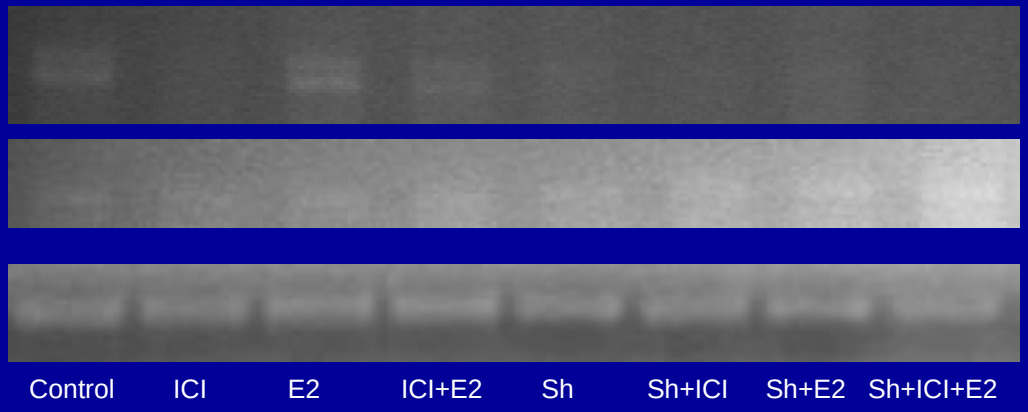
SRA
PELP1
TRAP220
PGC1
RIP140
DAX1
REA
Me (Arg17)
H3
Ac
H3
(Nucleosome)
MEF2c
TAFs
TF2H
TF2A
TF2F
TBP
RNA POL II
TF2E
P
P
P
Transcription

S. haematobium total antigen is an antagonist of estradiol

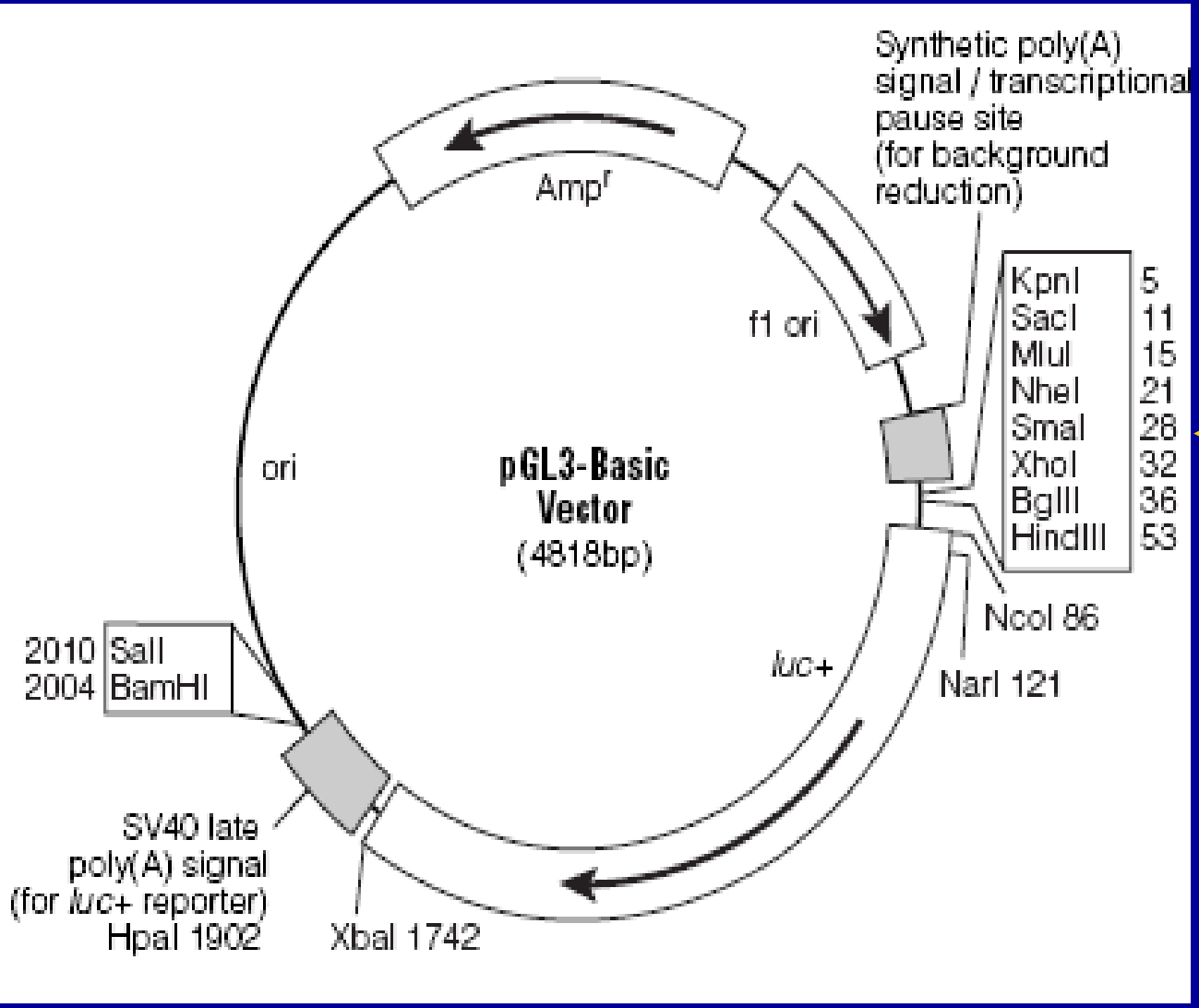


Estradiol production in supernatant of treated cells. Data represent means SEM from 3 independent experiments (* p<0,05 control vs. Sh).

S. haematobium total antigen down-regulates ER beta



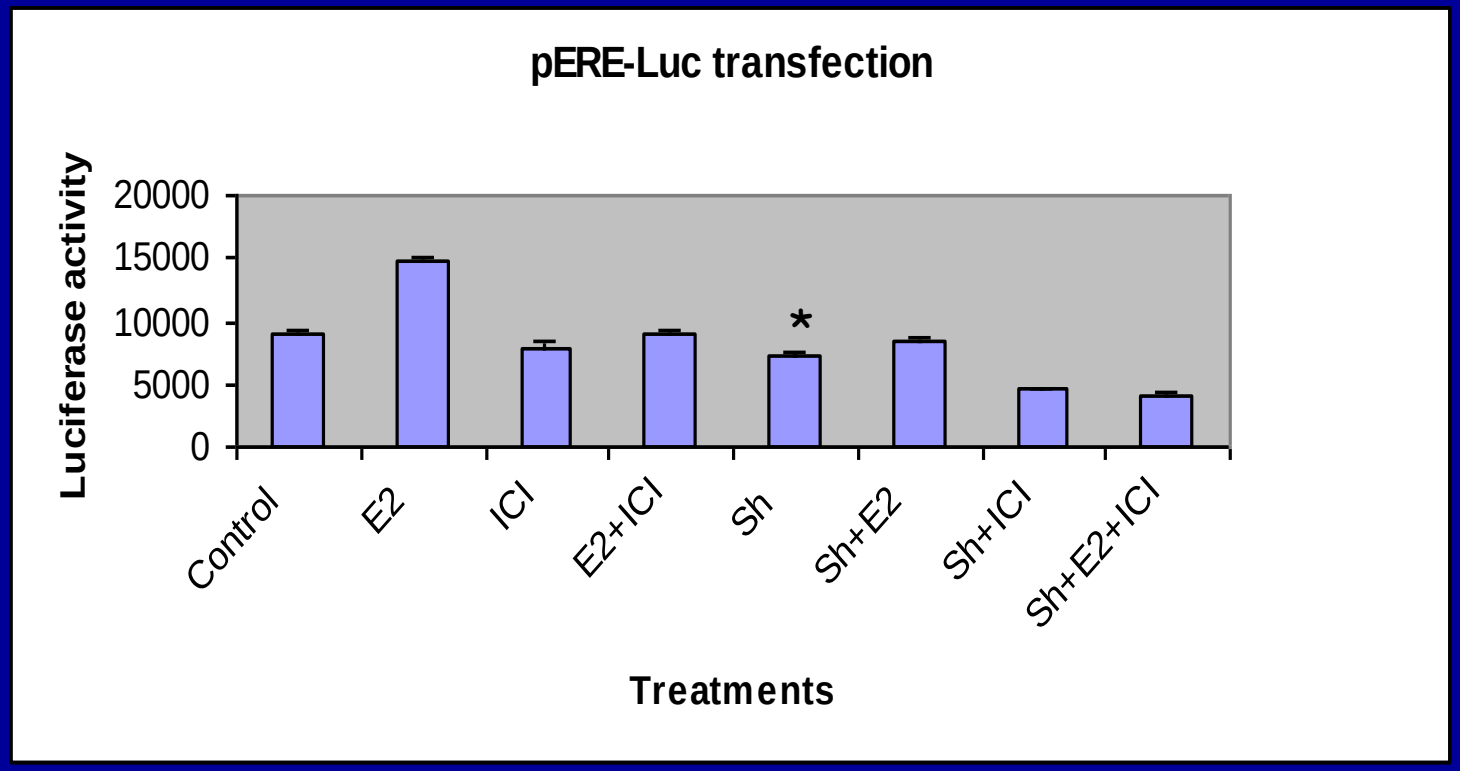
RT-PCR for ER alpha and ER beta in CHO cells.



Estrogen Responsive Element (ERE)

Hormones

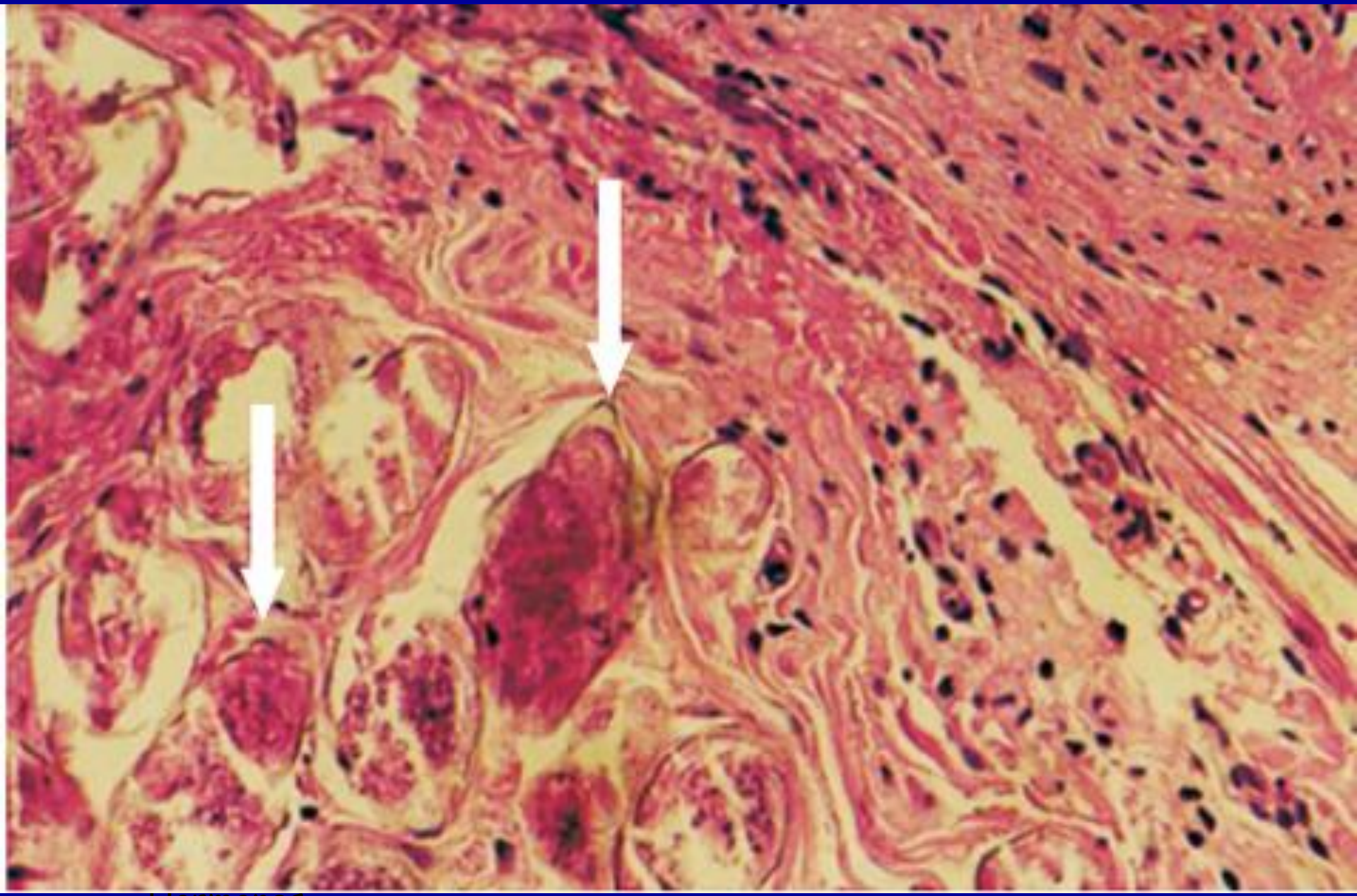
Inactivation of Estrogen Receptor signaling pathway by *S. haematobium* total antigen



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

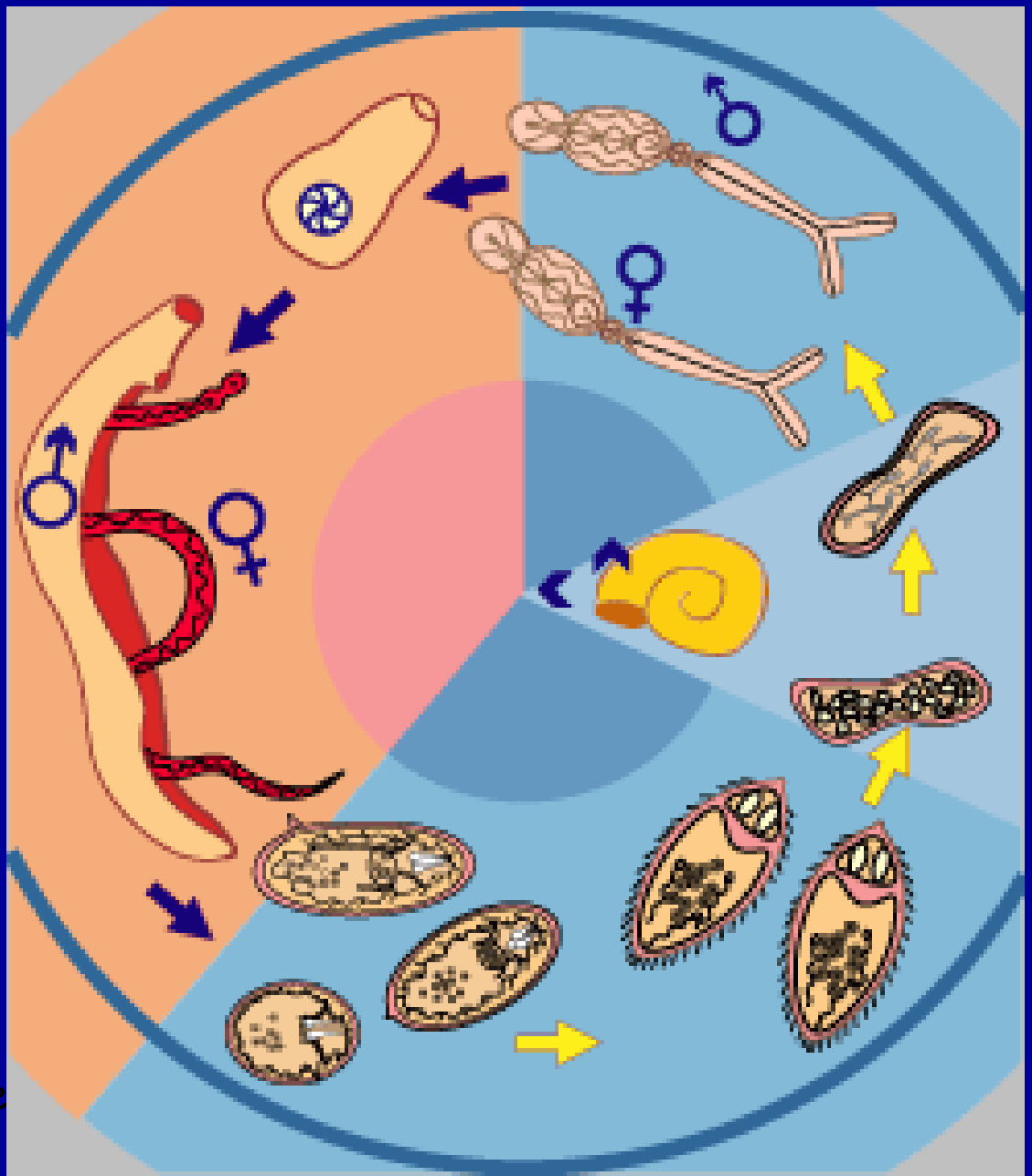
Hormones

Schistosomula

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

Egg production (10-30 per day)

Hormone



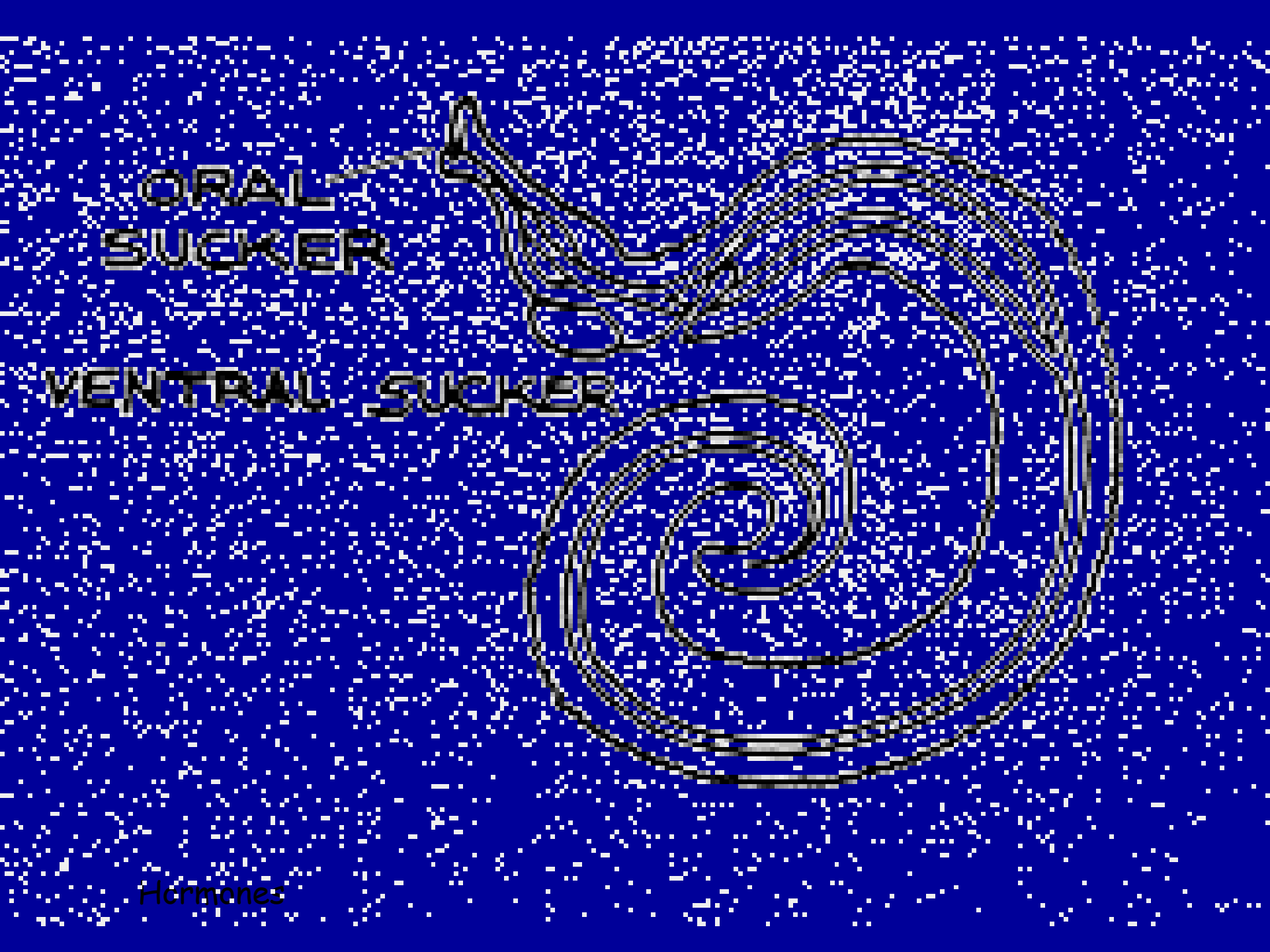
Cercaria (500 per year per snail)

Sporocysts (several per miracidium)

Miracidium in water

Hormones


Photo
John Walker

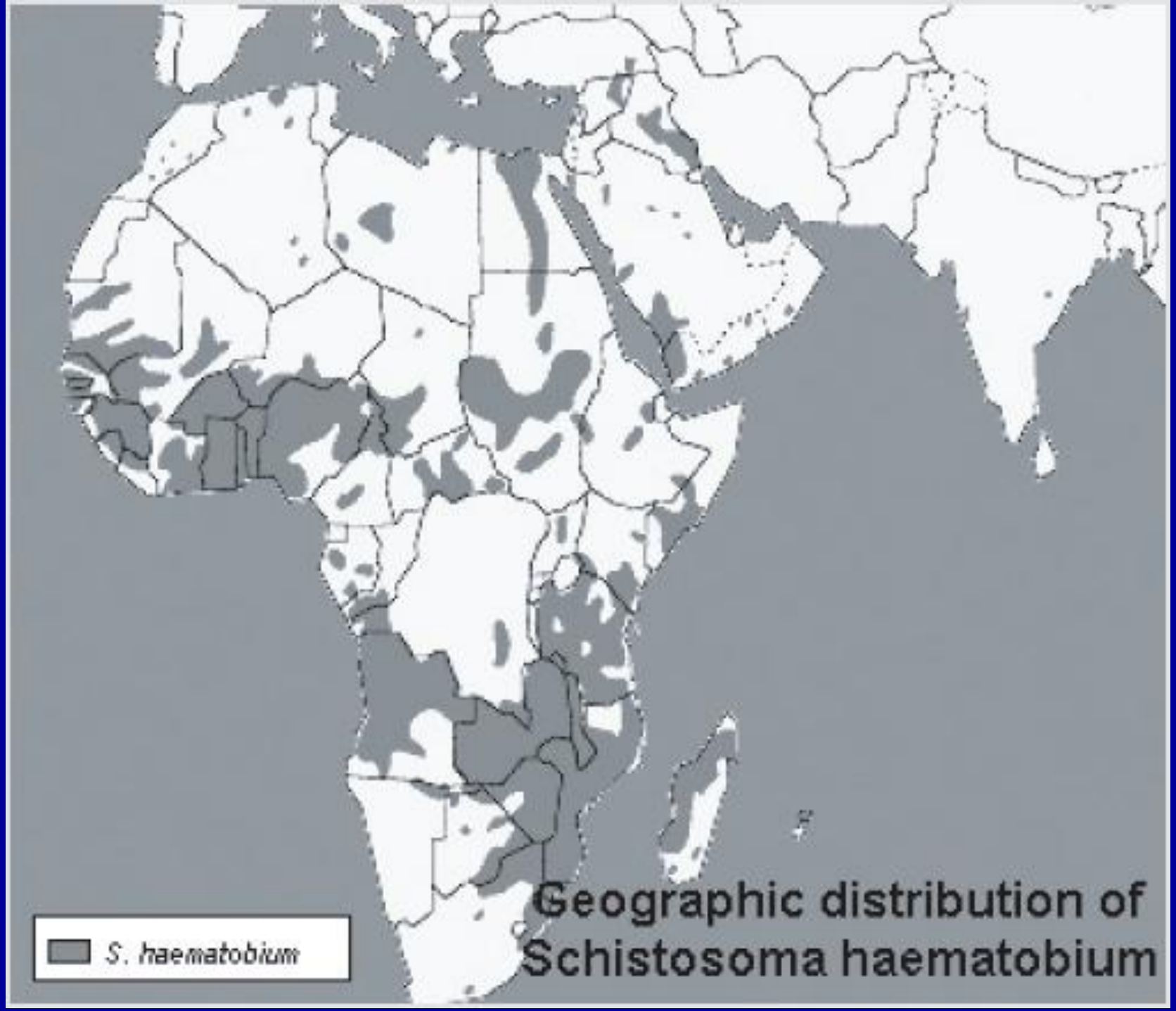


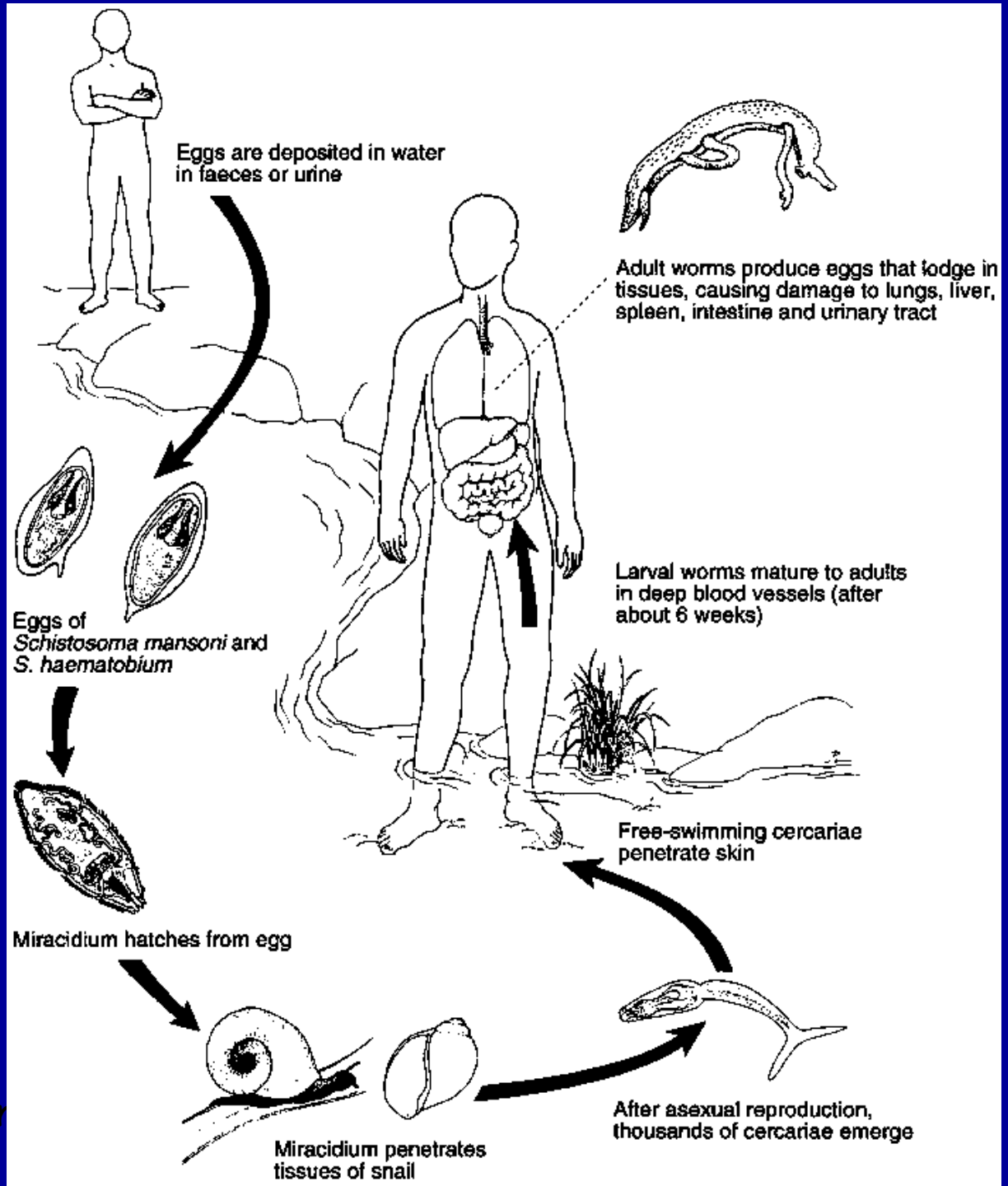
Hormones


oral sucker


Photo
John Walker

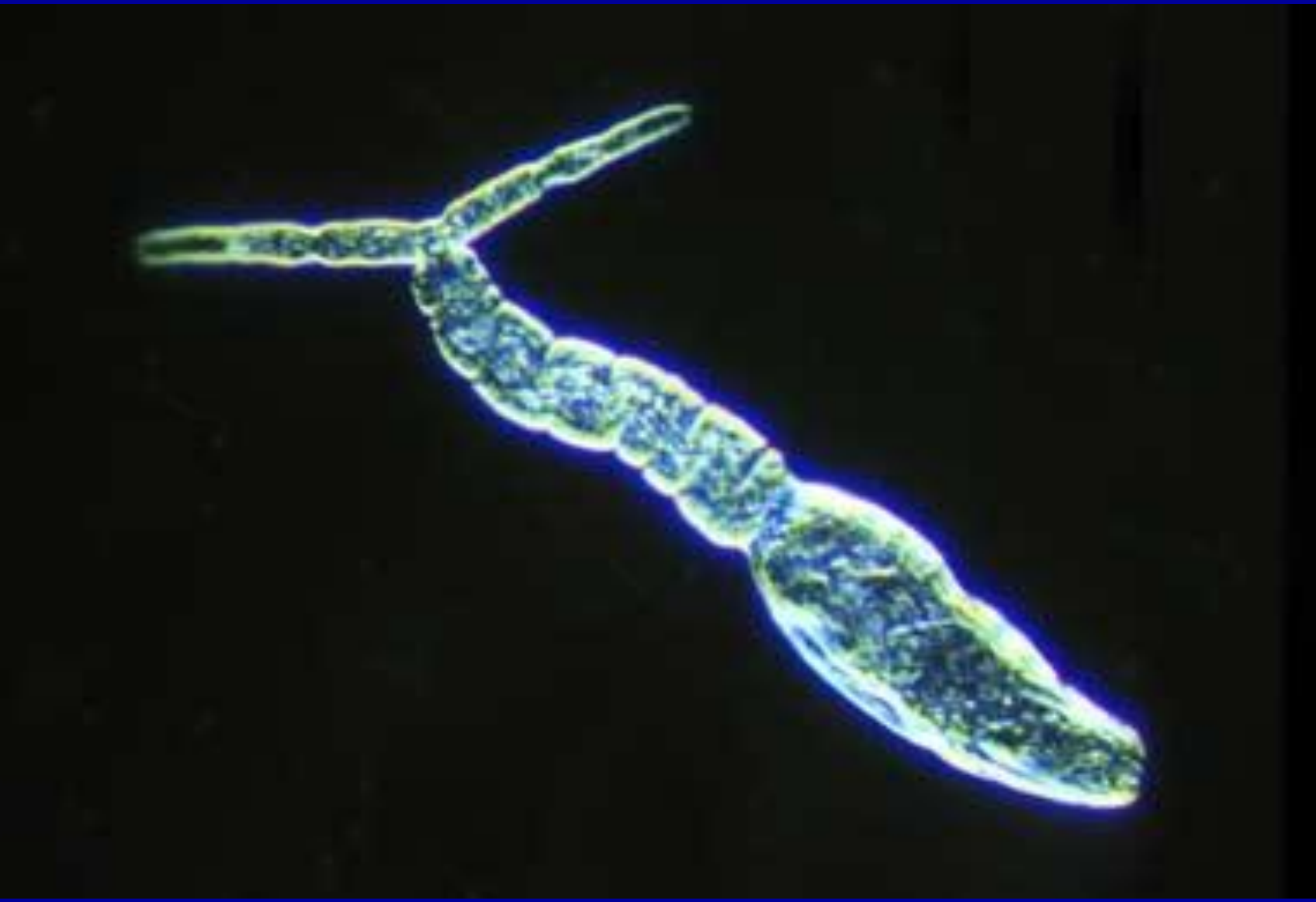
Hormones

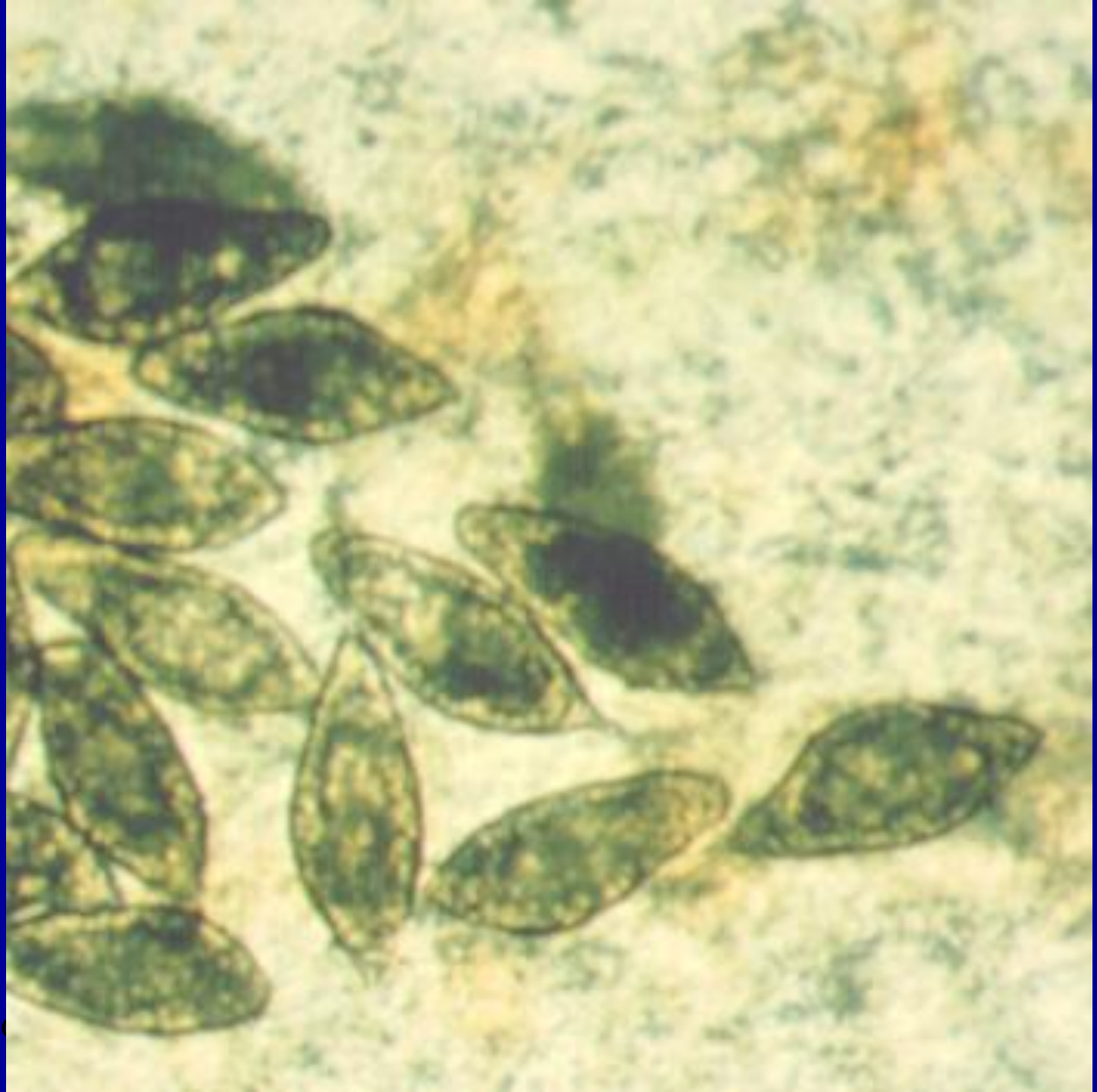

S. haematobium
Geographic distribution of
Schistosoma haematobium

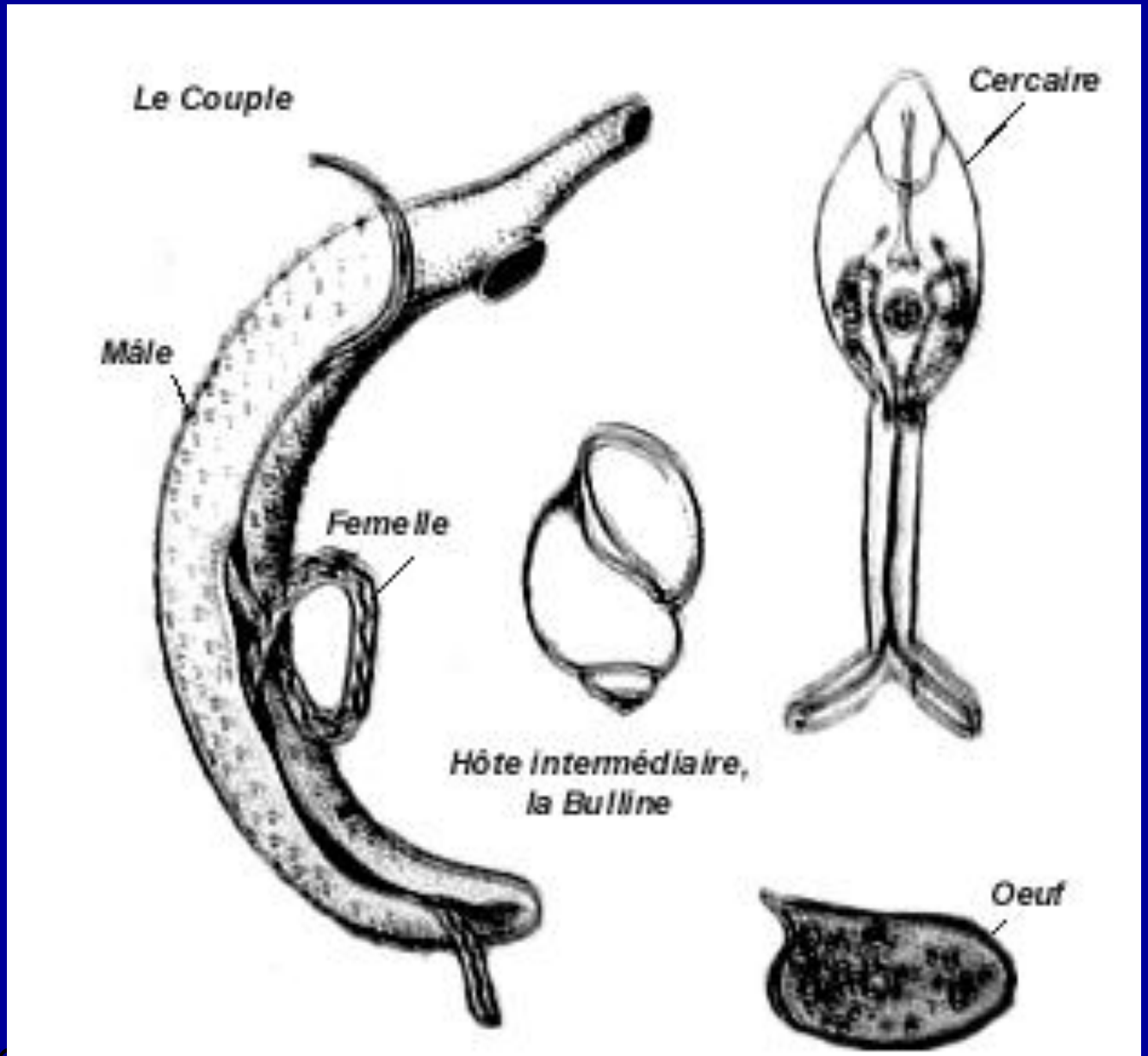

Eggs are deposited in water in faeces or urine
Adult worms produce eggs that lodge in tissues, causing damage to lungs, liver, spleen, intestine and urinary tract
Eggs of *Schistosoma mansoni* and *S. haematobium*
Larval worms mature to adults in deep blood vessels (after about 6 weeks)
Free-swimming cercariae penetrate skin
Miracidium hatches from egg
After asexual reproduction, thousands of cercariae emerge
Miracidium penetrates tissues of snail

Hormor

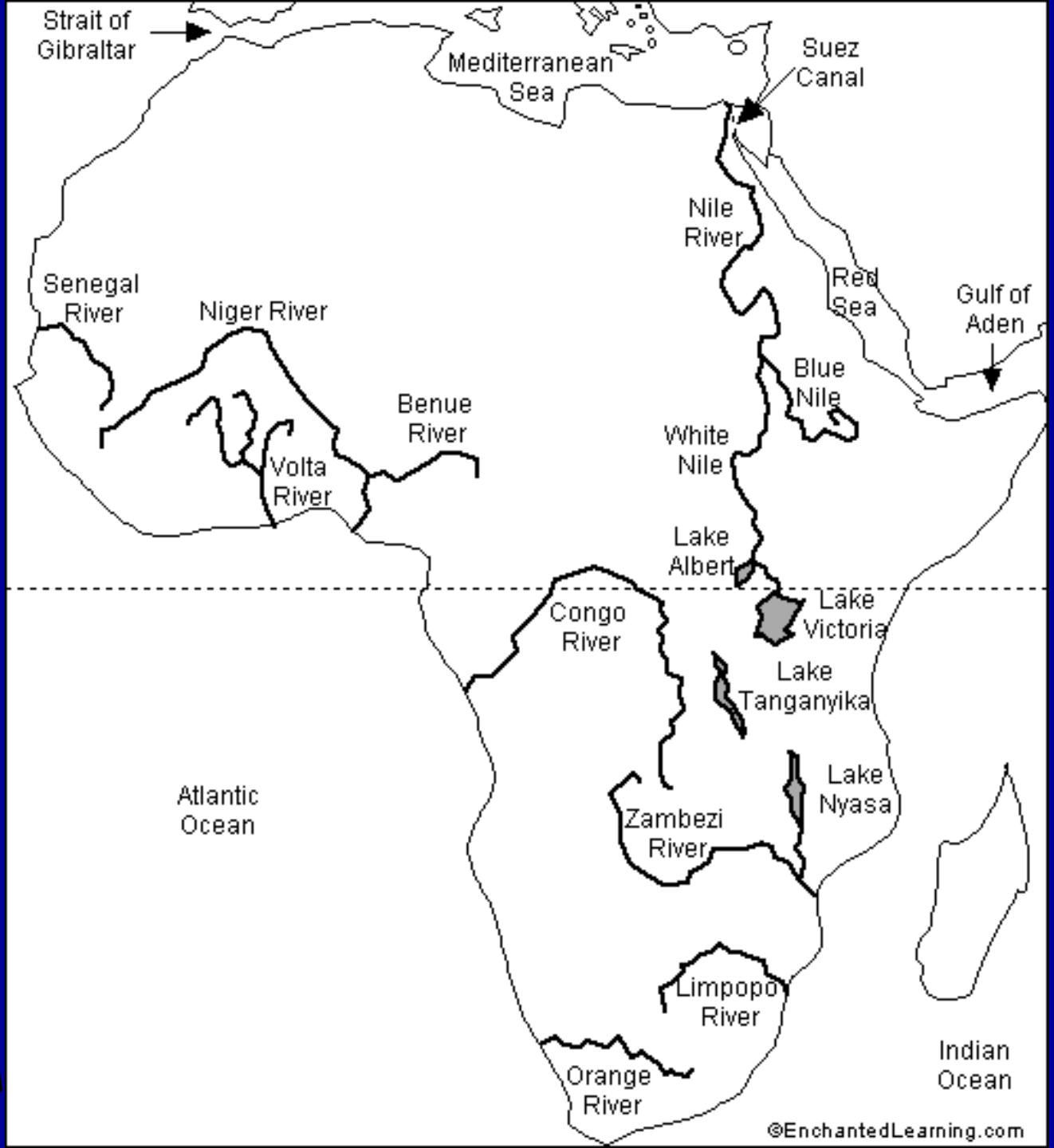
Hormones






Le Couple
Cercaire
Mâle
Femelle
Hôte intermédiaire,
la Bulline
Oeuf
Schistosoma Haematobium

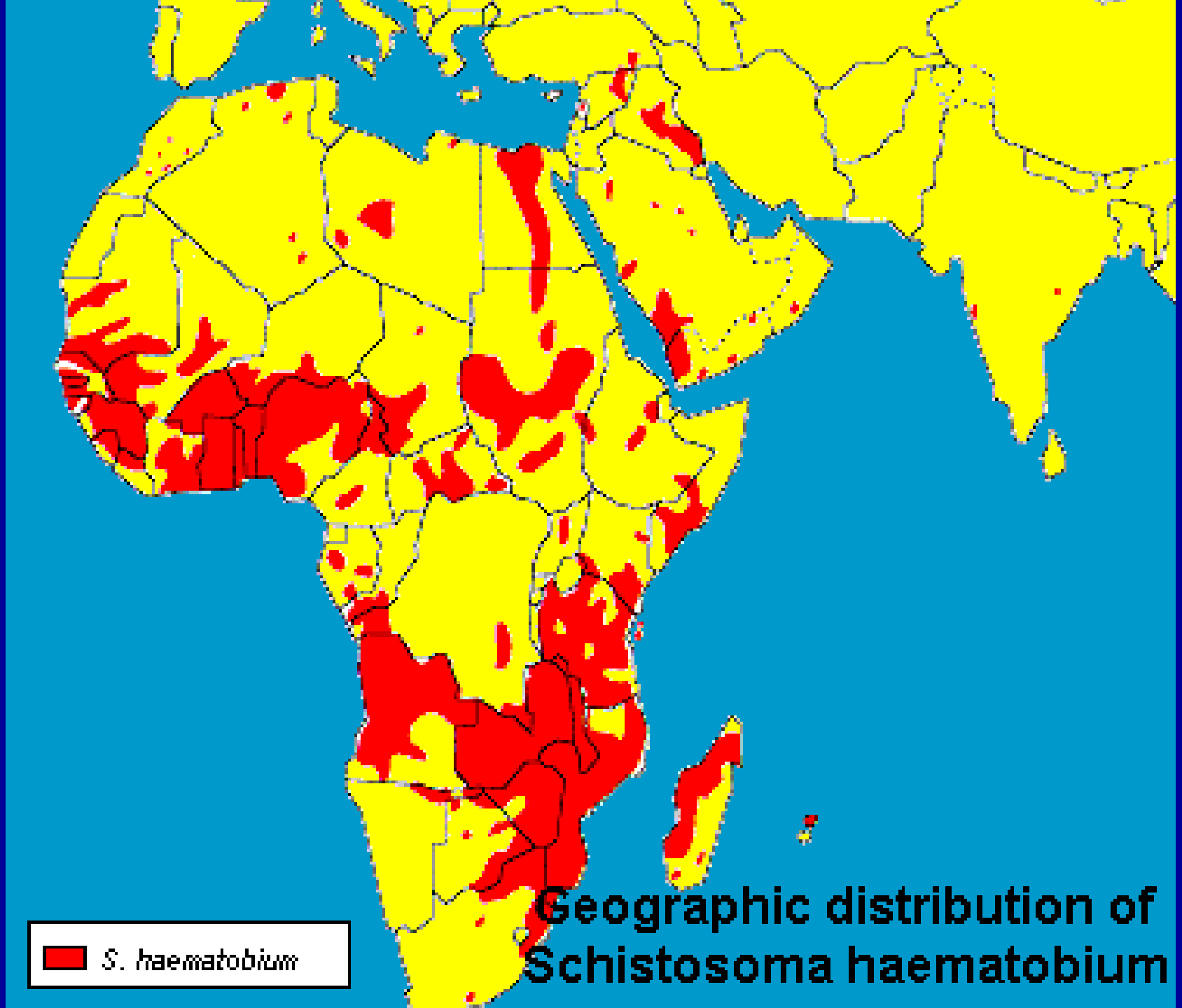
Horr


Strait of Gibraltar
Mediterranean Sea
Suez Canal
Nile River
Red Sea
Gulf of Aden
Senegal River
Niger River
Blue Nile
Benue River
White Nile
Volta River
Lake Albert
Lake Victoria
Congo River
Lake Tanganyika
Lake Nyasa
Atlantic Ocean
Zambezi River
Limpopo River
Indian Ocean
Orange River
©EnchantedLearning.com

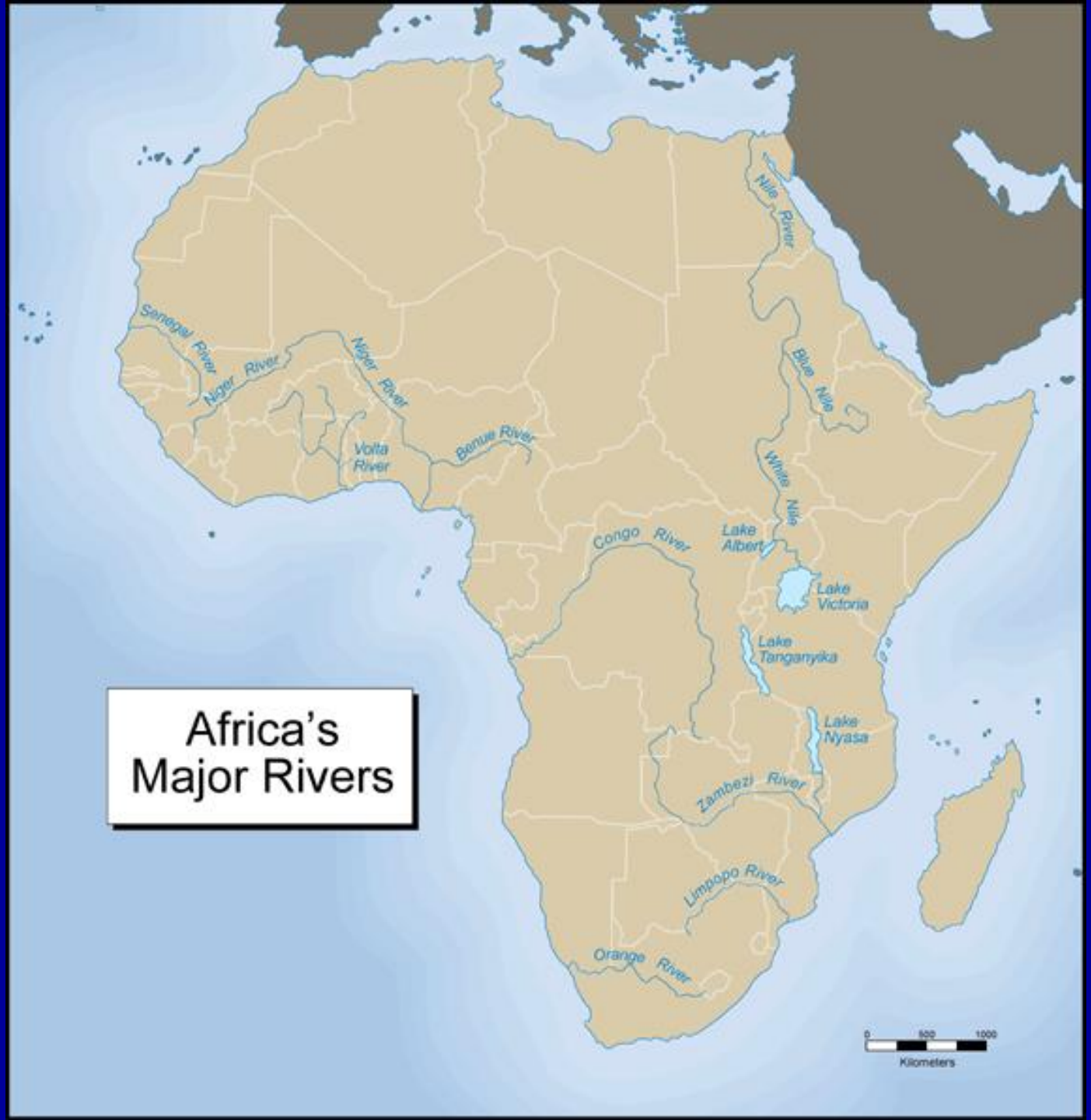
Horm


EUROPE
Tunisia
Mediterranean Sea
ASIA
Morocco
Atlantic Ocean
ATLAS MTS.
Persian Gulf
Algeria
Libya
Libyan Desert
Nile River
Western Sahara
SAHARA
Egypt
Ahaggar Mts.
Tibesti Mts.
Mauritania
Niger
Nubian Desert
Red Sea
Djibouti
Arabian Sea
Cape Verde
Senegal
Mali
SAHEL
Eritrea
Sudan
Gambia
Burkina Faso
Benin
Chad
Horn of Africa
Guinea-Bissau
Guinea
Nile River
Ethiopian Highlands
Togo
Nigeria
Indian Ocean
Sierra Leona
Cote D'Ivoire
Central African Rep.
Uganda
GREAT RIFT VALLEY
Liberia
Ghana
Gulf of Guinea
Cameroon
Somalia
Congo
Congo River Basin
Eq. Guinea
Ethiopia
Sao Tome & Principe
Dem. Rep. of the Congo
Kenya
800 mi
Rwanda
Lake Victoria
Gabon
Burundi
Tanzania
800 km
Seychelles
Cabinda (Angola)
AFRICA
Angola
Comoros
Zambia
Malawi
Namibia
Zimbabwe
Mauritius
Kalahari Desert
Namib Desert
Mozambique Channel
N
Atlantic Ocean
Madagascar
Botswana
South Africa
Mozambique
Swaziland
Indian Ocean
Lesotho
Cape of Good Hope
©GraphicMaps.com

Ho



Geographic distribution of
Schistosoma haematobium


Africa's
Major Rivers
Nile River
Blue Nile
White Nile
Lake Albert
Lake Victoria
Lake Tanganyika
Lake Nyasa
Congo River
Senegal River
Niger River
Niger River
Volta River
Benue River
Zambezi River
Limpopo River
Orange River
0
500
1000
Kilometers

"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)**

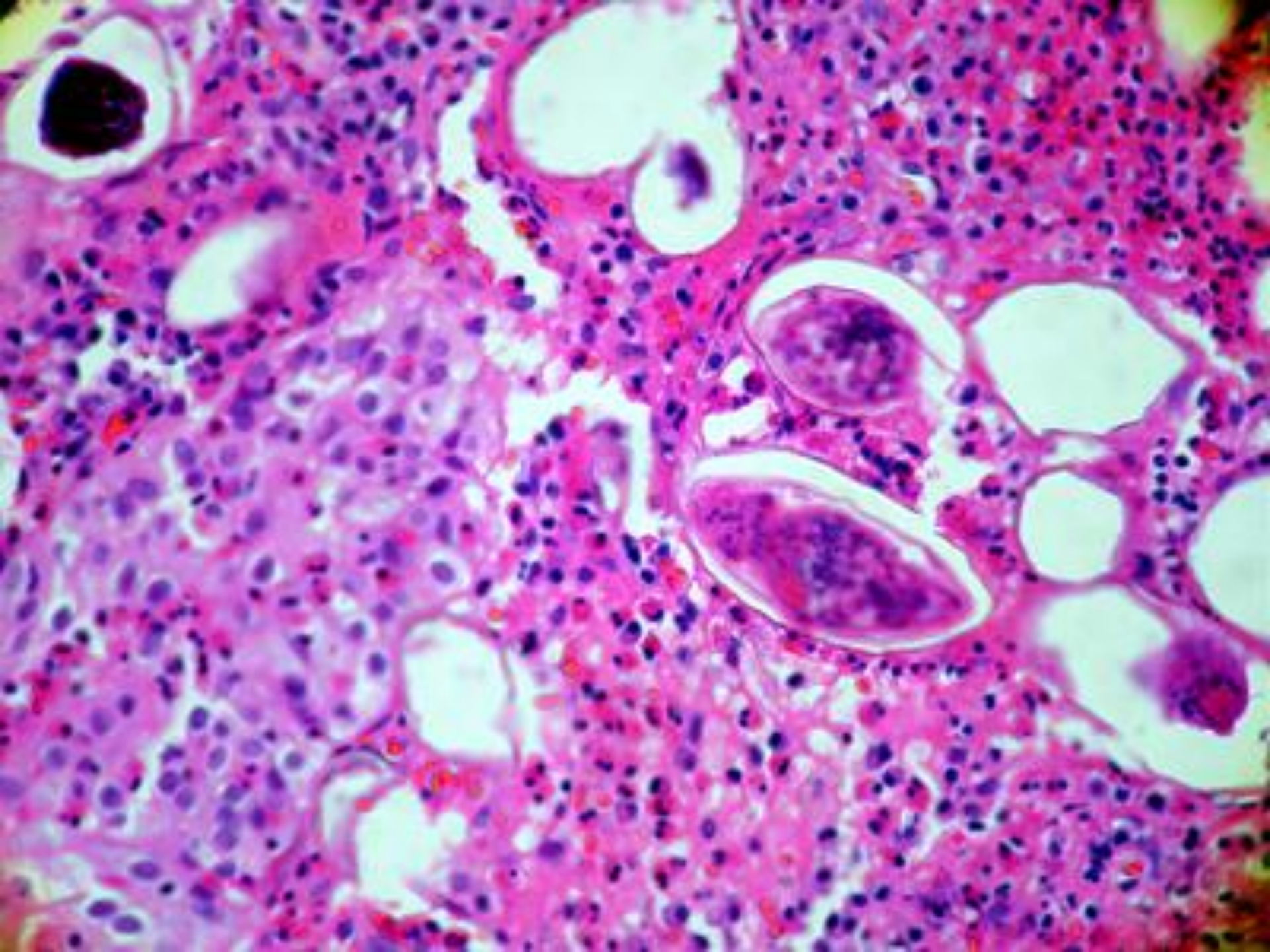
- *Giardia lamblia* (the most common intestinal protozoan in the United States)
- *Naegleria fowleri* (facultative parasite causing amoebic meningitis)
- *Entamoeba histolytica* (causes Amebiasis, common in developing countries)
- *Trypanosoma* (sleeping sickness or Chagas disease)
- *Plasmodium* (malaria)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

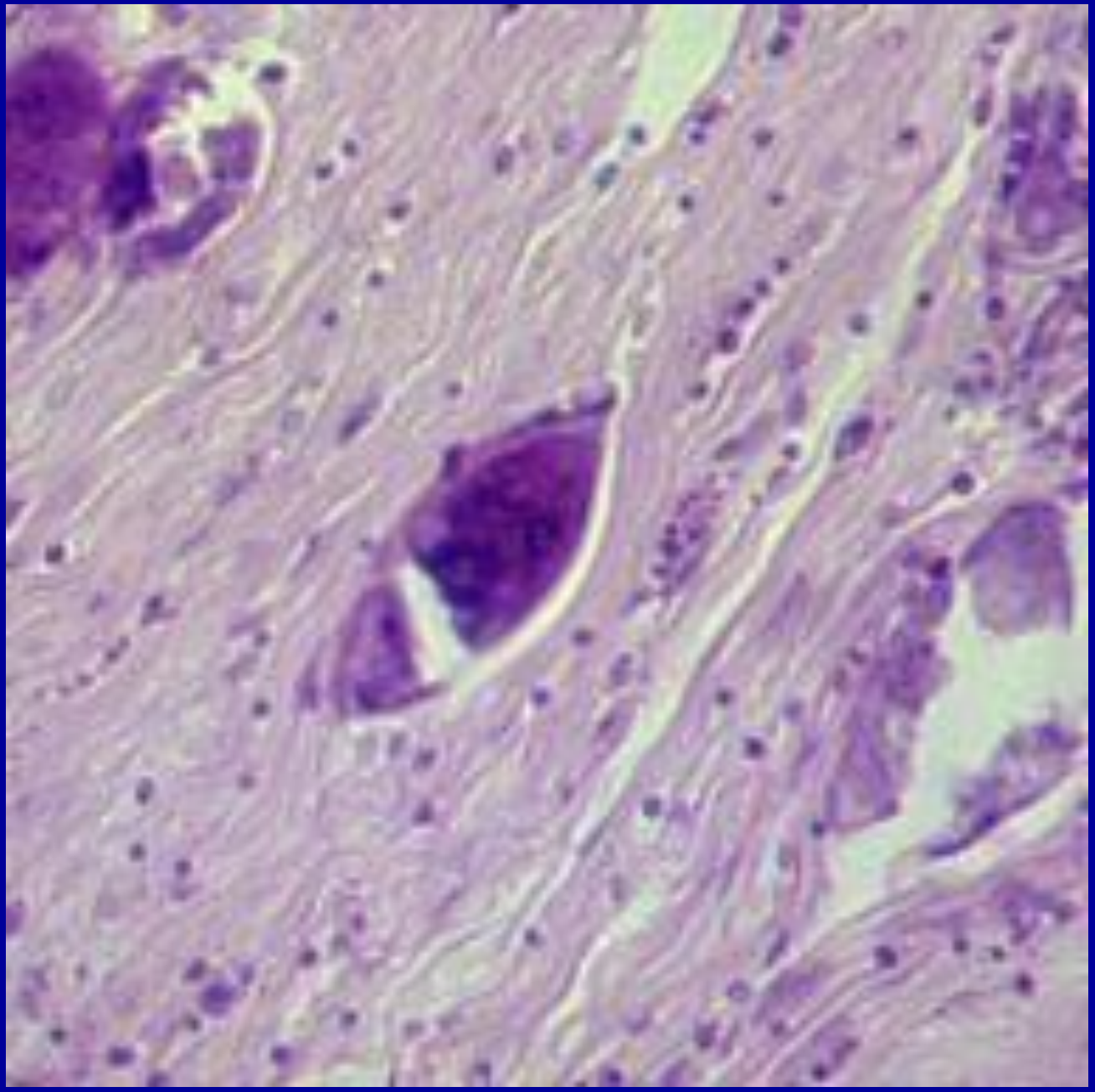
- **Helminths**

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

Hormones

- *Schistosoma haematobium*





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

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