

# Mechanism of action of thyroid hormones in bovine pre-implantation embryo *in vitro*

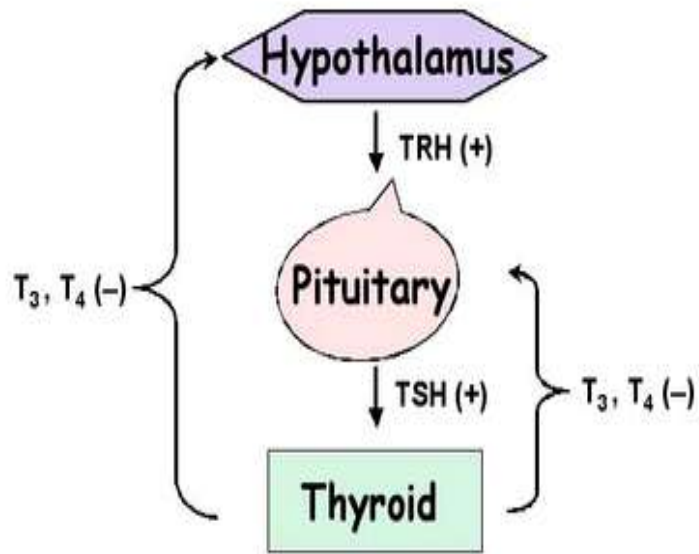
**EmbryoGene AGM**

Date: June 7-9<sup>th</sup> 2011

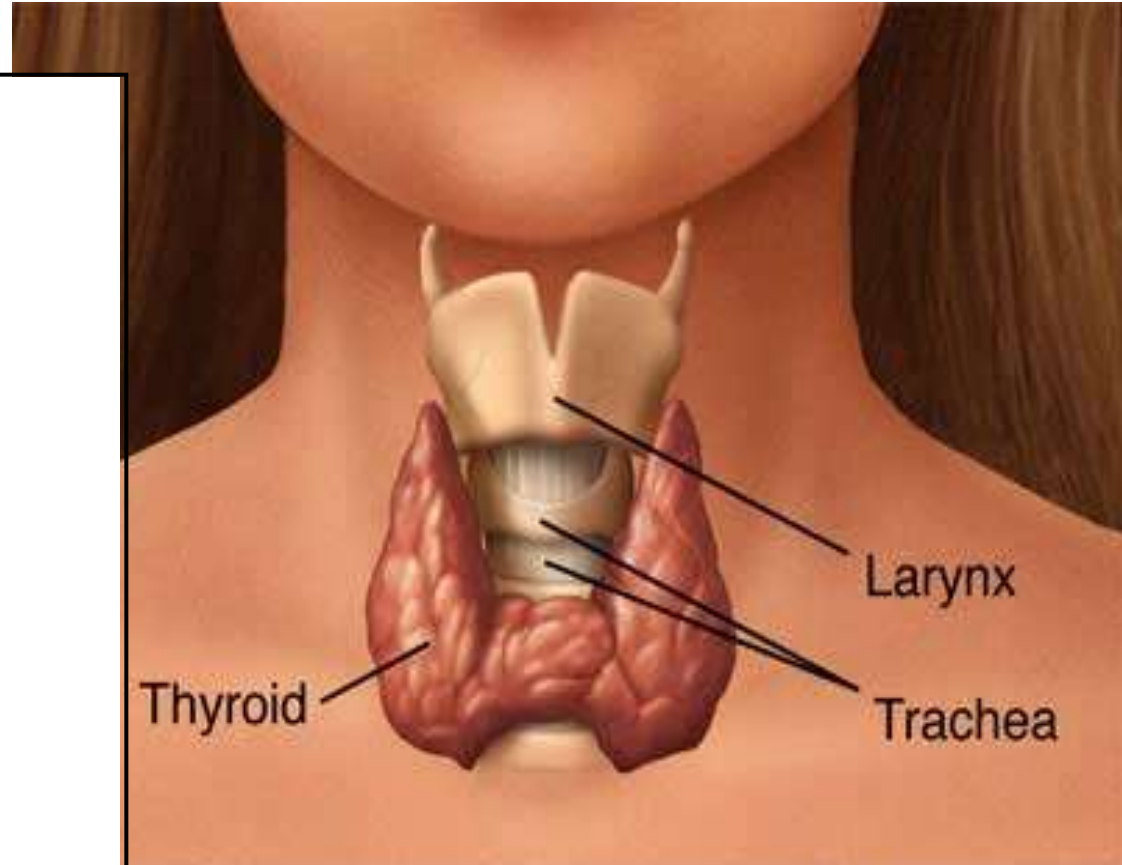
**By: Fazl A. Ashkar**

**Advisor: Dr. W. Allan King**

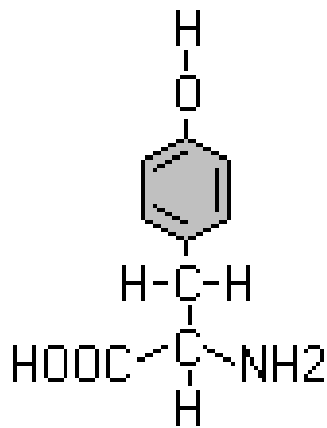
# Thyroid gland



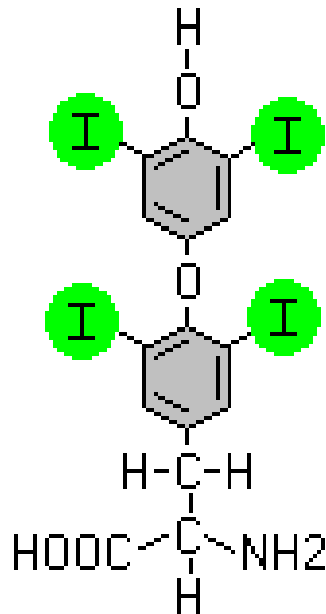
(-) = inhibition; (+) = stimulation



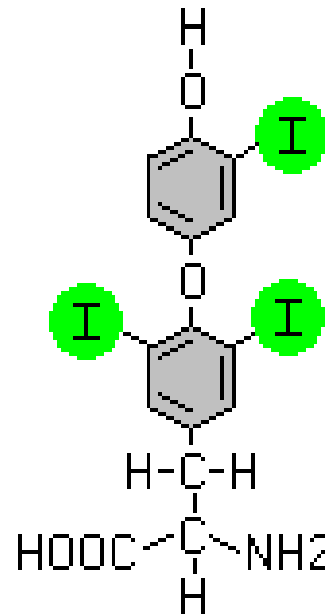
# Biochemistry of Thyroid Hormones



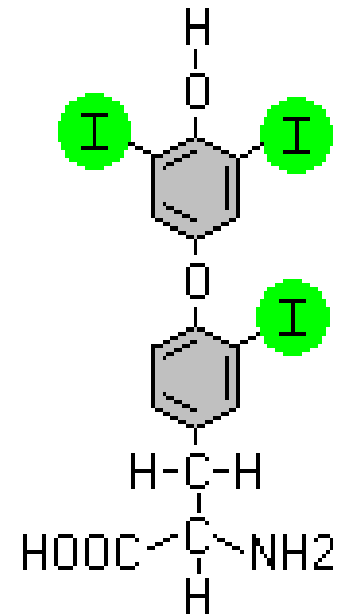
**Tyrosine**



**Thyroxine (T<sub>4</sub>)**

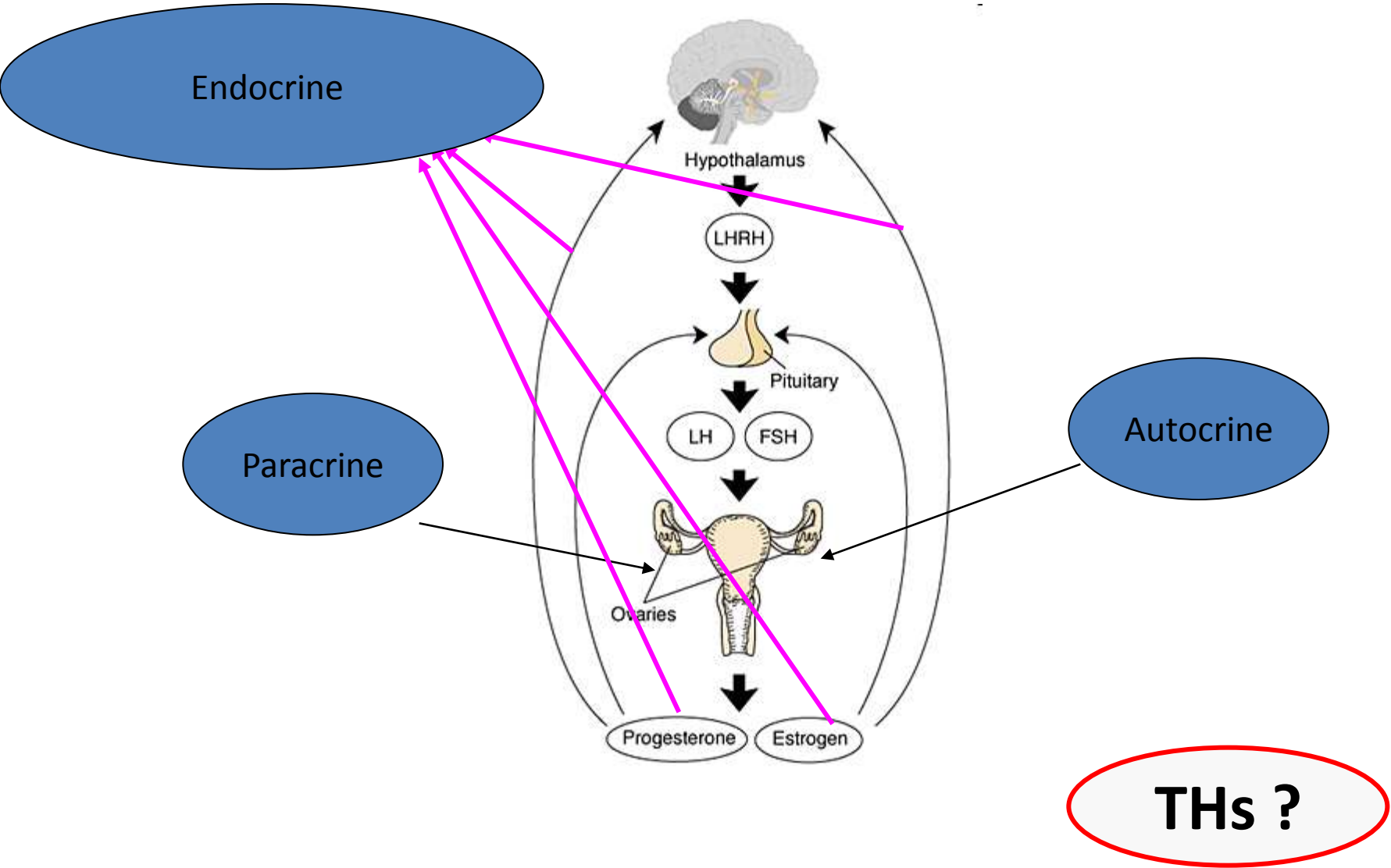


**Triiodothyronine (T<sub>3</sub>)**

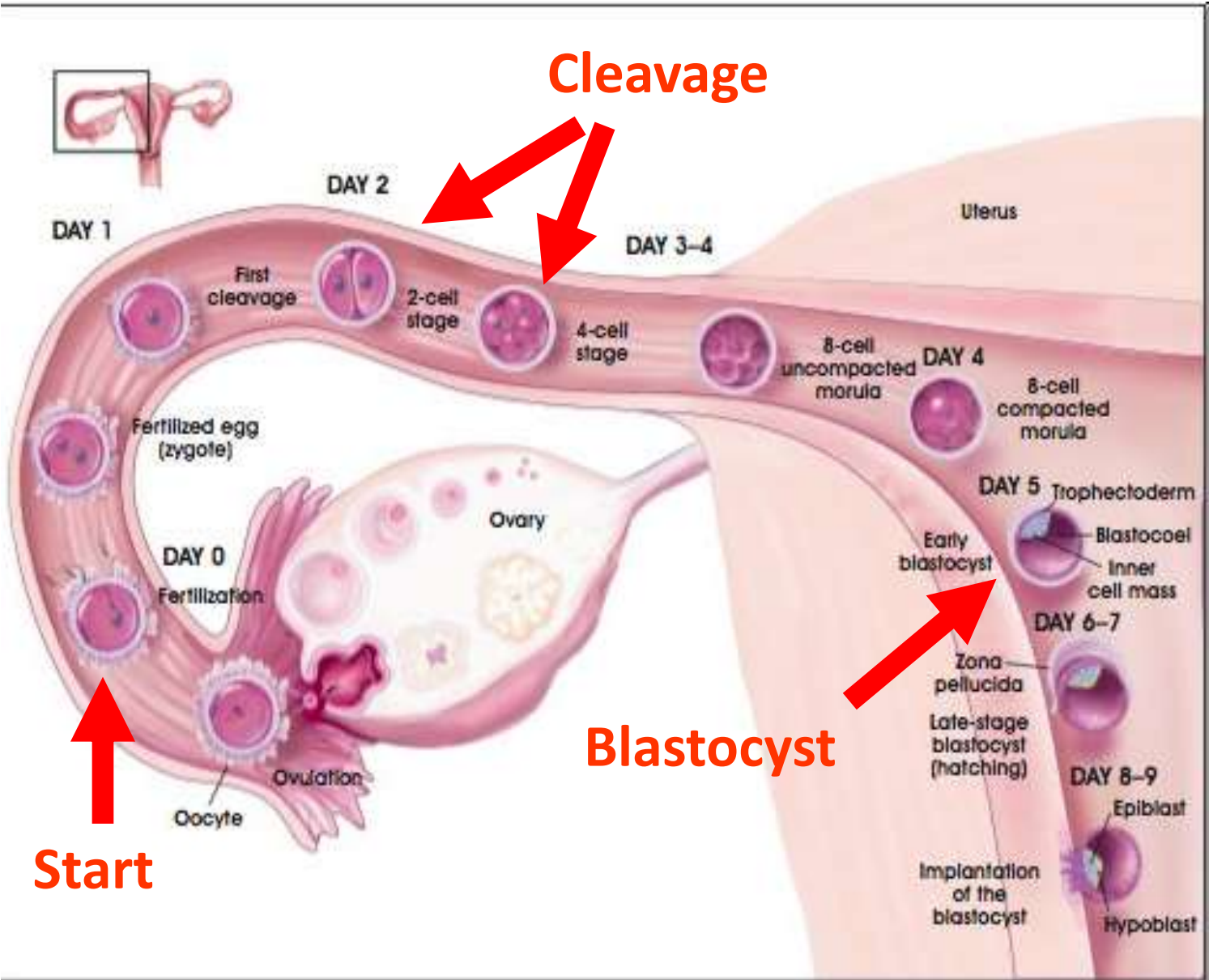


**"Reverse T<sub>3</sub>" (inactive)**

# Hormonal regulation of reproductive system



# Early embryo development



Rationale

# Our previous findings :Thyroid hormones in the serum and the reproductive system of cattle

Table 1 Summary of concentrations of total (T) and free (F) fractions of thyroid hormones (T<sub>3</sub>-triiodothyronine and T<sub>4</sub>-thyroxin; mean ± SEM) in peripheral serum and follicular fluid of cows in Experiment 1 (n = 15)

Thyroid hormone fraction and ratios/ time of sampling and source	TT <sub>4</sub> (nmol/L)	TT <sub>3</sub> (nmol/L)	TT <sub>4</sub> :TT <sub>3</sub>	FT <sub>4</sub> (pmol/L)	FT <sub>3</sub> (pmol/L)	FT <sub>4</sub> :FT <sub>3</sub>
Day 5 serum	47.3 ± 2.7ab	1.5 ± 0.08a	32:1	15.6 ± 0.9b	1.5 ± 0.1	12:1
Day 5 FF (DFs)	40.3 ± 2.2b	1.1 ± 0.06bc	36:1	13.6 ± 0.9bc	ND	–
Day 5 FF (SFs)	31.2 ± 3.3c	0.8 ± 0.2c	39:1	12.6 ± 1.1c	ND	–
Day 12 serum	55.5 ± 3.9a	1.5 ± 0.09a	36:1	18.4 ± 1.2a	1.7 ± 0.1	11:1
Day 12 FF	48.7 ± 3.7a	1.3 ± 0.09b	39:1	12.9 ± 0.8bc	ND	–

Within columns, means denoted by different letters are significantly different (*P* < 0.05)  
ND, not detected (hormone concentrations below the assay sensitivity); FF, follicular fluid; DFs, dominant follicles; SFs, subordinate follicles

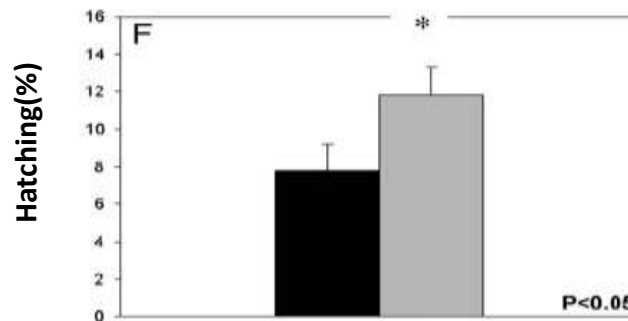
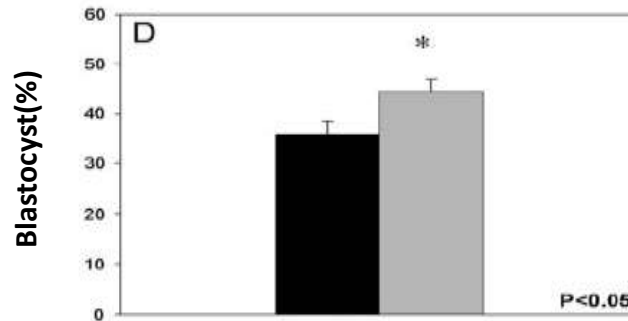
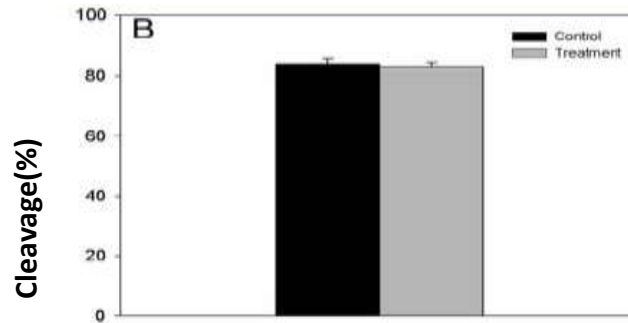
Ashkar et al,2010,  
*Experimental Biology and Medicine*

**Table 1** Thyroid Hormone (TH) detection in dissected bovine oviducts and uterine horns (*n* = 10 each)

TH fraction (concentration)/Source	TT <sub>4</sub> (nmol//l g*)	TT <sub>3</sub> (nmol//l g)	T T <sub>4</sub> :tT <sub>3</sub>	FT <sub>4</sub> (pmol//l g)	FT <sub>3</sub> (pmol//l g)	FT <sub>4</sub> :fT <sub>3</sub>
Flushing of oviduct	ND	ND	–	ND	ND	–
Oviduct-homogenates	7.0 ± 2.8	1.0 ± 0.2	7:1	31.6 ± 4.6	6.9 ± 1.4	5:1
Flushing of uterine horn	ND	ND	–	ND	ND	–
Endometrium-homogenates	11.0 ± 0.8	0.9 ± 0.1	12:1	9.1 ± 0.7	ND	–

\*Expressed per 1 g of tissue; ND: non-detectable.

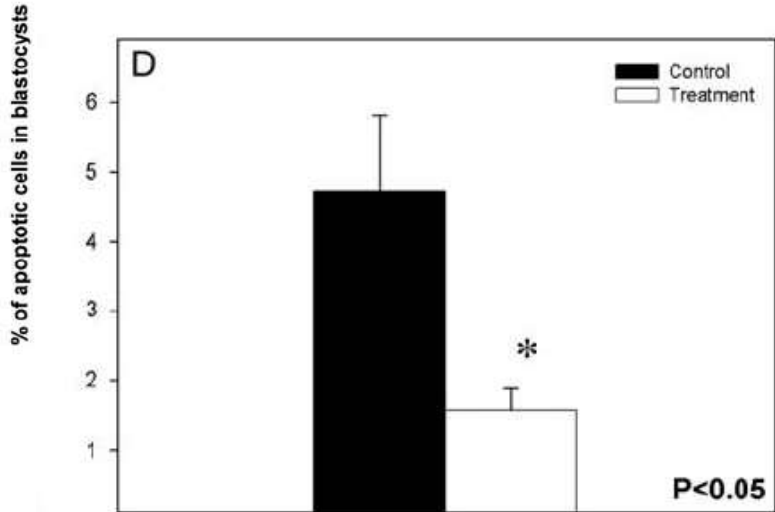
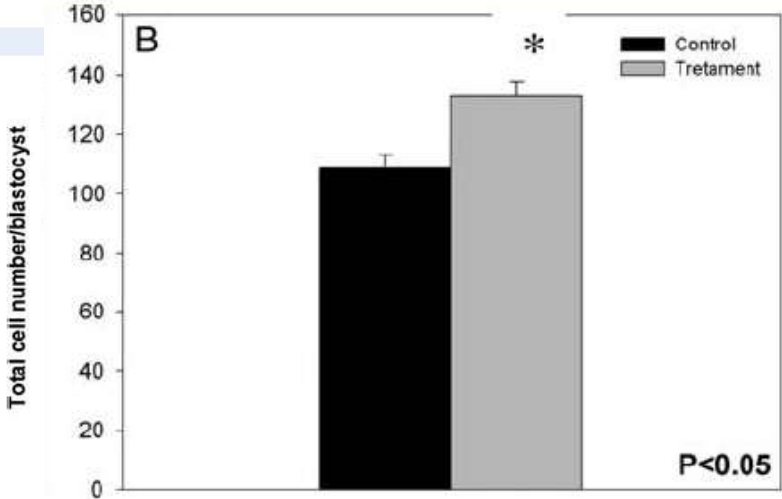
# Thyroid hormones improve bovine early embryo development *in vitro*



C

Ashkar et al,2009  
Human reproduction

# THs and Embryo quality



Ashkar et al,2009  
Human reproduction

# Hypothesis and objectives

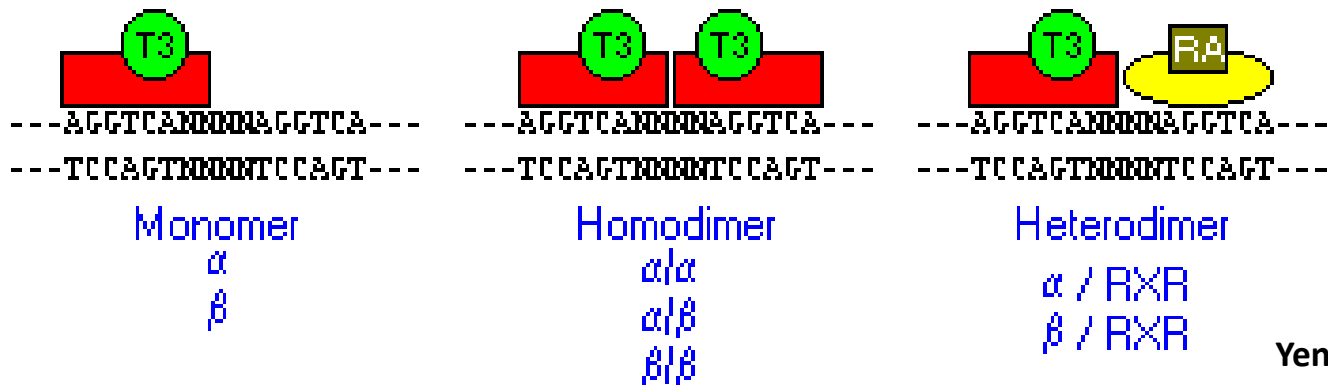
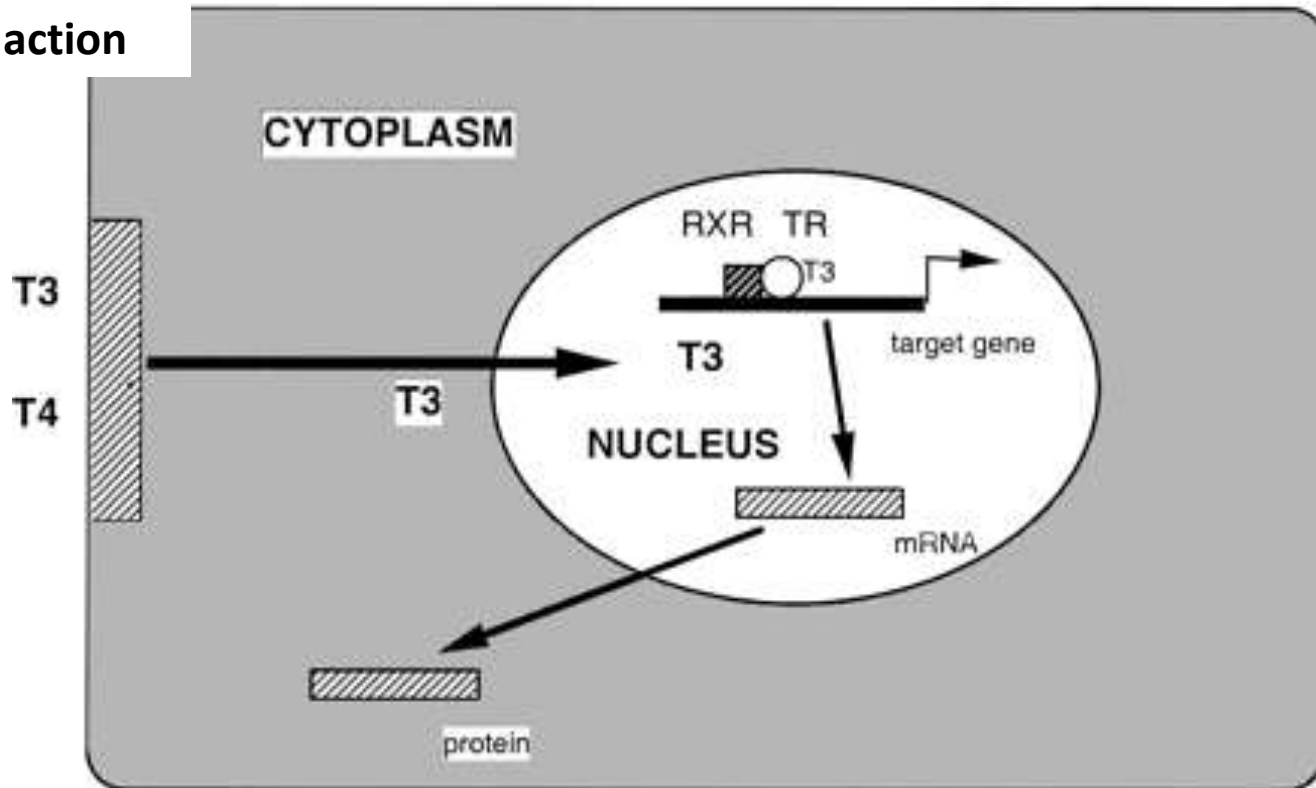
Thyroid hormones have exerts their modulate early embryo development via their receptors

To test the hypothesis we are looking at:

- mRNA expression of TRs
- Level of the expression in different stages of pre-implantation
- TRs protein

# Mechanism of action of THs

- Genomic action

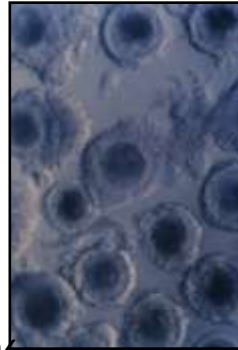


# *In vitro* embryo production (IVP)



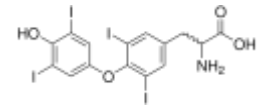
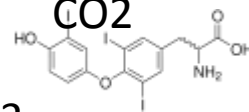
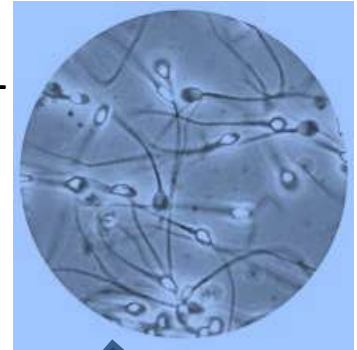
Selection of COCs

IVM 22-24h  
@ 38.5°C, 5%  
CO<sub>2</sub>



*In vitro*  
fertilization  
5X 10<sup>6</sup> / mL  
sperm

16-18h @  
38.5°C, 5%  
CO<sub>2</sub>



RT and qPCR ← RNA extraction

Freezing  
-80



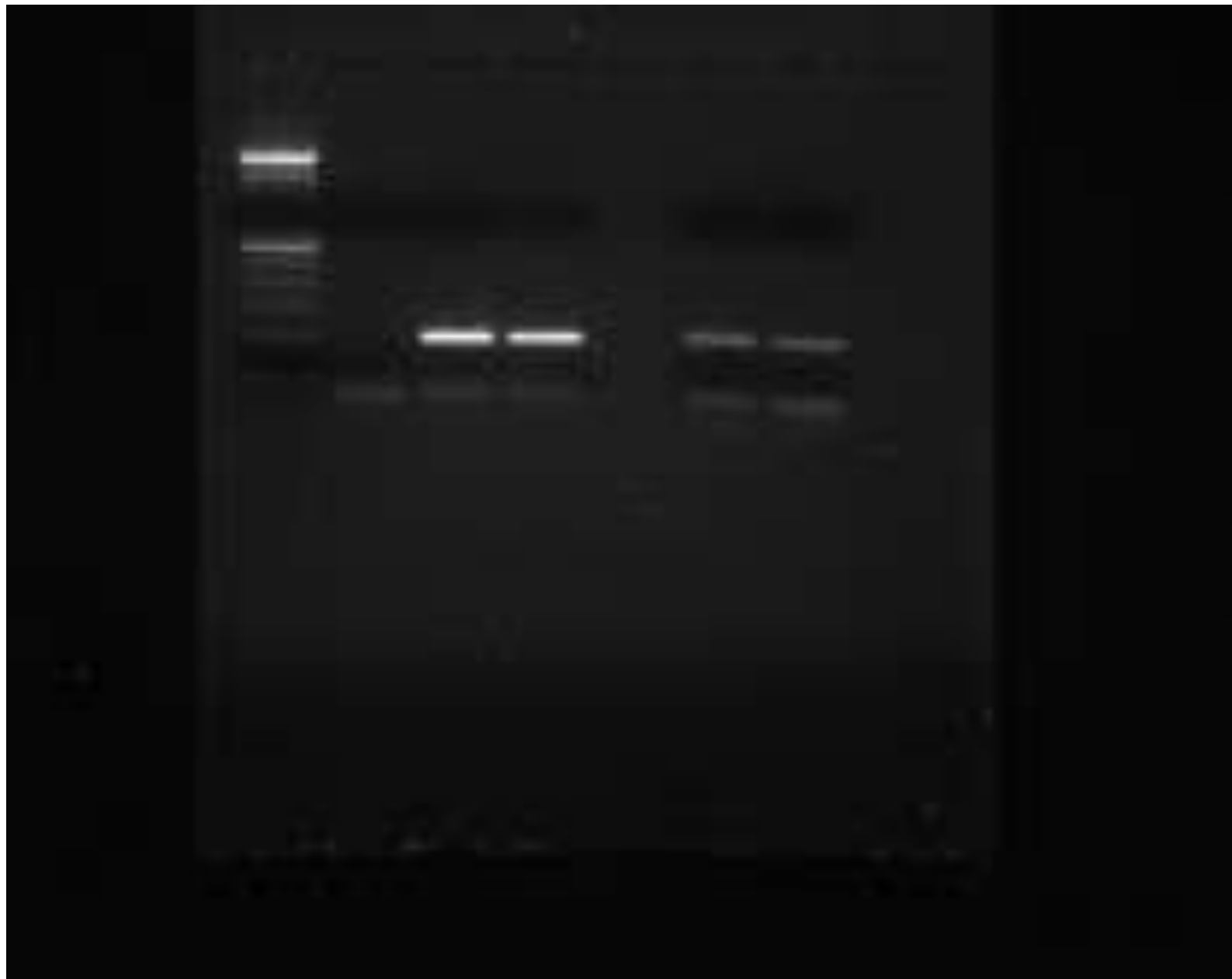
Fixation in PFA

Immunofluorescence staining

Results

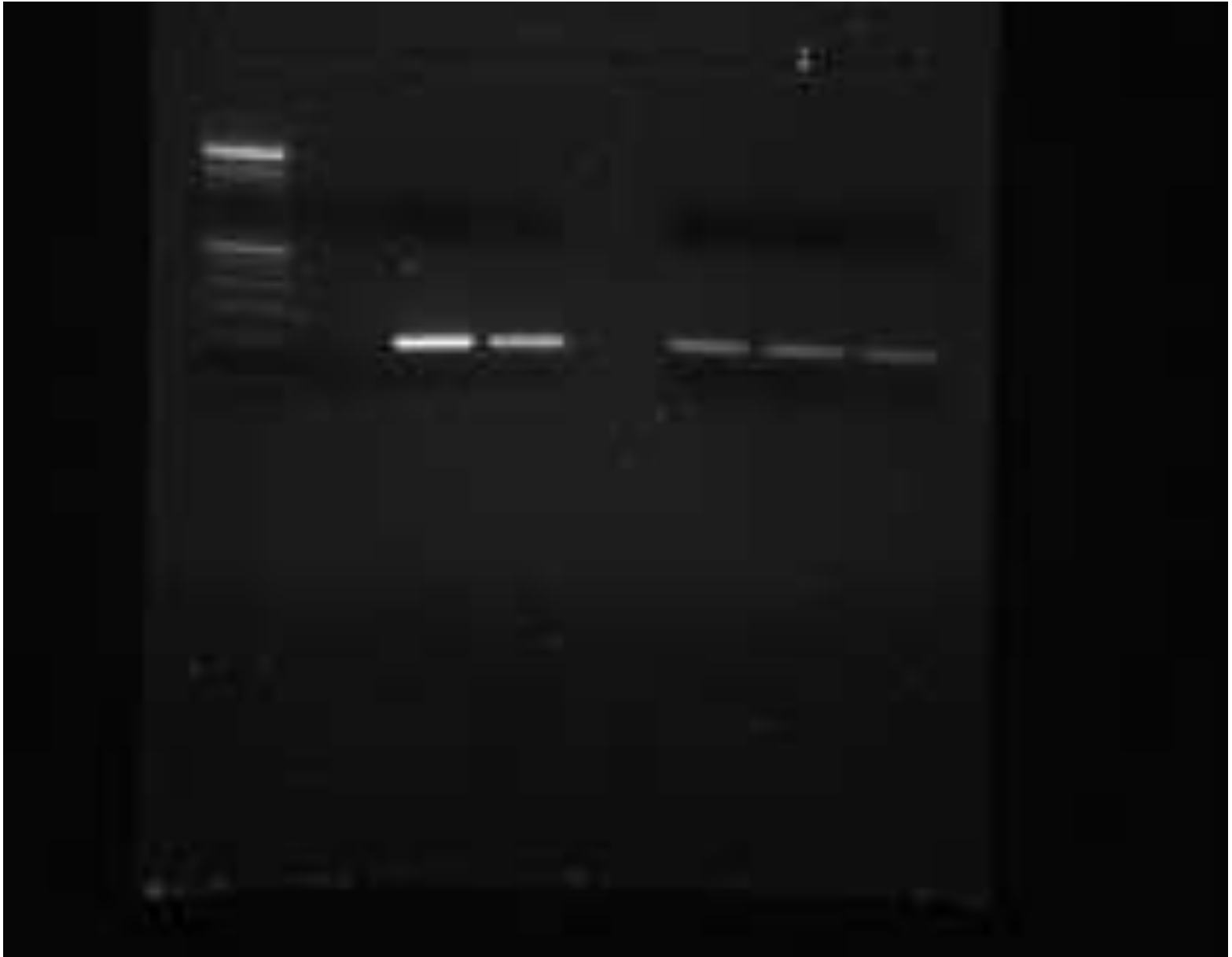
# TR $\alpha$

-ve C   +ve C   BLK   T   C

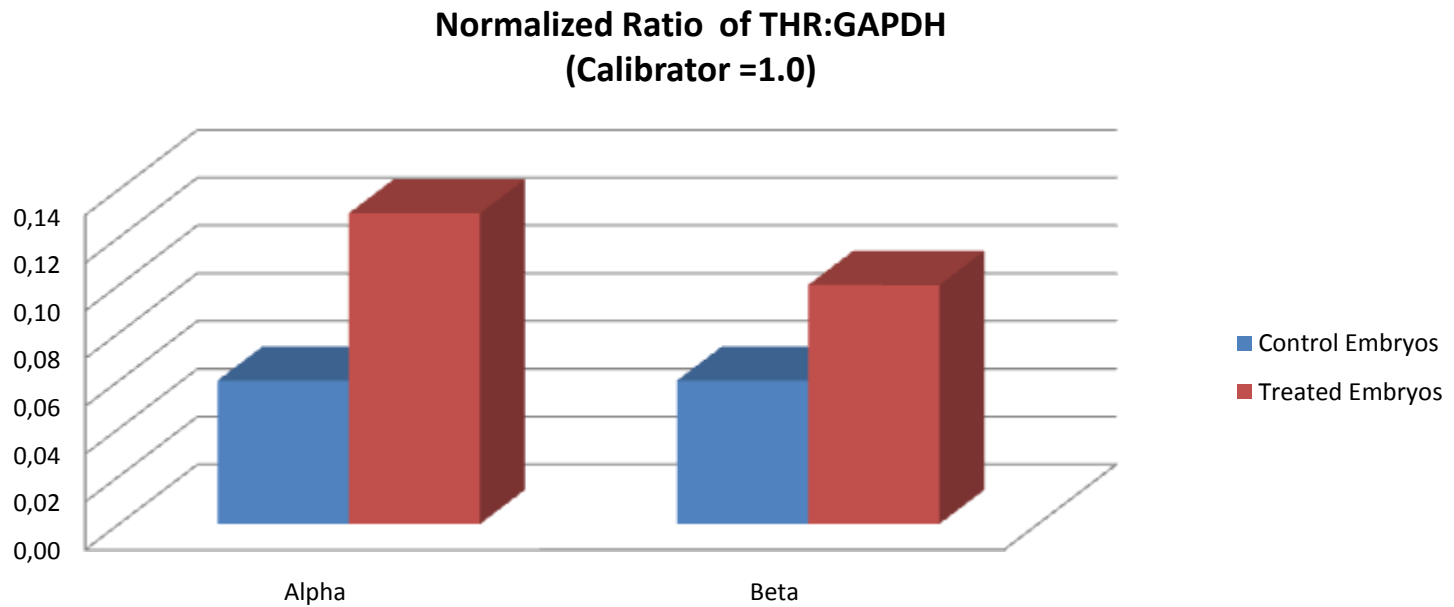


# TR $\beta$

-ve C +ve BLK T C T



# Expression of TR $\alpha$ and $\beta$ in blastocyst stages

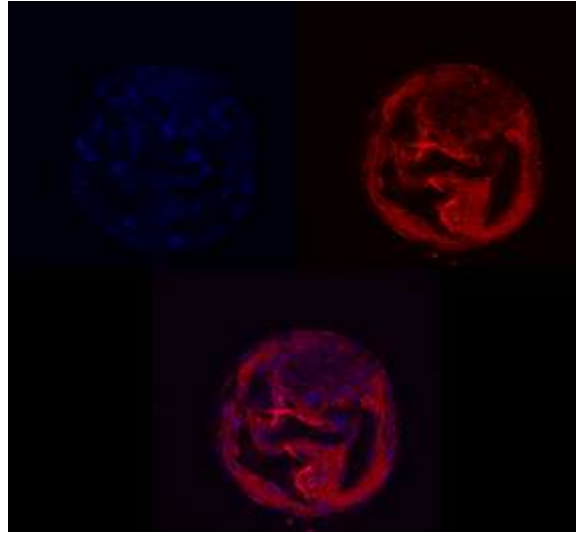


# Thyroid hormone receptor detection

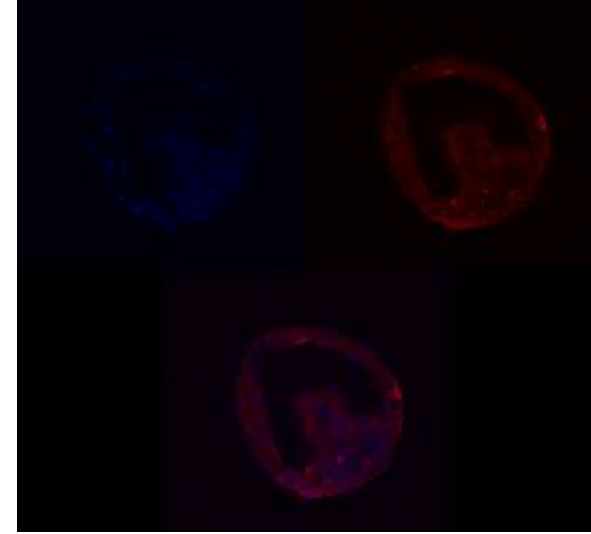
-ve control



Non-treated



Treated



# Future directions

- Looking for the expression of TRs in different stages of pre-implanted embryos
- Detecting genes associates with metabolism and mitochondrial function
- Micro-array analysis using Embryogne platform
- Looking for deiodinases enzymes in the embryos

# ACKNOWLEDGEMENTS

**My supervisor:**

Dr. Allan King

**My Advisory committee:**

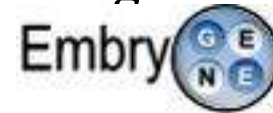
Drs. Pawel Bartlewski, Tracy Chenier,  
Roger Moorehead and John Leatherland

**Dr. King's lab:** Drs. Laura Favetta, and  
Pavneesh Madan. Esther semple, Ed  
Reyes, Liz St. John, Lucy

**Department of Biomedical  
Science**

Family & Friends

**Funding:**



NSERC

# Why Thyroid Hormones?

- Cellular differentiation, growth and metabolism are mostly governed by thyroid hormones in vertebrates.
- It has been documented that T4 treatment of rat follicles and equine chorionic gonadotropin (eCG) increased the number of ovulated eggs (Sato E et al 2001).
- T3 and T4 in bovine granulosa cells cause an increase in net estrogen production (L.J Spicer 2001).
- T3 synergizes with FSH to induce differentiation of granulosa cells in porcine follicles (Maruo et al 1987).

# Expression of TR $\alpha$ in blastocyst stages of early embryo

TR alpha expression

