Stallion spermatozoa: putative targets for estrogens

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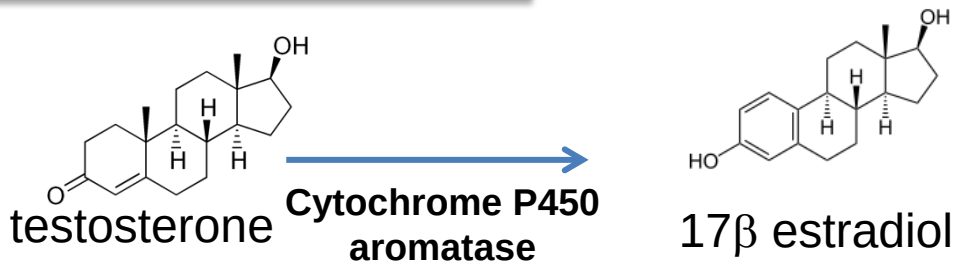
Stallion and estrogen production

- Seasonal breeder with increase of DSP in breeding season (Johnson et Thompson, 1983)
- Male producing largest amount of testicular estrogens (Raeside, 1969)
- Estrogen synthesis varies according to season (Lemazurier et al., 2002) with higher level in breeding season.

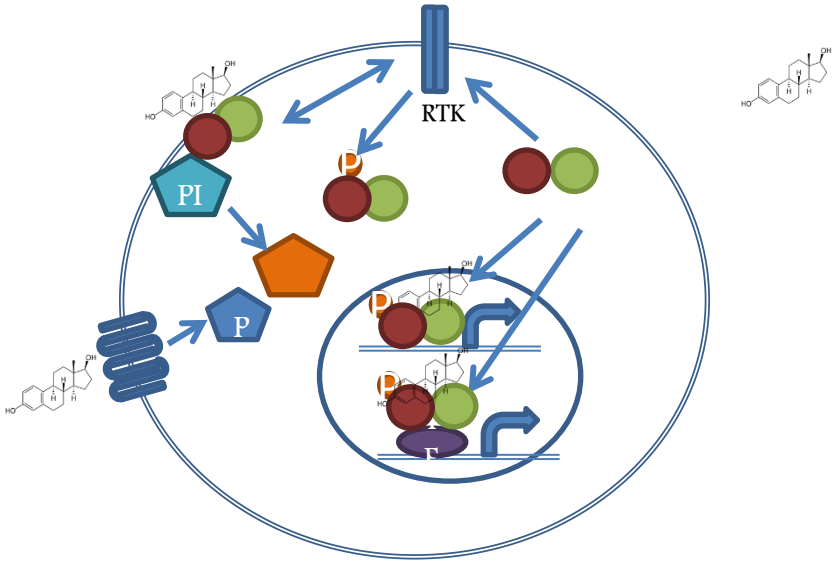
Stallion spermatozoa: putative targets for estrogens

Estrogens

- Estrogen **synthesis**: cytochrome P450 aromatase (encoded by *cyp19* gene) associated to NADPH reductase



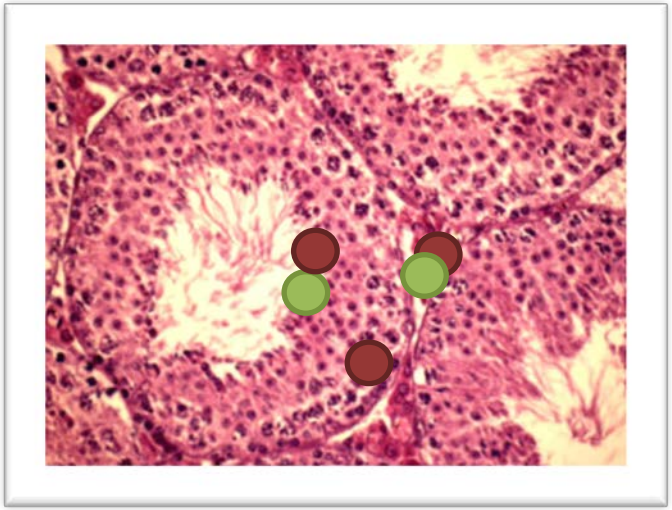
- Estrogen **action**: use of two nuclear receptors (**ESR1**or ERα and **ESR2** or ERβ) and a 7TM receptor **GPER** (or GPR30)



Stallion spermatozoa: putative targets for estrogens

Estrogens

- Synthetized mainly by Leydig cells (Almadhidi et al, 1995) and Sertoli cells (Sipahutar et al., 2003).
- Putative targets in testis: Leydig cells (ESR1-2), Sertoli cells (ESR1) and germ cells (ESR1-2) (Pearl et al., 2011).



Stallion spermatozoa: putative targets for estrogens

Estrogens and production of an active spermatozoon

reinitiation of spermatogenesis

(Zhang et al., 2010)

(Gancarczyk et al., 2010)

(Pak et al., 2002)

Epididymal maturation (ESR1KO)

(Joseph et al., 2010)

capacitation

(Ded et al., 2011)

(Adeoya Osiguwa et al., 2003)

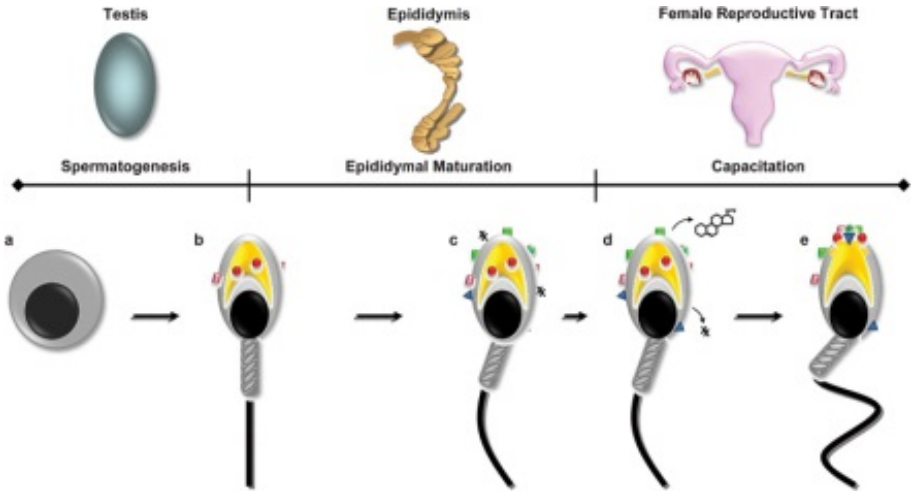
(Guido et al., 2011)

Protection against apoptosis

(Pentikainen et al., 2001)

Cell proliferation

(Lucas et al., 2008; Wahlgren et al., 2008)

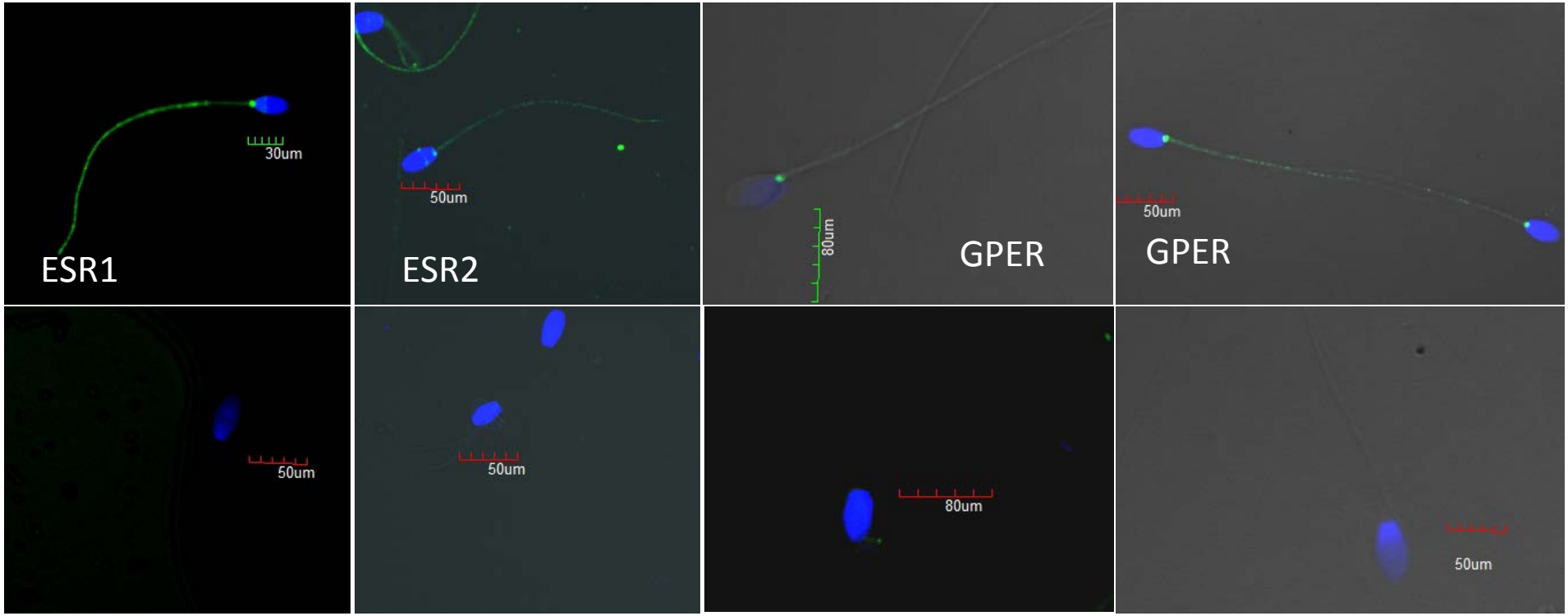


motility

Acrosomic reaction

(Luconi et al., 1999)

Estrogens and horse spermatozoa



Control without primary antibody

ESR1, 2 and GPER present on horse spermatozoa (Arkoun et al , submitted).

Could spermatozoon responds to estrogens?

- Identification of isoforms of ESR1 in spermatozoa
- Precise sub-localization in spermatozoon
- Seasonal quantification on ejaculated spermatozoa

Materials and methods

7 stallions
Jumenterie du Pin (IFCE)
(10 to 23 years)

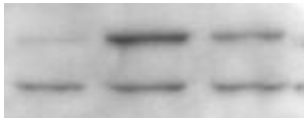
Collection of semen
2days/month, 12 months
May 2012 to april 2013

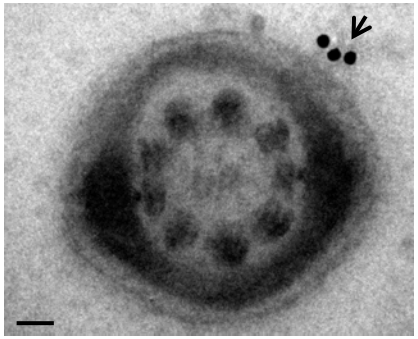
Fixation, 3,7%FA/PBS

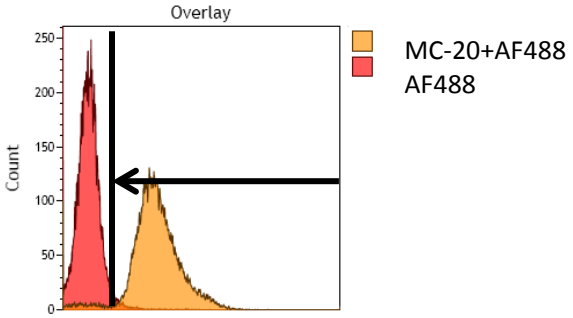
Identification of isoform by Western-blot

Localization by TEM and immunocytochemistry

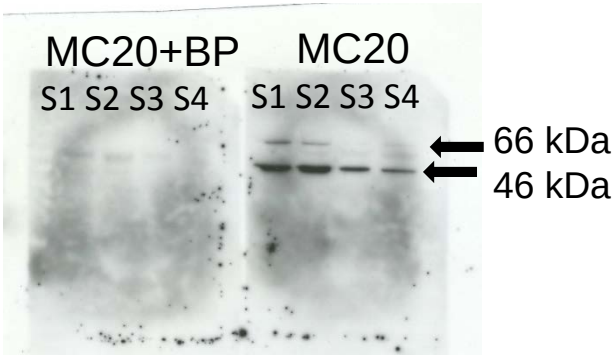
Quantification of population of spermatozoa ESR1+ by flow cytometry







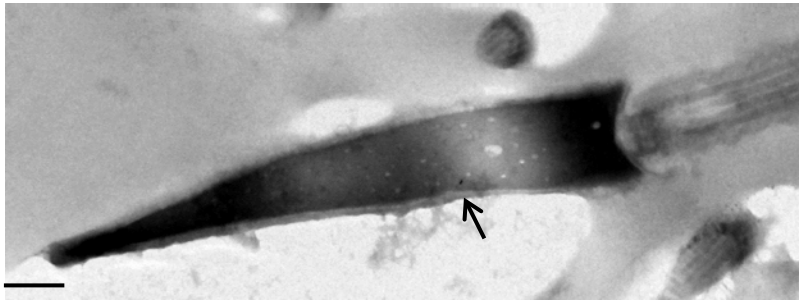
Results
Identification of ESR1 isoforms by Western-blot

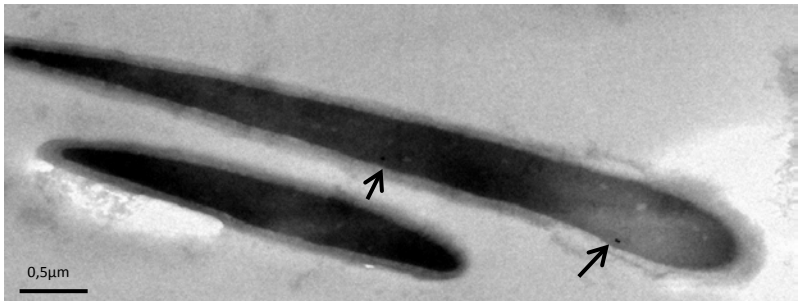


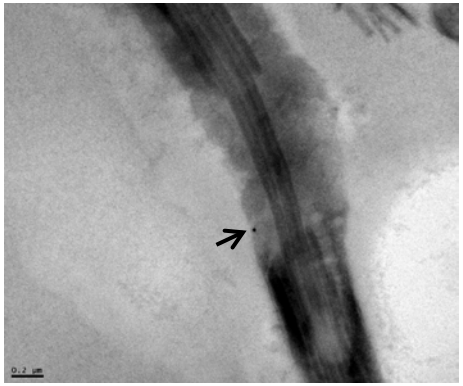
Two isoforms:

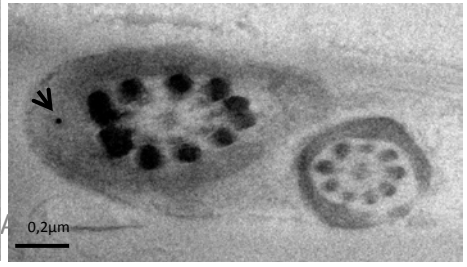
- 66 kDa, wild-type, complete receptor
- 46 kDa, truncated isoform, could be associated to membrane and rely rapid, non genomic, effects.

Results
Localization of ESR1 by TEM and immunocytochemistry

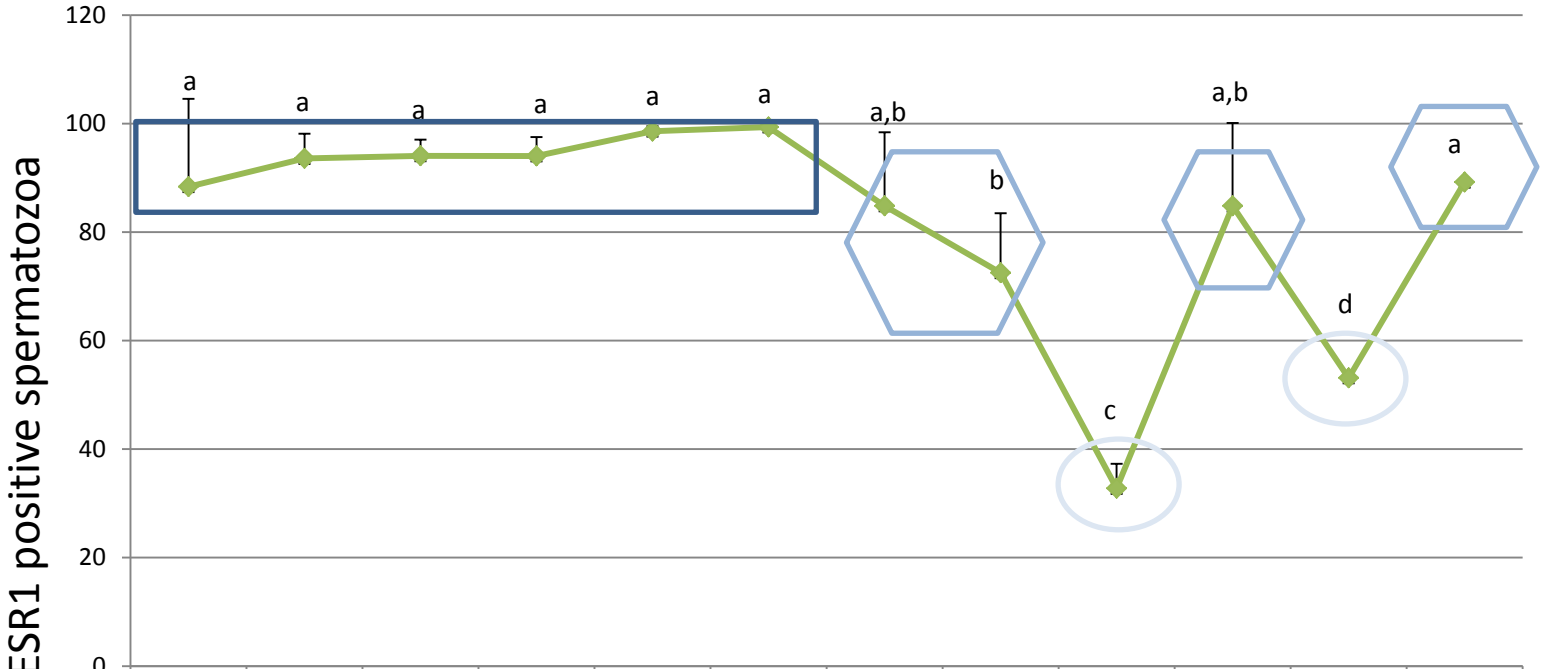








Results
Quantification of population of spermatozoa ESR1+ by flow cytometry



	May 2012	June 2012	July 2012	August 2012	Septemb er 2012	October 2012	Novembe r 2012	Decembe r 2012	January 2013	February 2013	March 2013	April 2013
ESR1	88,37	93,575	94,07364	94,0425	98,614	99,37375	84,834	72,509	32,762	84,825	53,13111	89,21

Discussion

Rate of ESR1 associated with spermatozoa decreases from October to December

Could this parameter be rely to decline of sperm quality (sperm motility, morphology, mitochondrial potential and membrane stability) observed in winter (Gamboa et al., 2010) or the best freezability described in winter (Magistrini et al., 1987)?

Discussion

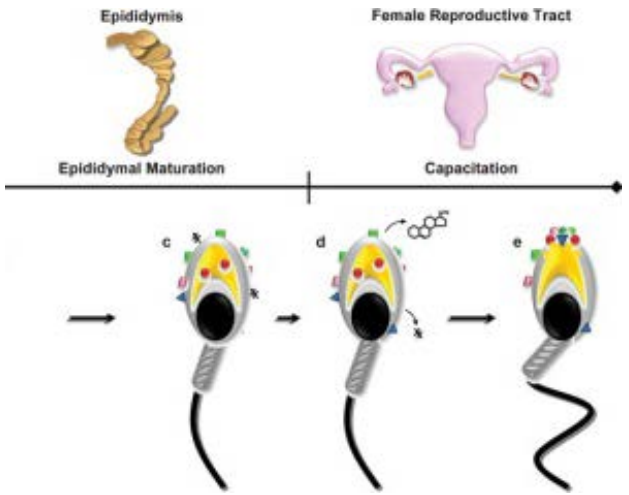
ESR1 and stallion spermatozoa:

- Presence of two isoforms with 46kDa isoform
- Localization almost near the membrane

two characteristics of estradiol rapid, non-genomic, effects

Perspectives

Could estradiol modulate spermatozoa post-testicular maturations?



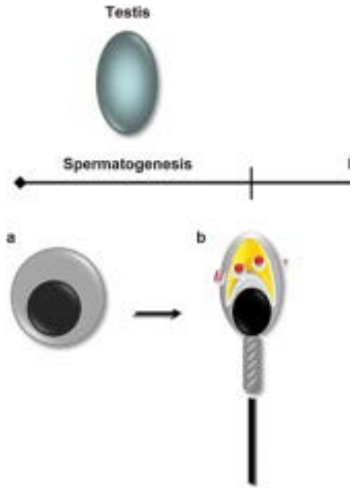
Could ESRs vary according to another parameters like

- sperm quality?
- Effort?
- Freezability?

Perspectives

What role play estradiol on testicular physiology:

- Somatic cells proliferation or differentiation?
- Spermatogenesis?



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