



Expression study of *ESR1*, *ESR2*, *CYP19* and *CYP3A* mRNA and protein in different reproductive tissues of breeding boars

Asep Gunawan⁽¹⁾⁽²⁾., K.Kaewmala⁽²⁾.,U.Cinar⁽²⁾ and K.Schellander⁽²⁾

⁽²⁾Institute of Animal Science
Animal Breeding and Husbandry group
University of Bonn, Germany

⁽¹⁾Faculty of Animal Science
Bogor Agriculture University

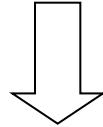
Outline

- Introduction
 - Boar Selection
 - Information of selected genes
- Objective
- Material and Methods
 - Work Flow of Expression Analysis
- Results and Discussion
 - mRNA Expression Analysis
 - Protein Expression Analysis
 - Localization of Protein
- Conclusion
- Prospective aspect

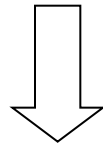
Introduction

Boar Selection

AI has a significant influence on the pig breeding and production



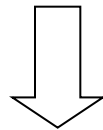
Fertility and sperm quality are important parameters for the selection of boars



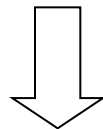
Difficult to perform direct selection: Low heritabilities

→ Fertility (LS=0.01 – 0.06)¹

→ Sperm quality (SC=0.14-0.18; SM=0.05-0.13)²



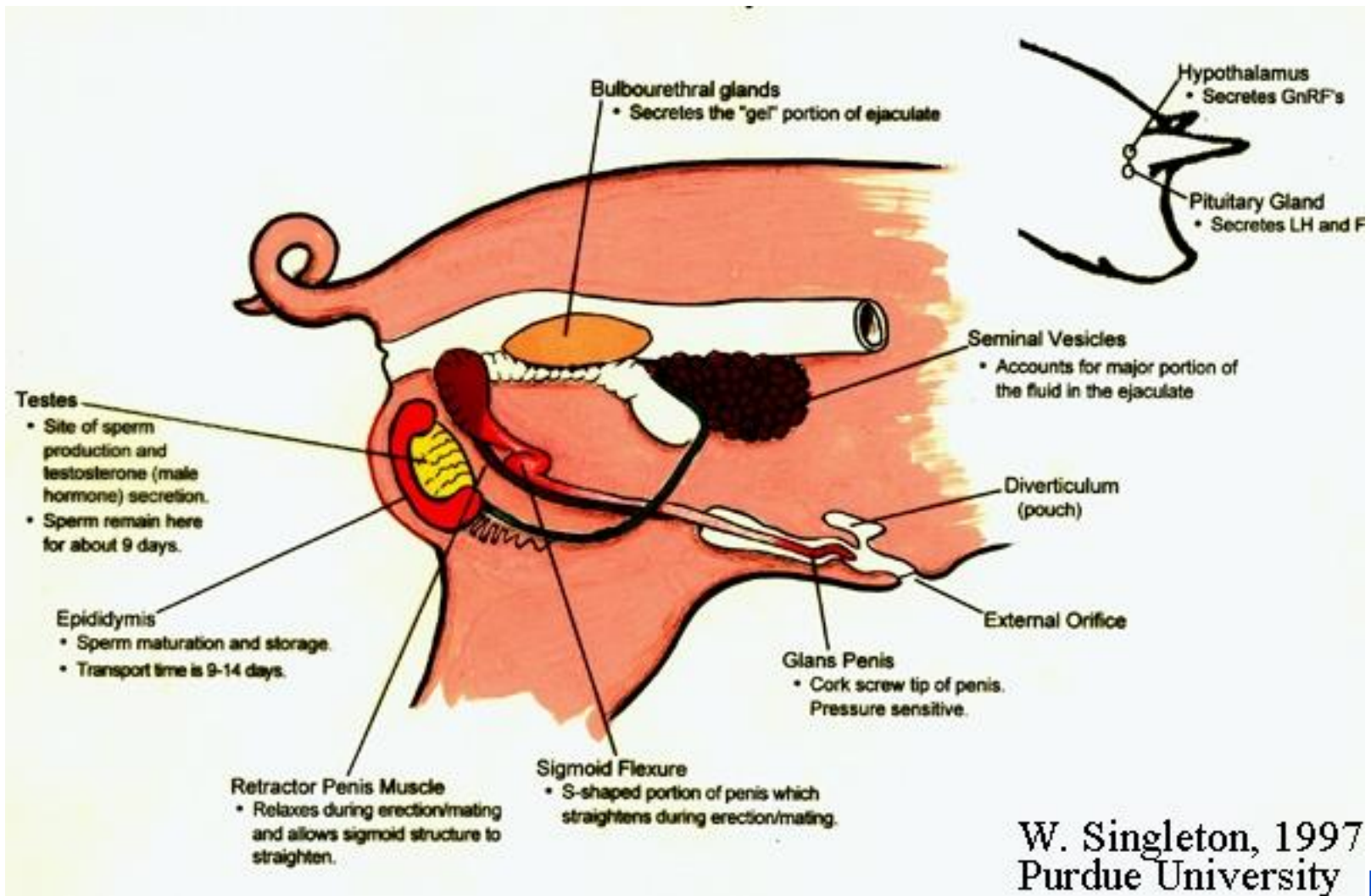
Marker Assisted Selection (MAS): candidate gene analysis



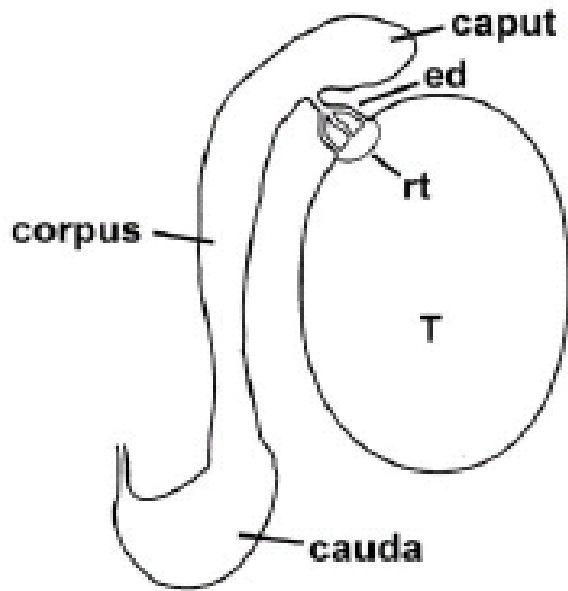
Effective way to improve male reproductive traits

¹See (2000); ²Brandt and Grandjot(2008)

Boar reproductive physiology



Reproductive tissues



Tissue	Function
Testes	<ul style="list-style-type: none"> - Leydig cells produce testosterone under LH stimulation - Sertoli cells produce estradiol under FSH stimulation
Caput	Early maturation in sperm <ul style="list-style-type: none"> - Translocation of cytoplasmic droplet - Change in membrane phospholipid
Corpus	Continued maturation of sperm <ul style="list-style-type: none"> - Change in membrane sterol:phospholipid - Increased permeability of plasma membrane
Cauda	Final maturation and storage of sperm <ul style="list-style-type: none"> - Increased intracellular pH - Decrease intracellular Ca^{2+} - Efficient energy production - Improve pregnancy ensurance - Normal fertility and birth rate

Literature studies

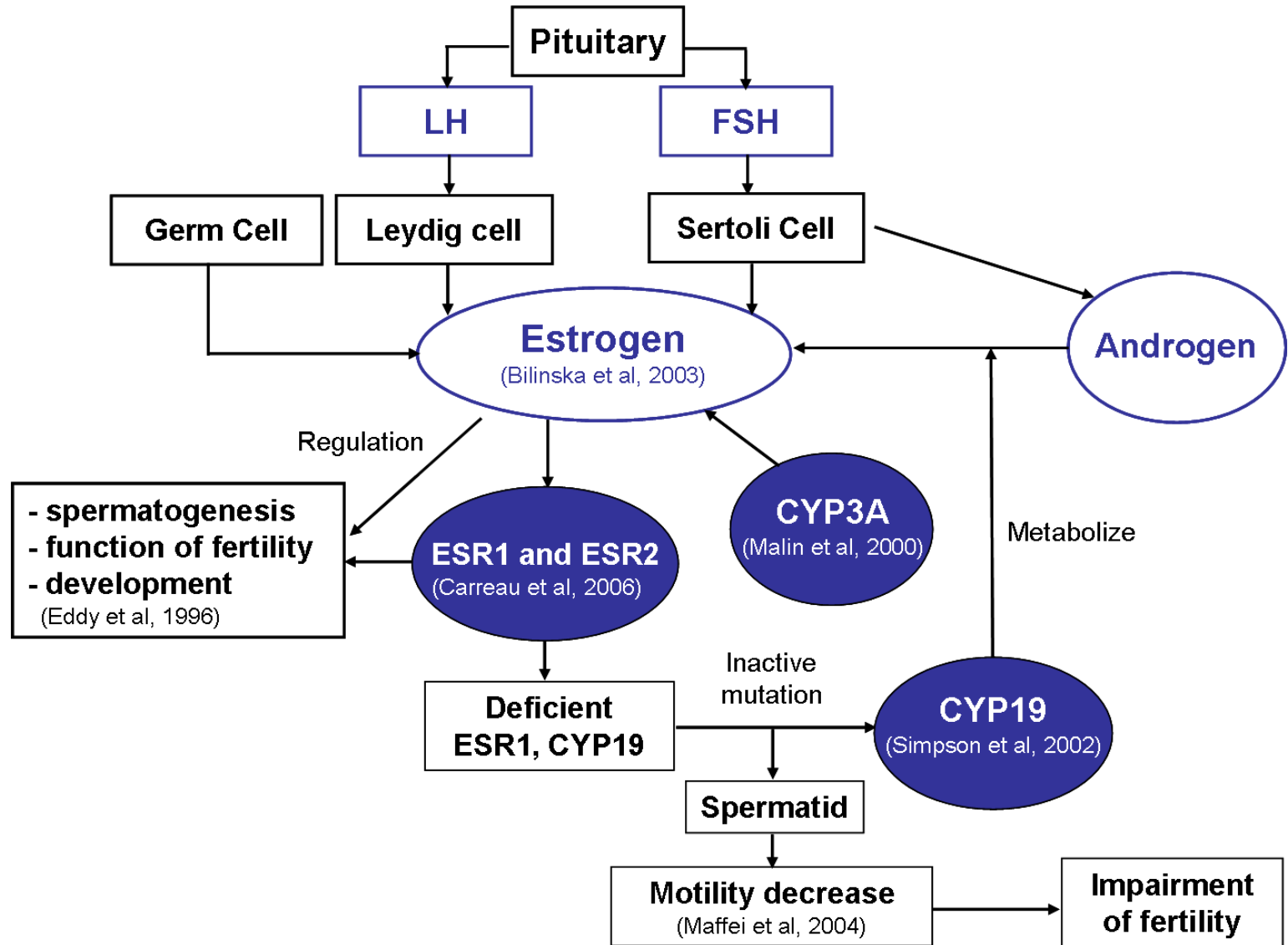
- Large number of genes and protein involved in the mechanism and process of fertilization, but there a few report with an influence in sperm quality and fertility (Giesecke et al. 2009)
- Fertility traits
 - Three genes ACTN1, ACR and ONPin6 had significantly effect on boar fertility traits: non return rate and number of piglets born alive (Wimmer et al.2005)
- Sperm quality traits
 - Fourteen genes FSHB, PRL, ACR, INHA, INHBA, INHBB, FST, RLN, RBP4, AR, ACTG2, GnRHR, OPNin6 and OPNpro significantly affected sperm quality traits: sperm concentration, semen volume per ejaculate, motility, plasma droplets rate and abnormal sperm rate (Lin et al. 2006a ; 2006b)

Selected genes function in male reproductive trait

Gene	Function	Key tissue	Reference
ESR1	<ul style="list-style-type: none"> - Association with semen traits : sperm number per ejaculate and sperm motility; - Lack of either ESR1 result infertility in adult life - Spermatogenesis and sperm maturation 	Testis Epididymis	Terman et al., 2006; Guarducci et al.,2006; Eddy et al, 1996 Ren et al, 2008a Rohrer et al (1996)
ESR2	<ul style="list-style-type: none"> - Association genotype ESR2 gene to male infertility - Deficient ESR2 in male mice are reported to be fertile - Process of differentiation and maturation of testis 	Testis, Epididymis	Aschim et al (2006); Lambard et al (2004); Munoz et al (2002)
CYP19	-Catalyze for estrogen synthesis from androgen; CYP19 deficiency caused progressive infertility in adult mice and reduced sperm production and sperm motility in humans	Testis	Furbass et al, 1997 Carani et al1997; Robertson et al, 1999; Herrmann et al, 2002, Tiwari et al 2008
CYP3A	-Sperm maturity, sperm storage	Testis, Epididymis	Ren et al, 2008



Function of selected genes in reproductive tract



Objective of the study

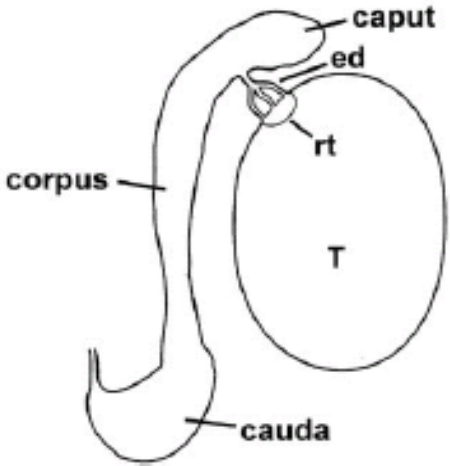
- To study the mRNA expression and protein expression in testis and epididymis between good and bad sperm quality of boar
- To localize selected proteins in testis and epididymis

Material and methods

Sampling



4 good sperm quality

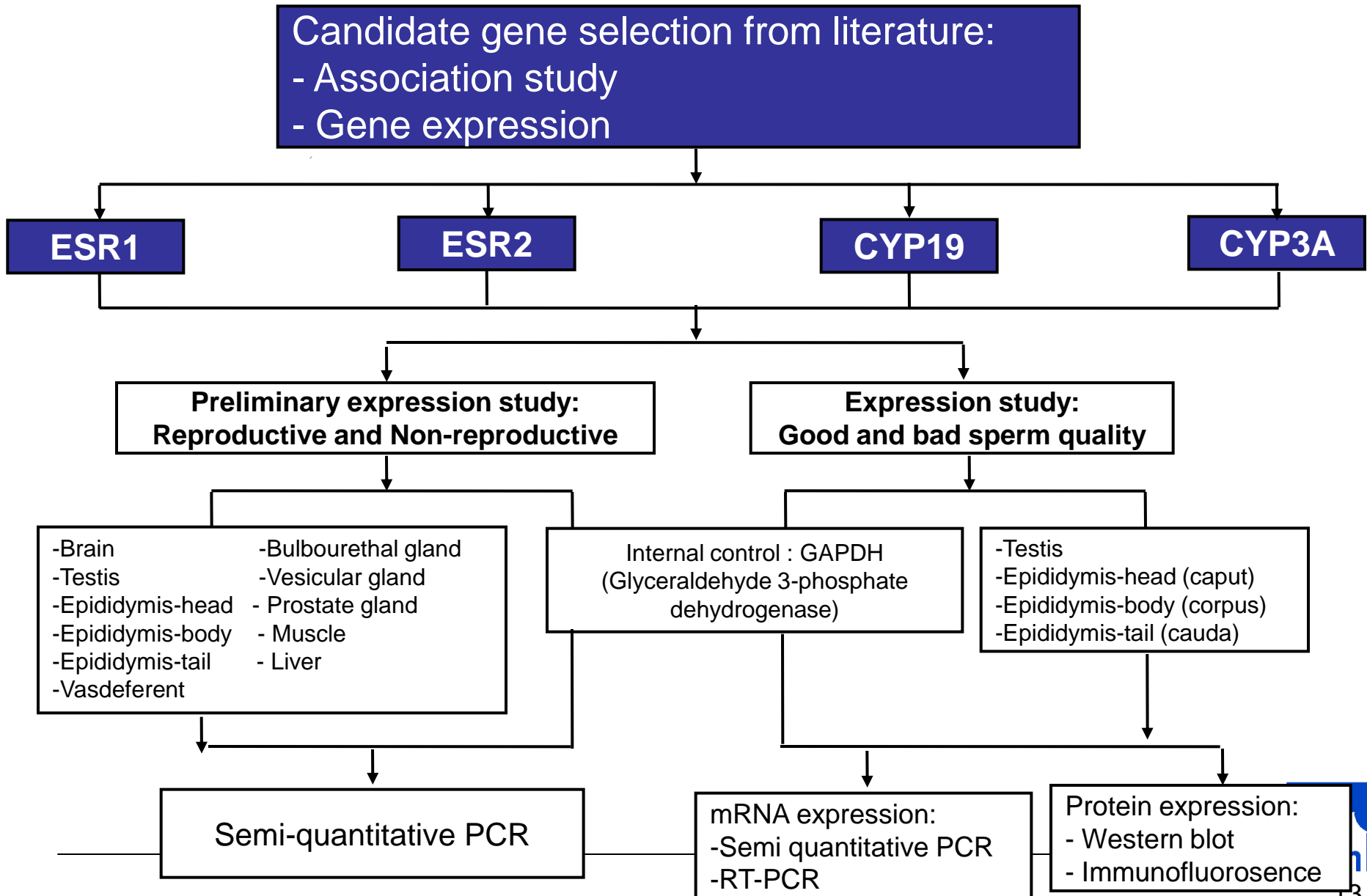


4 bad sperm quality

AI station declaration
-sperm concentration
-sperm volume
-sperm motility

Reproductive tissues

Workflow



Gene expression analysis

- Gene expression analysis by SAS version 9.2, using the Generalized Linear Model:

$$Y = \mu + a + e$$



Where

Y : the expression of phenotype;

μ : the overall mean

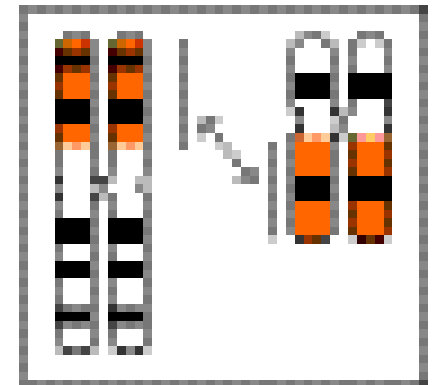
a : the fix effect of phenotype (reproductive tissues:testis and epididymis good and bad sperm quality of boar)

e_{ij} : the random residual error

Comparative mapping

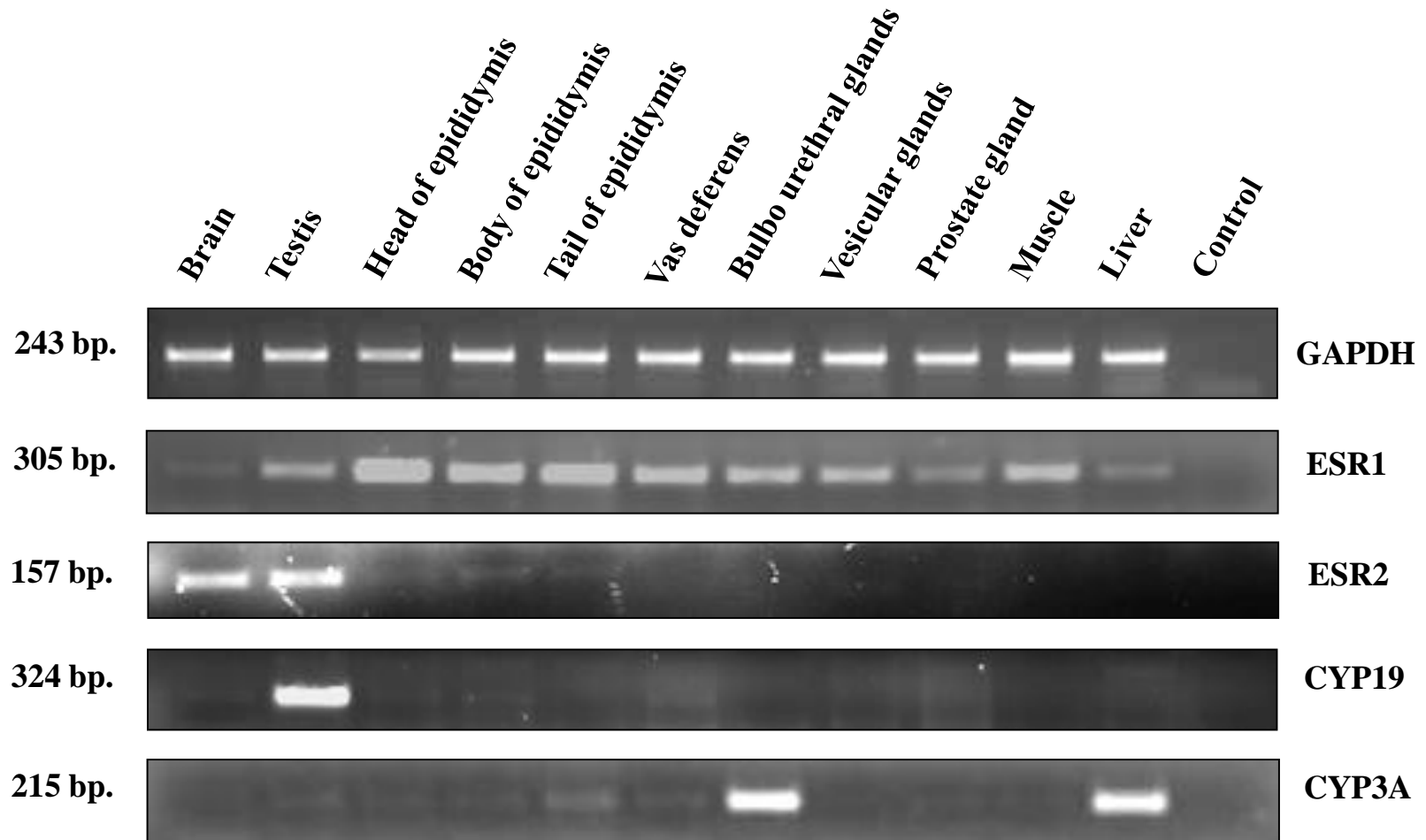
Sus scrofa <> *Homo sapiens*

- Comparative mapping of selected genes *ESR1*, *ESR2*, *CYP19* and *CYP3A* were identified using the INRA-Minnesota 7000 rad radiation hybrid panel (IMpRH) (Yerle et al. 1998)
- Comparative maps analysis were performed using available software database <https://www.lgc.toulouse.inra.fr/pig/compare/compare.htm> for chromosome assignment

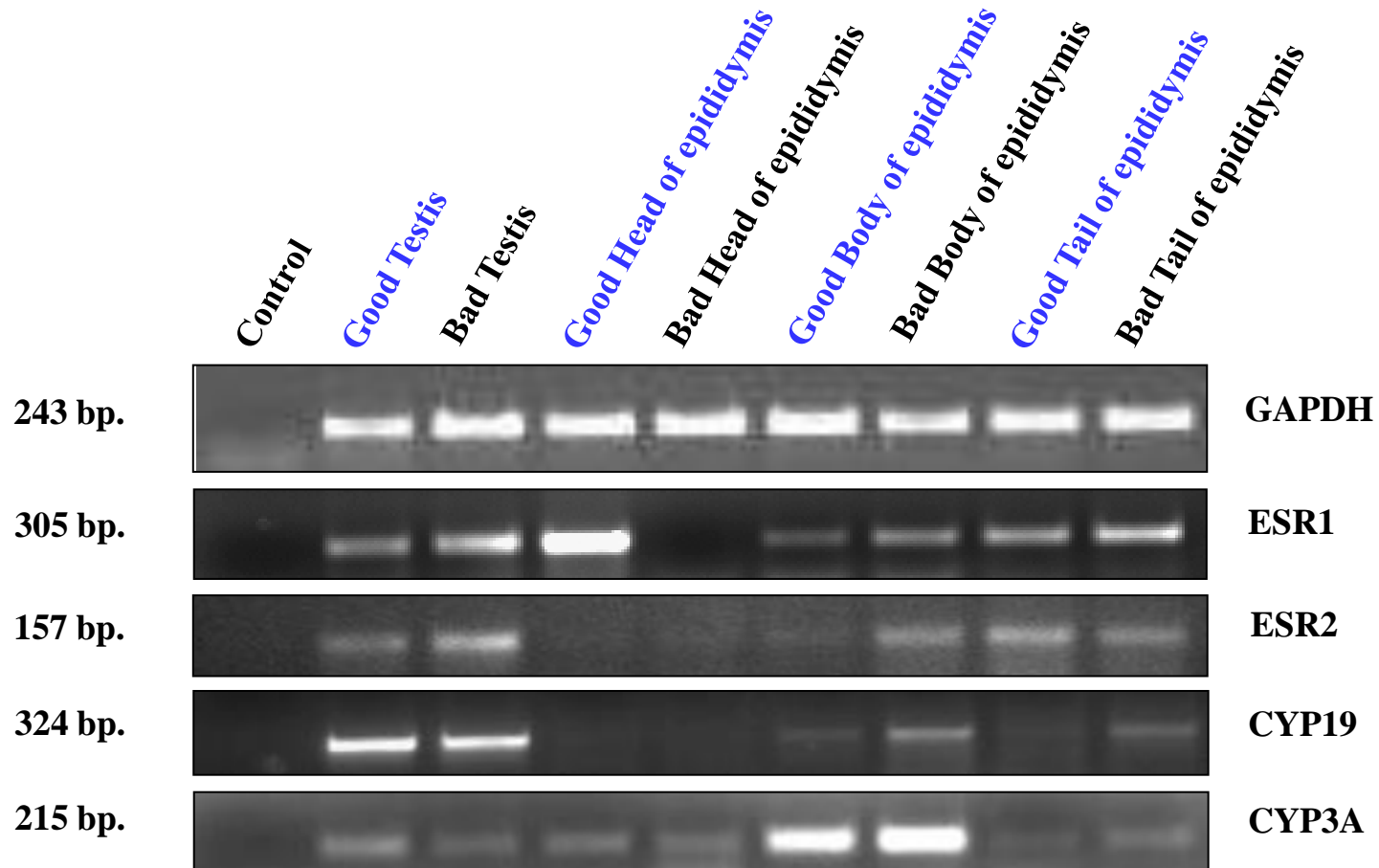


Results and Discussion

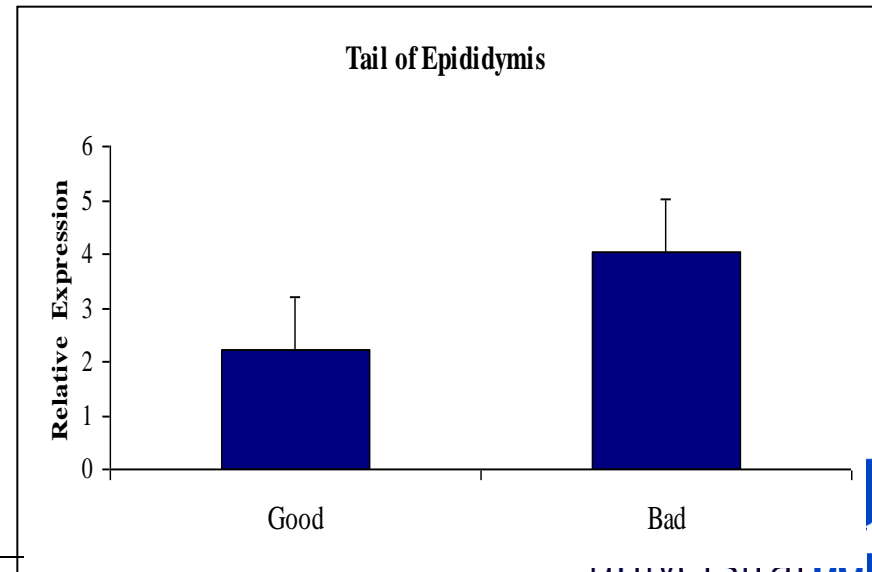
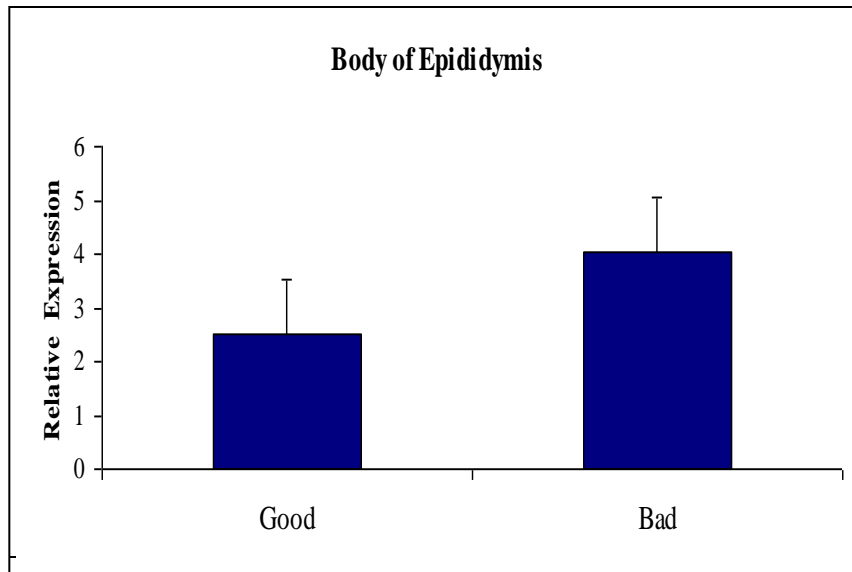
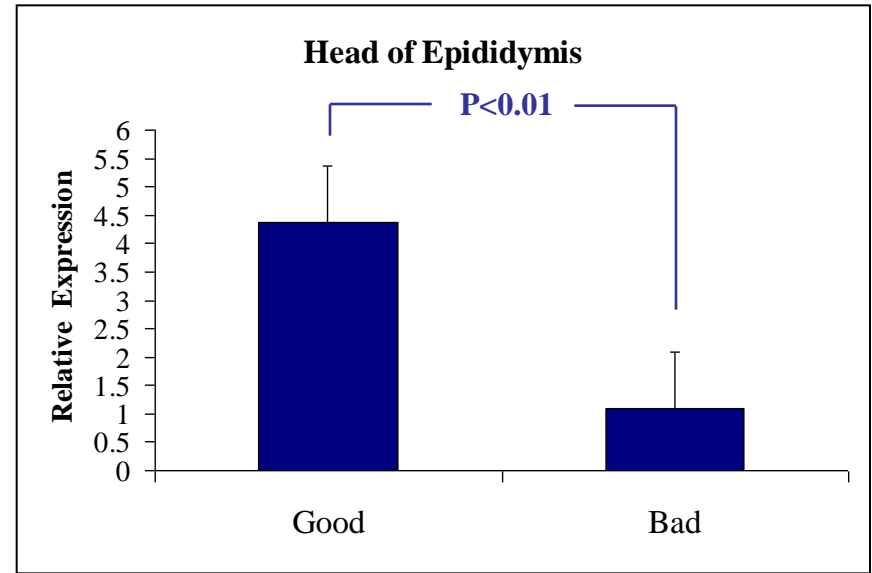
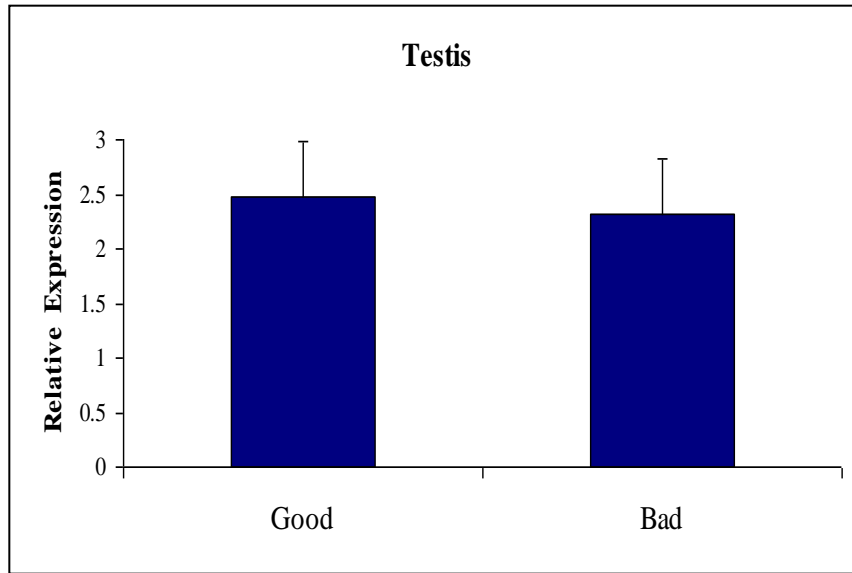
The expression of selected genes in reproductive and non reproductive tissues



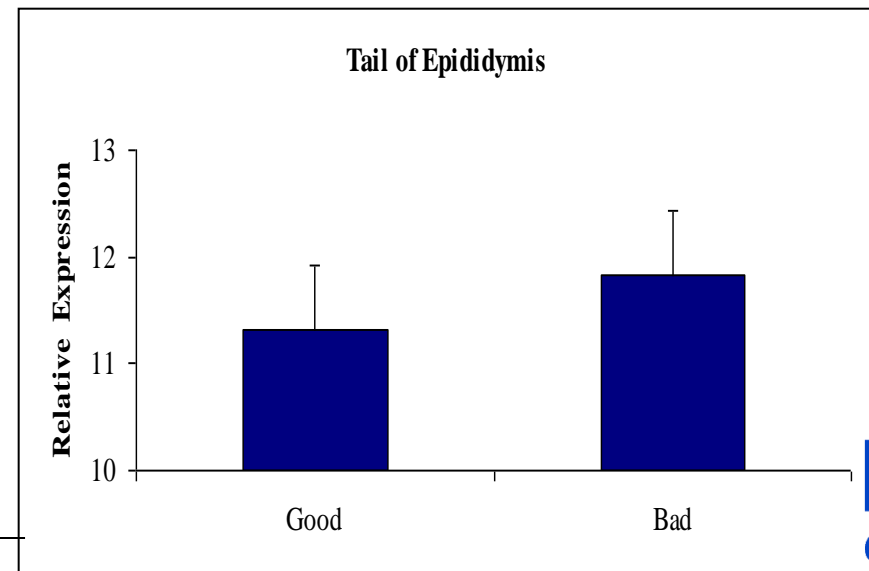
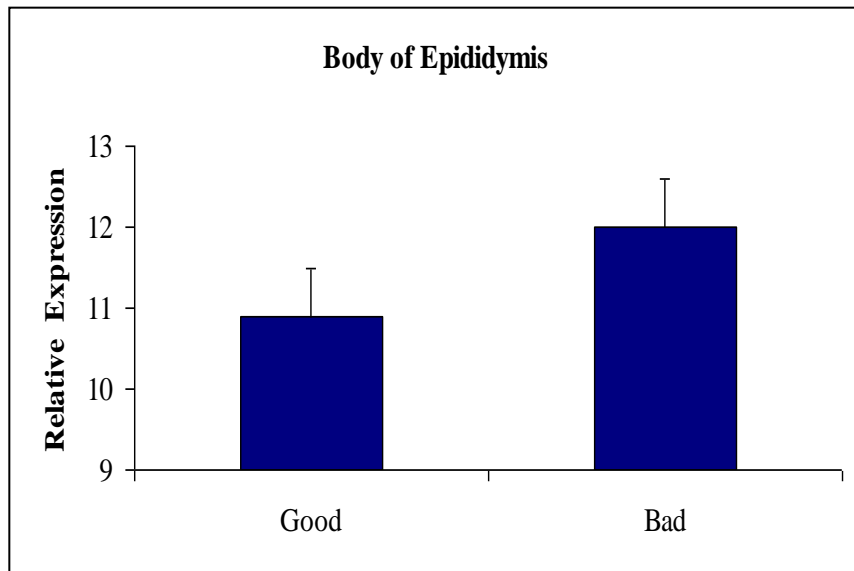
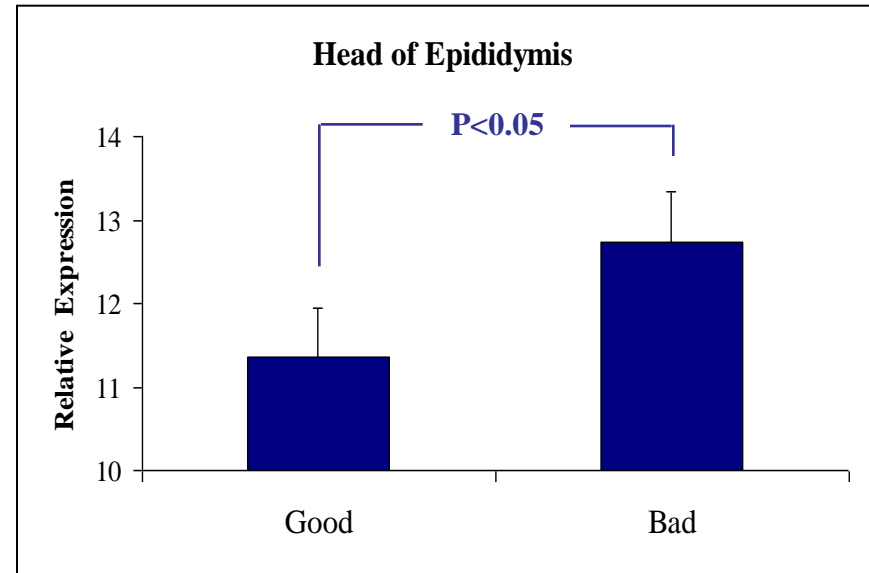
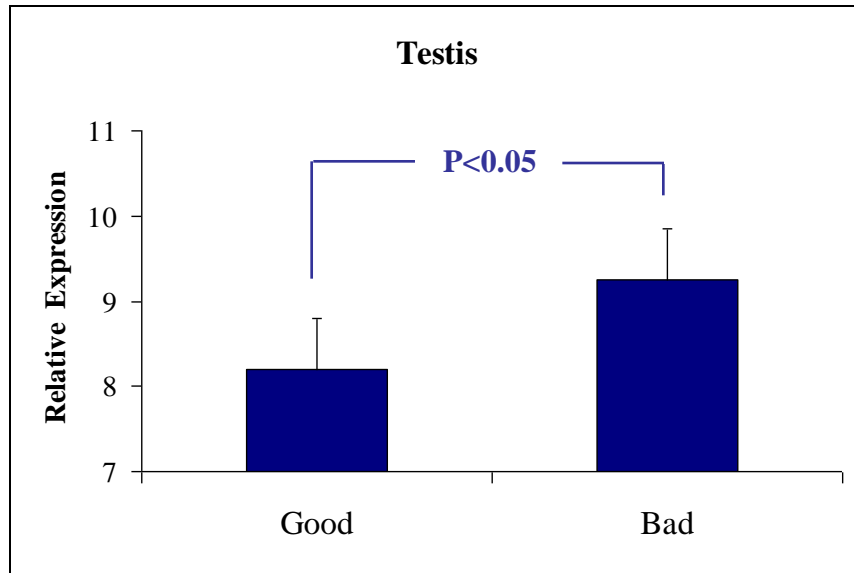
The expression of selected genes in good and bad sperm quality boars



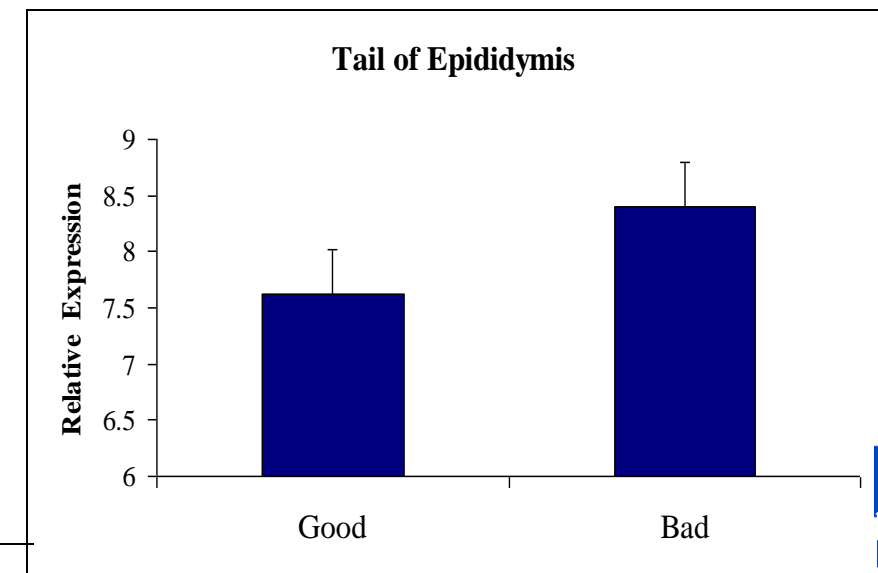
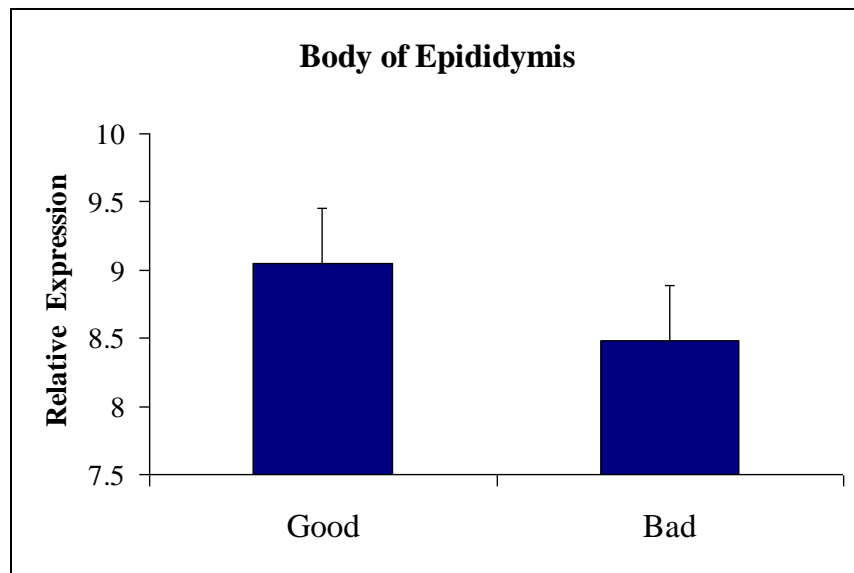
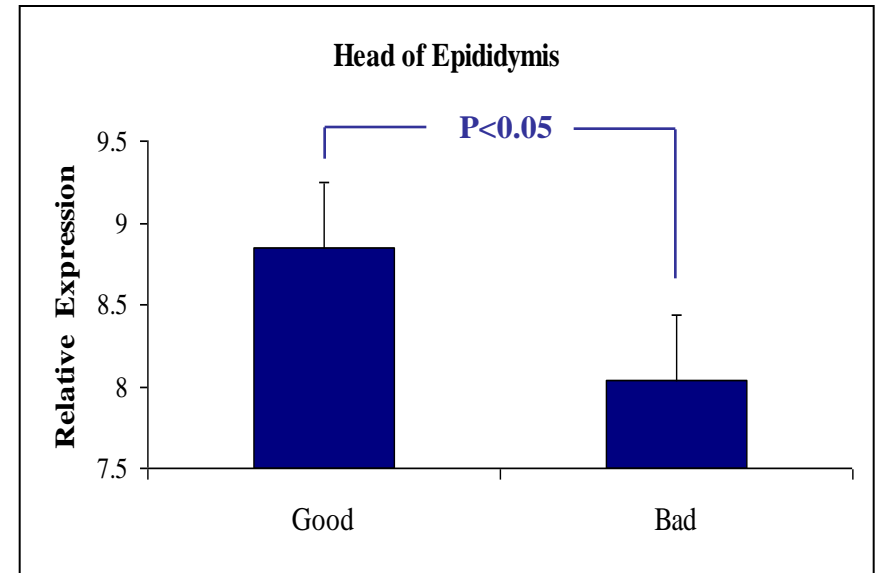
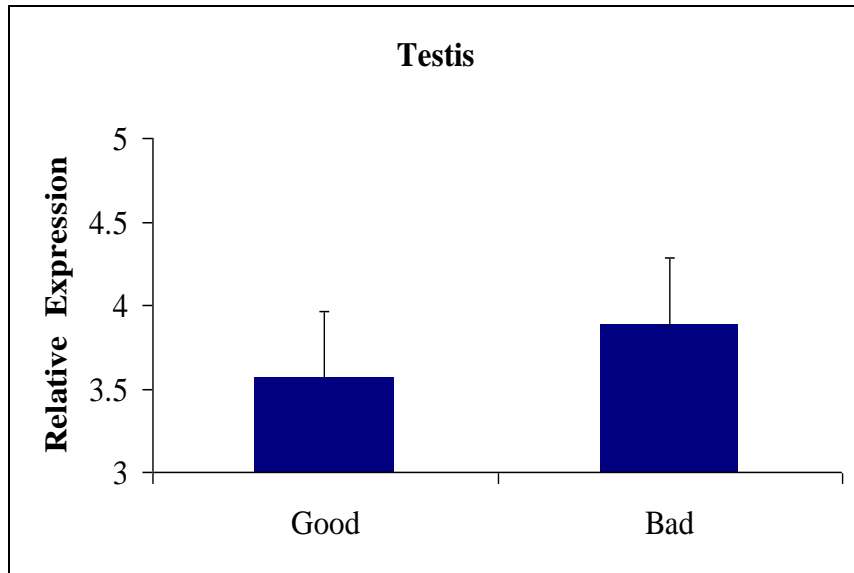
The expression profile of ESR1 in good and bad sperm quality boars



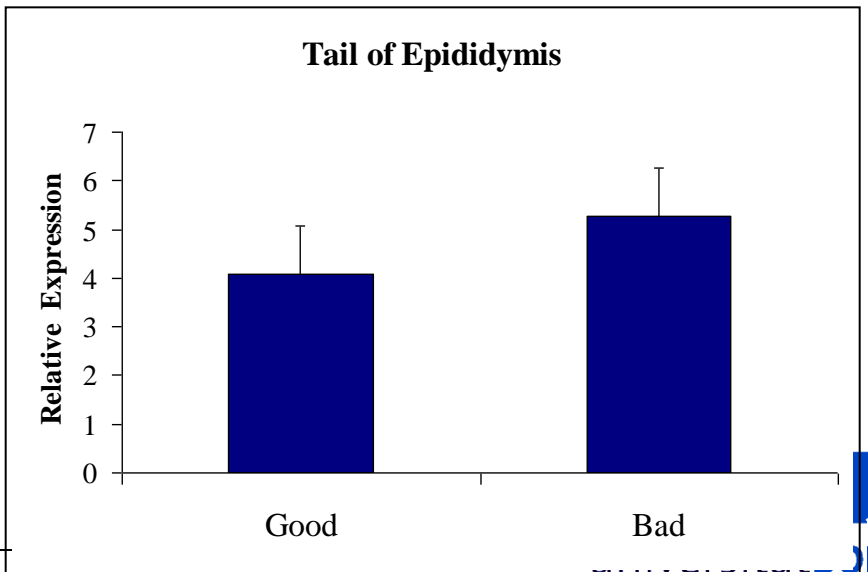
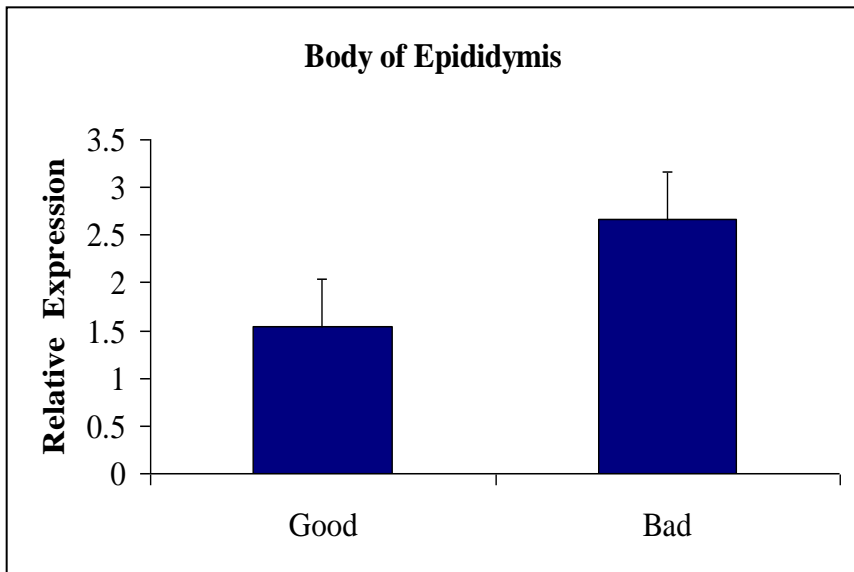
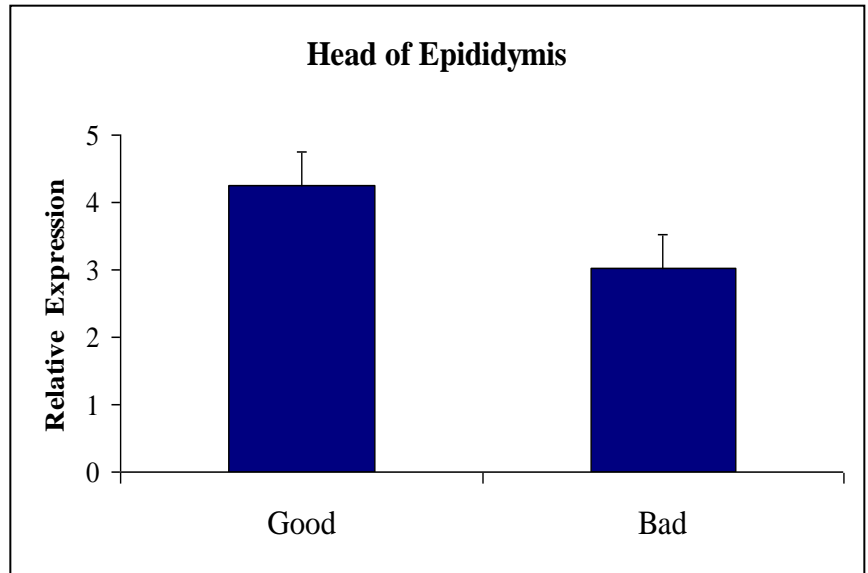
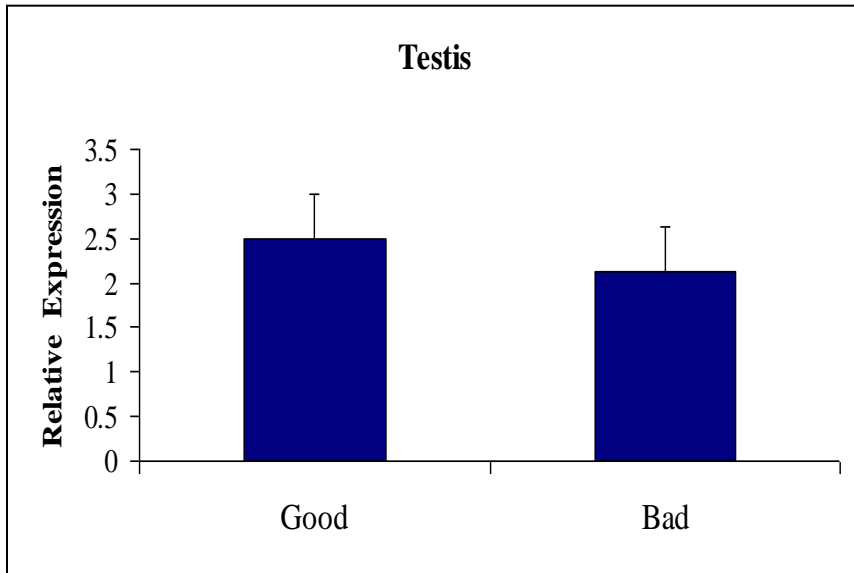
The expression profile of ESR2 in good and bad sperm quality boars



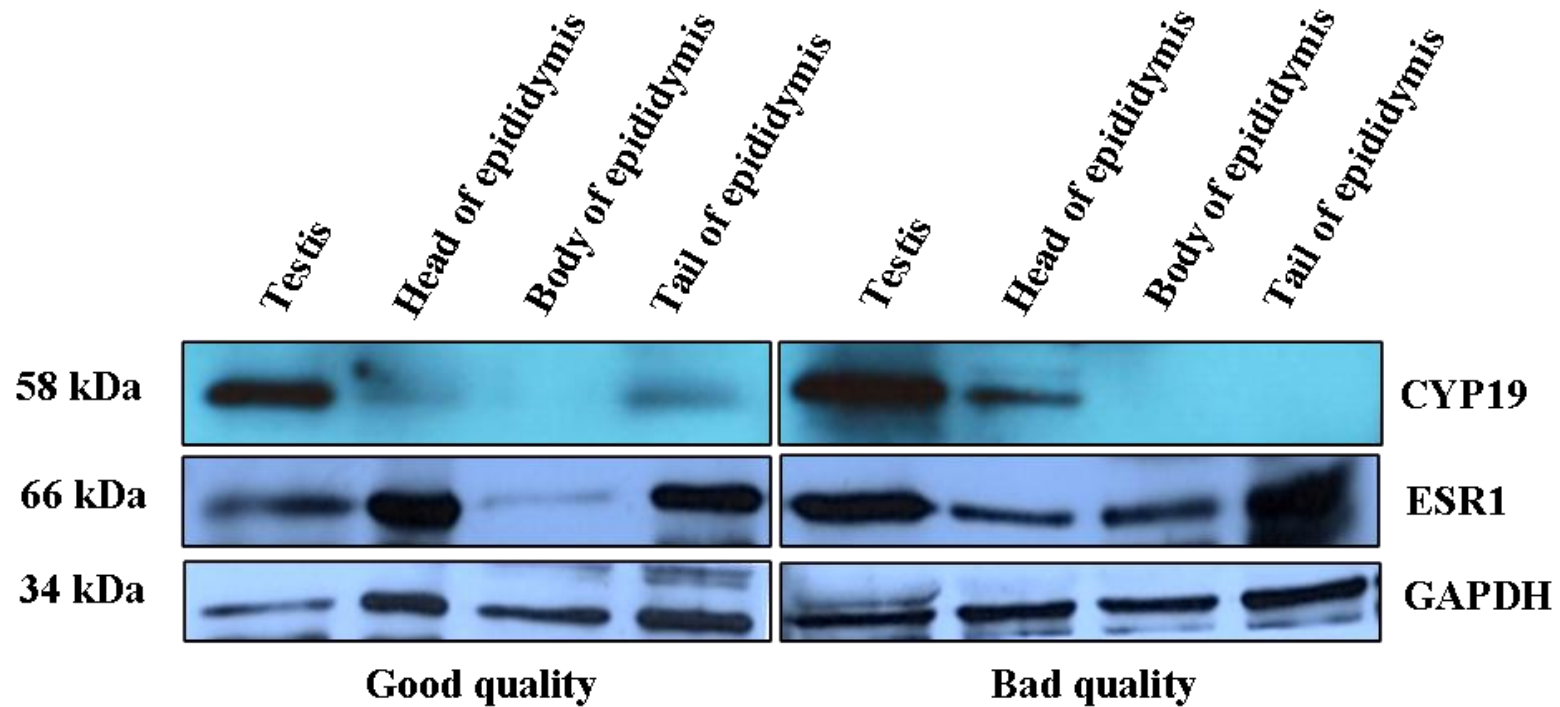
The expression profile of CYP19 in good and bad sperm quality boars



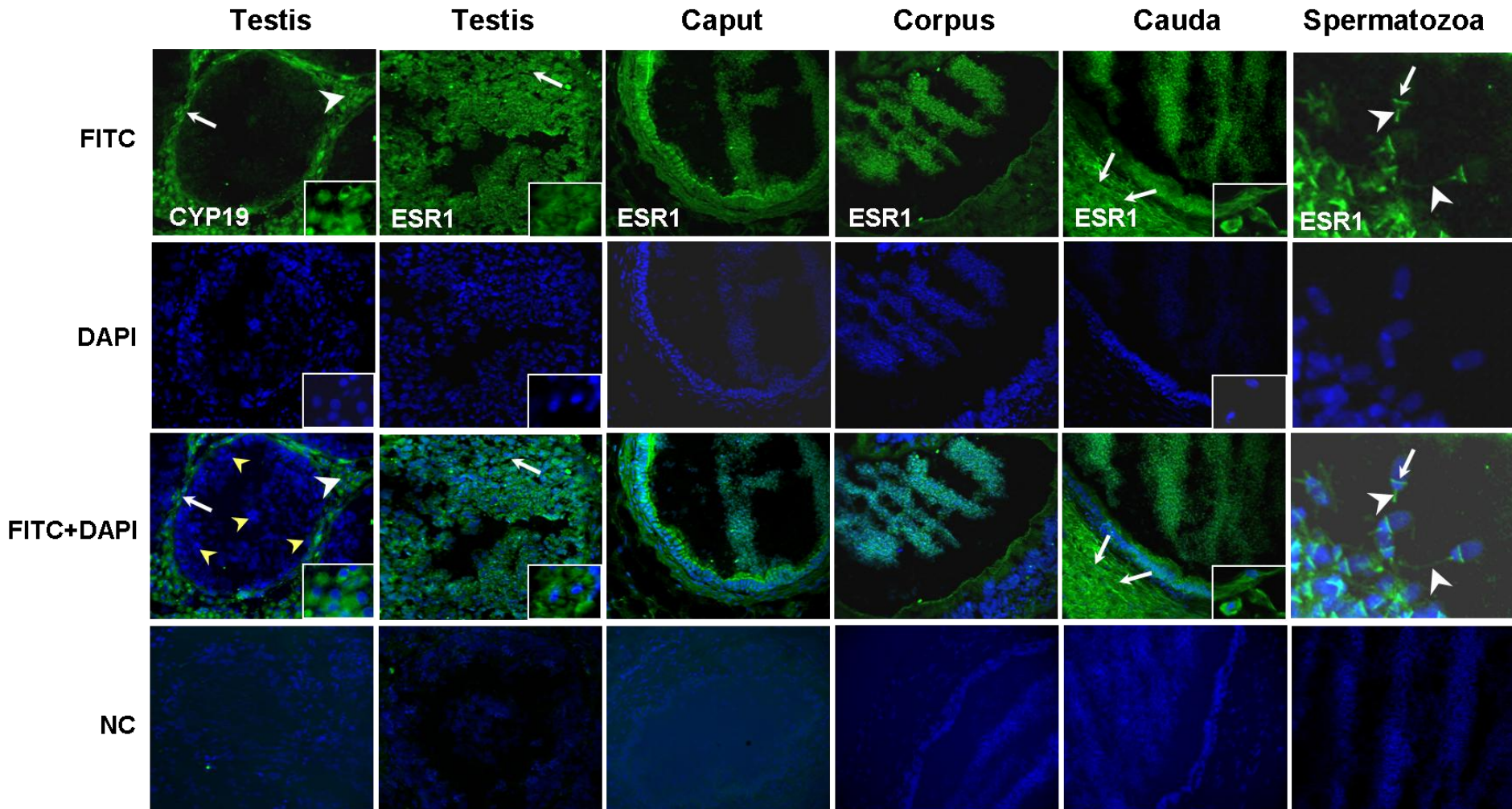
The expression profile of CYP3A in good and bad sperm quality boars



The expression protein of ESR1 and CYP19 in good and bad sperm quality boars



Localization of ESR1 and CYP19



FITC = Immunostain for Fluorescein Isothiocyanate (green)

DAPI = Cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (blue)

NC = Negative Control

Discussion

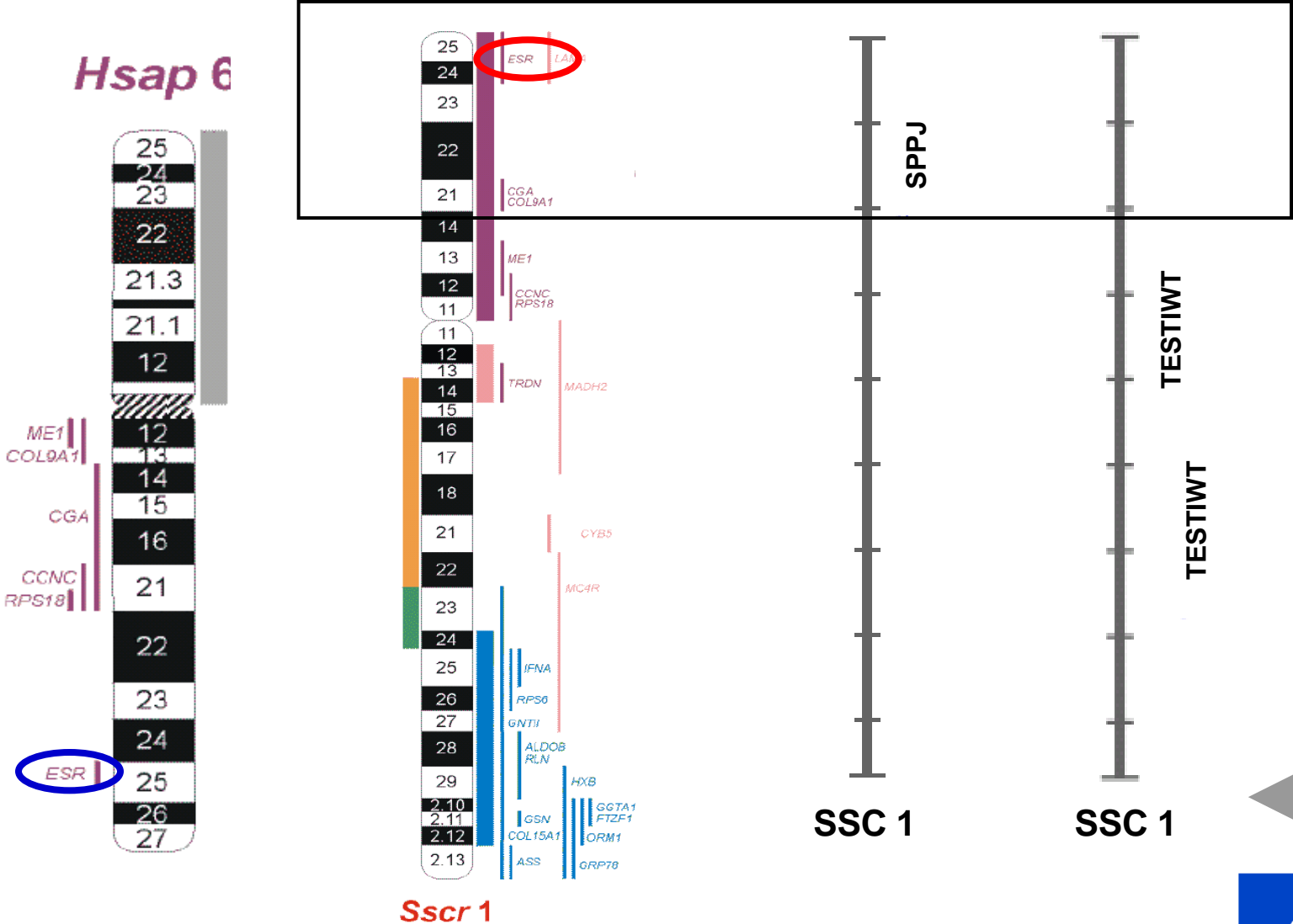
- Up regulation of *ESR1* was found in head of epididymis in boar with good sperm quality
 - The highest concentration of estrogen receptor in the male reproductive tract is found in the head of epididymis in mouse and macaques (West et al, 1990; Shayu et al, 2005)
- Down regulation of *ESR2* was shown in testis and head of epididymis in boar with good sperm quality
 - splicing of *ESR2* gene might have specific function in spermatogenesis (Forsti et al, 2003)
- Up regulation of *CYP19* was revealed in head of epididymis in boar with good sperm quality
 - high level of *CYP19* was detected in head of epididymis of rhesus monkey epididymis (Martinez et al. 2007)

Discussion

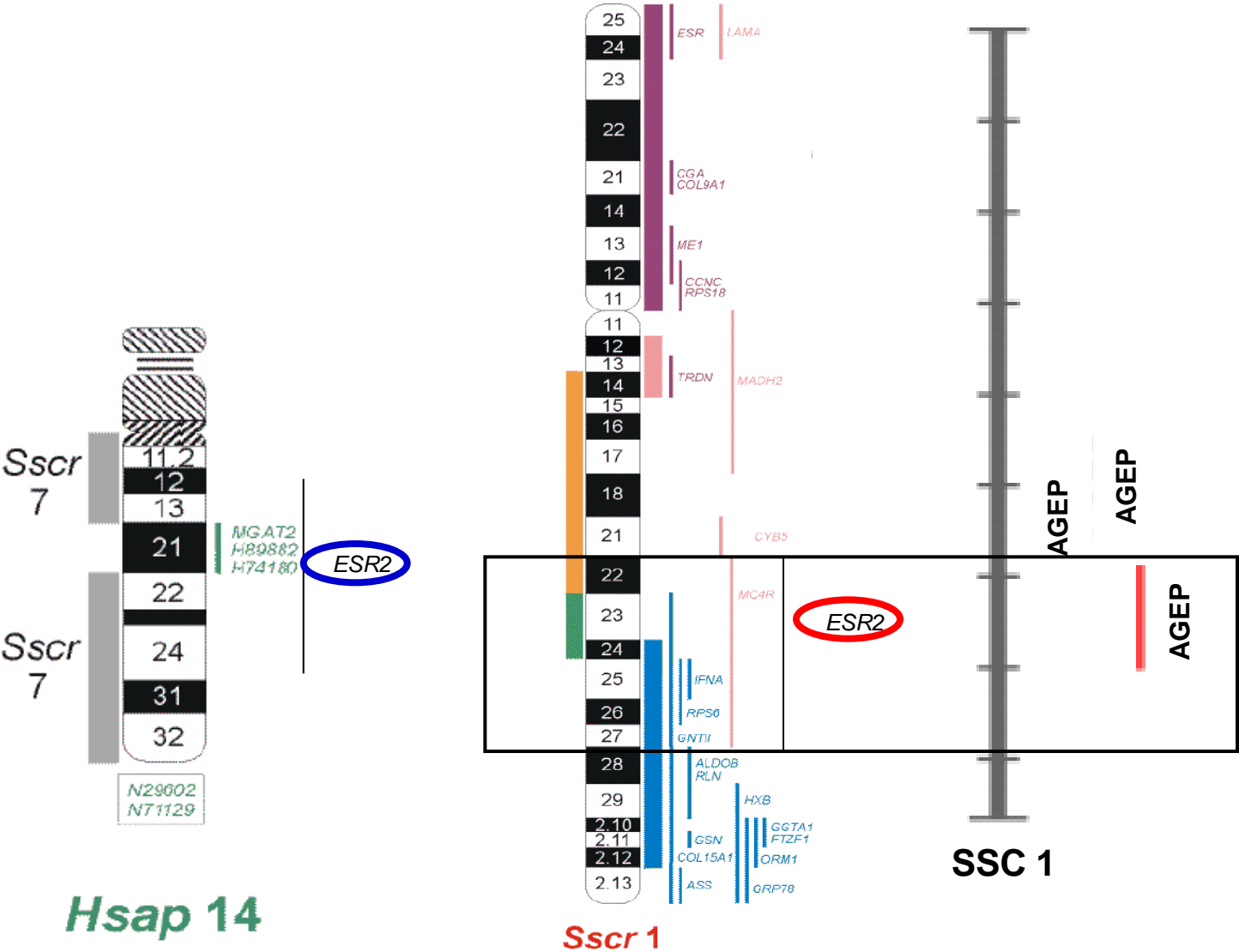
- Localization of CYP19 was observed in leydig and epithelial cells
→ The highest CYP19 activity and proliferation occurred in the Leydig cells during puberty to adulthood (Hess et al, 2004)
- ESR1 localization was detected in different part of epididymis
→ indicate important for regulating protein secretion and to be involved in the initiation of sperm motility (Pearl et al, 2007)
- Localization of ESR1 was localized in the post acrosomal region
→ ESR1 was localized in post acrosomal region of the sperm head (Solakidi et al. 2005)
- ESR1 was found to be localized on tail of sperm
→ involve in cell survival and motility (Aquila et al (2004)

¹Hess et al (2004); ²Pearl et al (2007); ³Ramalho-Santos et al (2002); ⁴Aquila et al (2004)

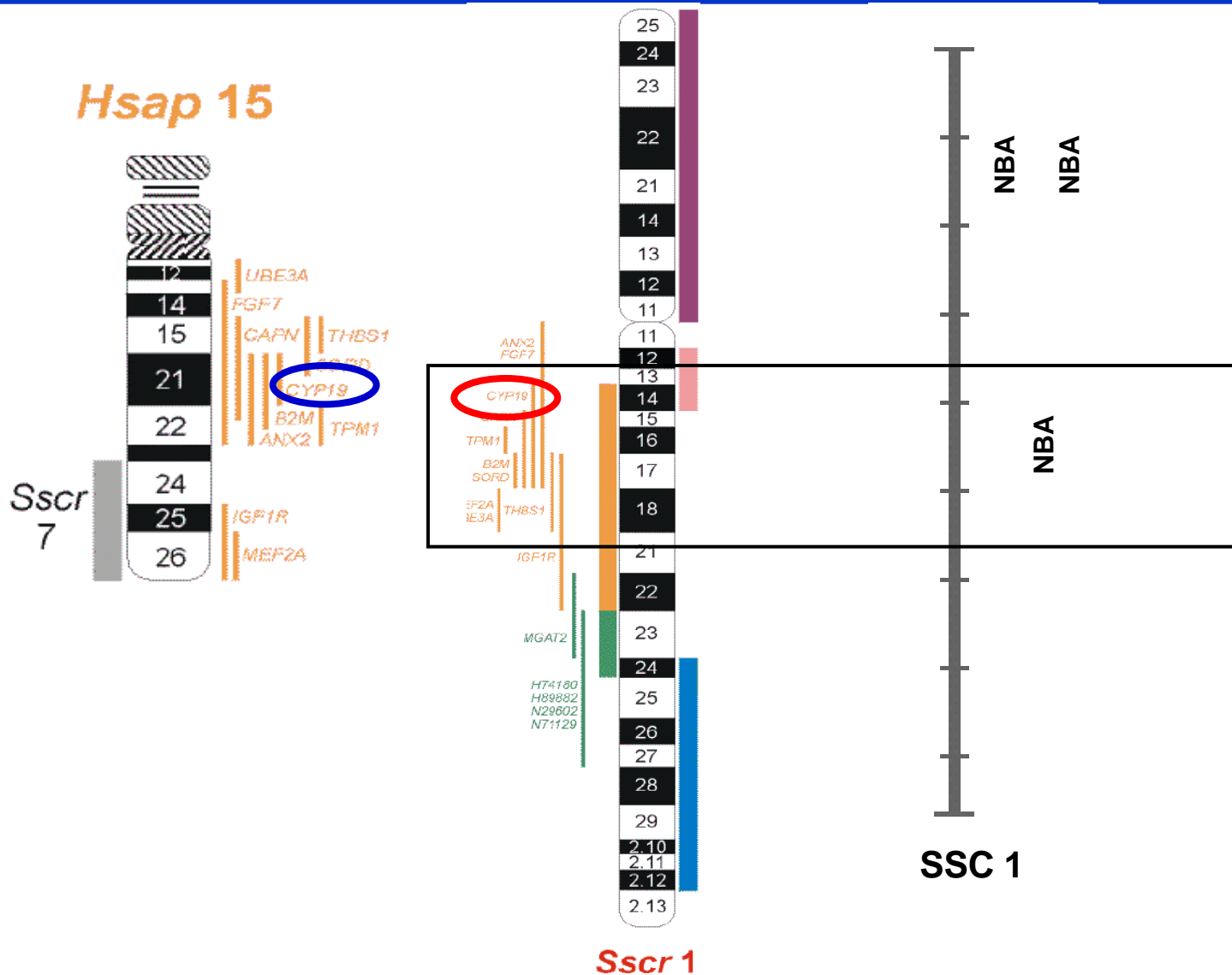
QTL for male reproduction trait



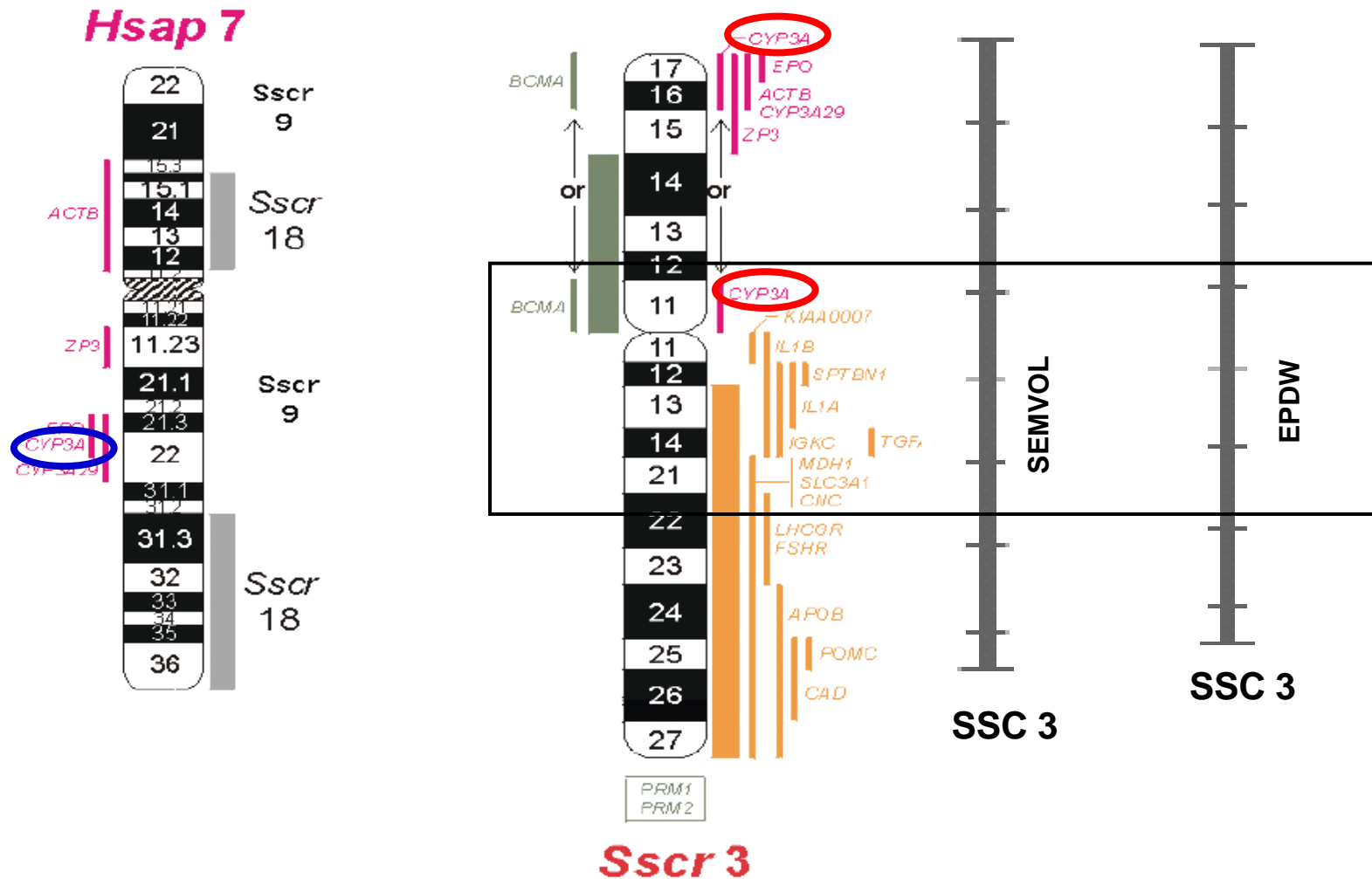
QTL for male reproduction trait



QTL for male reproduction trait



QTL for male reproduction trait



Discussion

- *ESR1* gene mapped on SSC1p24-25
 - QTL for total sperm per ejaculate and close to the QTL for sperm, testicular weight (Xing et al, 2008; Ren et al, 2008) ►
- *ESR2* gene mapped on SSC1q22-27
 - QTL affecting reproductive traits for number of nipples and age at puberty (Cassedy et al, 2001) ►
- The assignment of the porcine *CYP19* gene mapped SSC1 q14-17
 - *CYP19* to be a candidate gene affecting fertility performance in farm animals : bovine → 10q26; buffalo → 11q26 ; goat → 10q32.; sheep → chr 7 (Ianuzzi et al, 2001) ►
- *CYP3A* gene mapped on SSC3, in the region p16-p17 or p11
 - QTL effect associated with semen volume and epididymal weight (Ren et al, 2008) ►

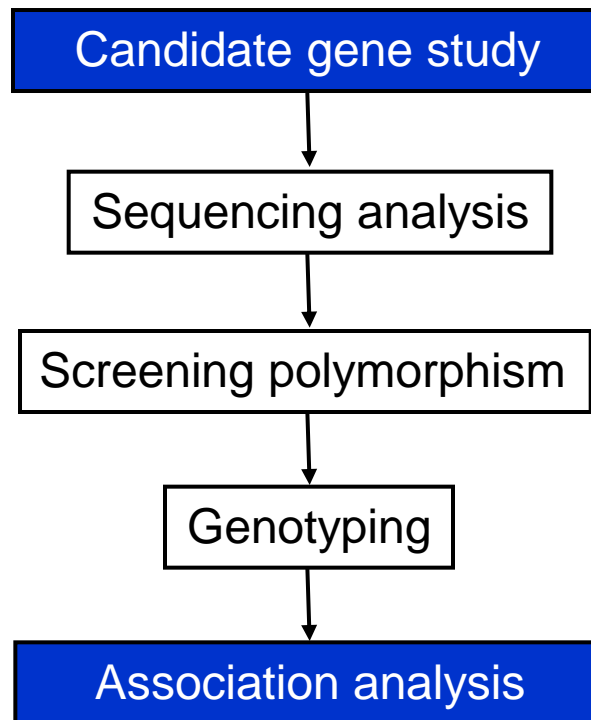


Conclusion

- We suggest that *ESR1*, *ESR2* and *CYP19* might be good candidate genes for the sperm quality and fertility which could be used in boar selection

Prospective aspect

SNPs analysis need to be done for ESR1, ESR2 and CYP19 as candidate genes for sperm quality and fertility traits



Acknowledgement

- DAAD
- Prof Dr. Karl Schellander
- Dr. Dawit Tesfaye, Dr. C. Phatsara, Mr. M. Ulas Cinar
- Dr. Ralf Nolten
- Prof. Dr. Mathias Becker, Mrs Susanne Hermes and Mrs Britta Chaffik
- Ms. Kanokwan Kaewmala
- Member of Animal Science

Thank you for your attention!

