Différenciation des cellules germinales mâles *in vitro*

13è journée sur l'AMP de l'Hôpital Américain de Paris
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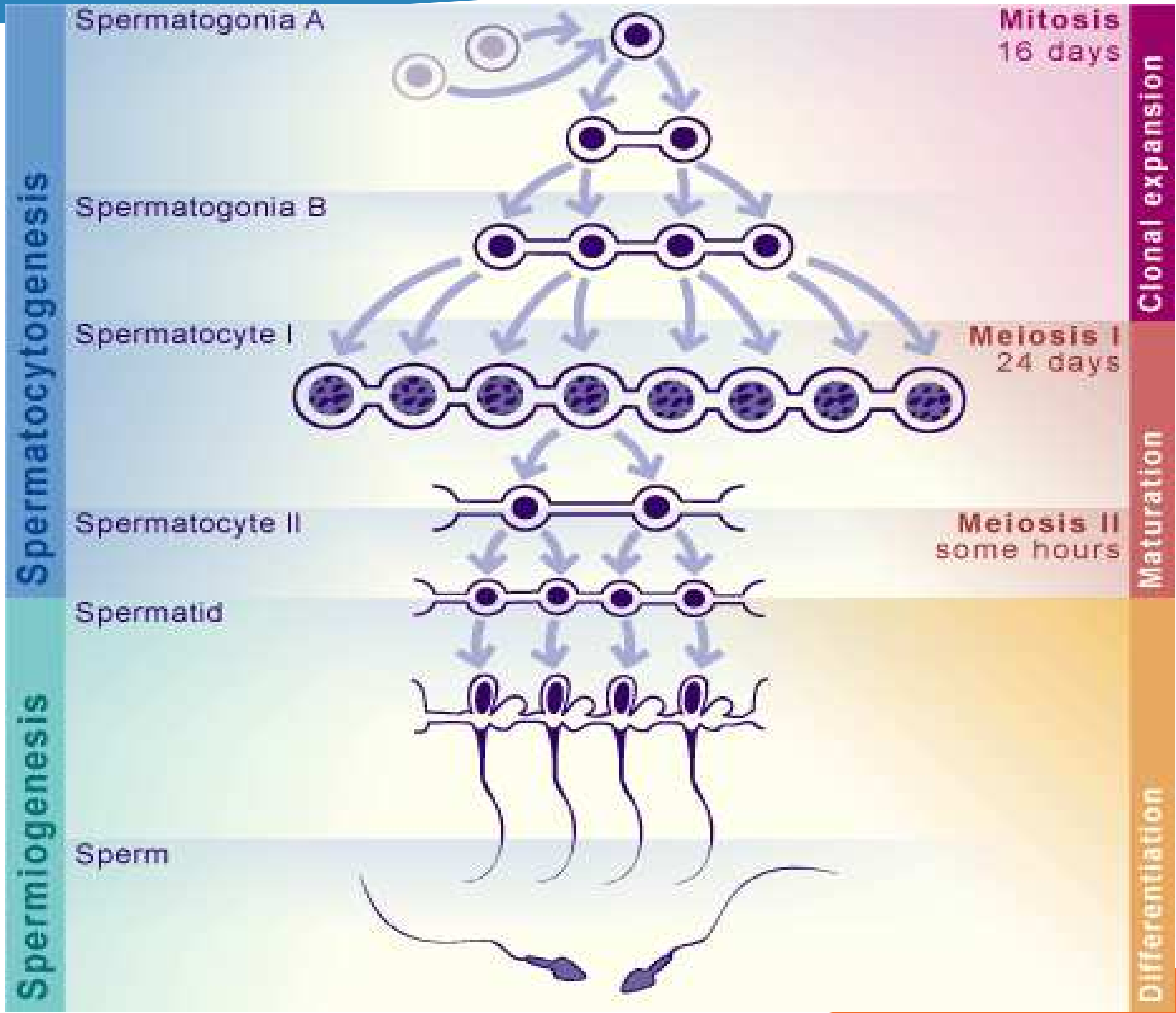
Spermatogenesis



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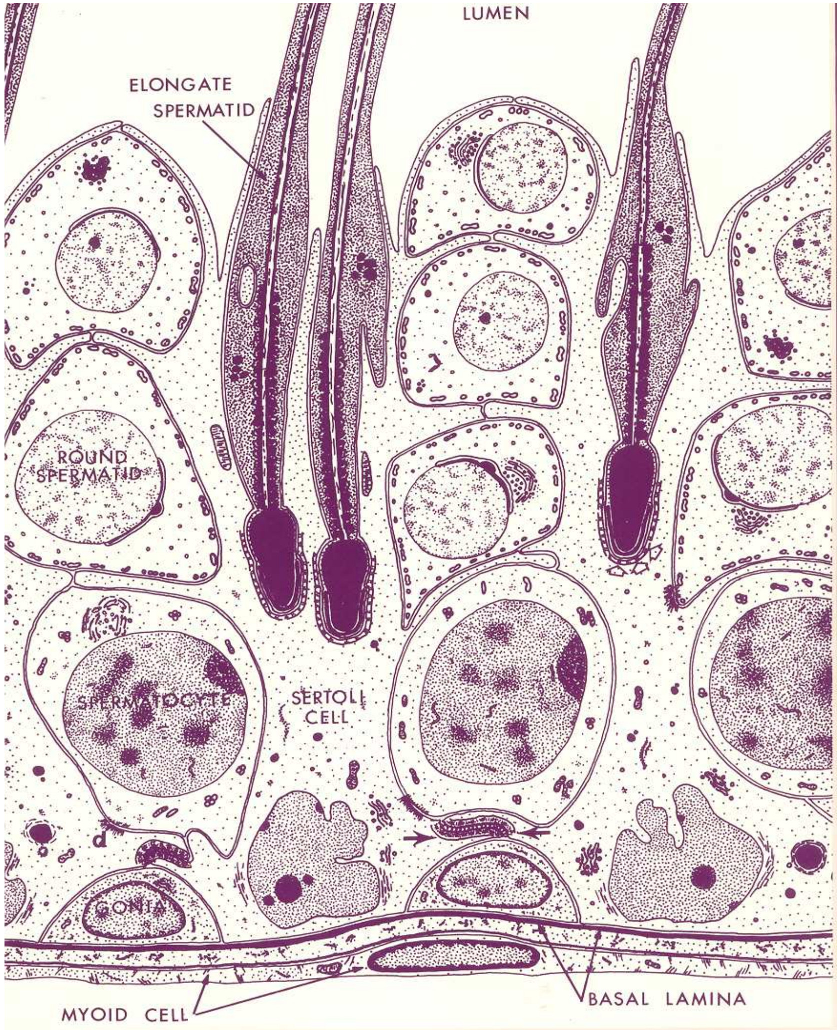
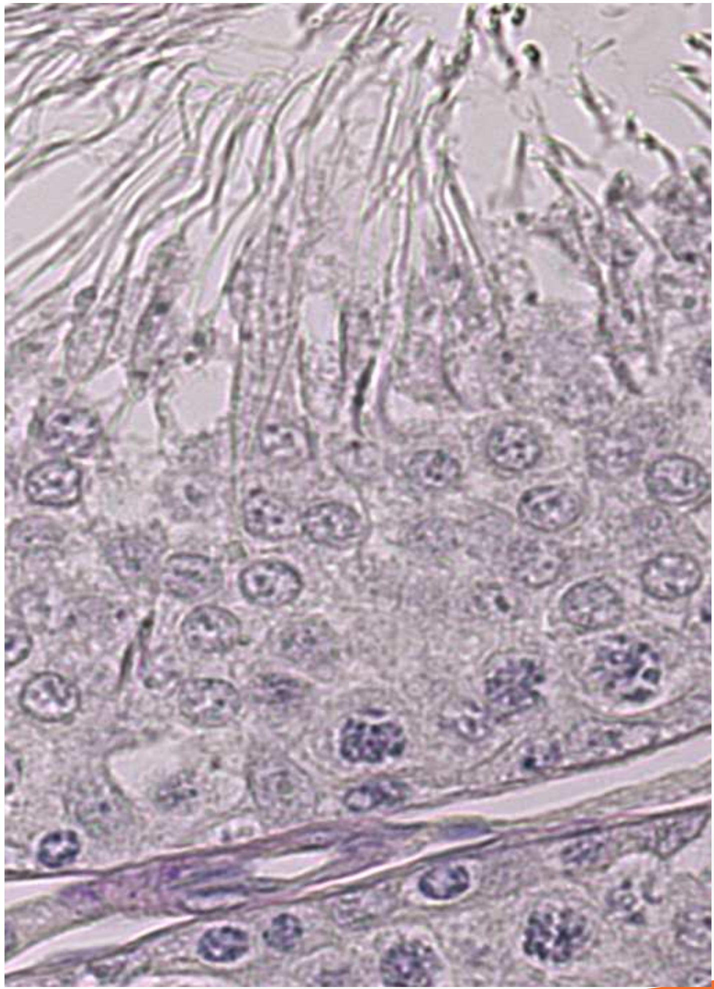
http://www.embryology.ch/francais/cgametogen/spermato03.html

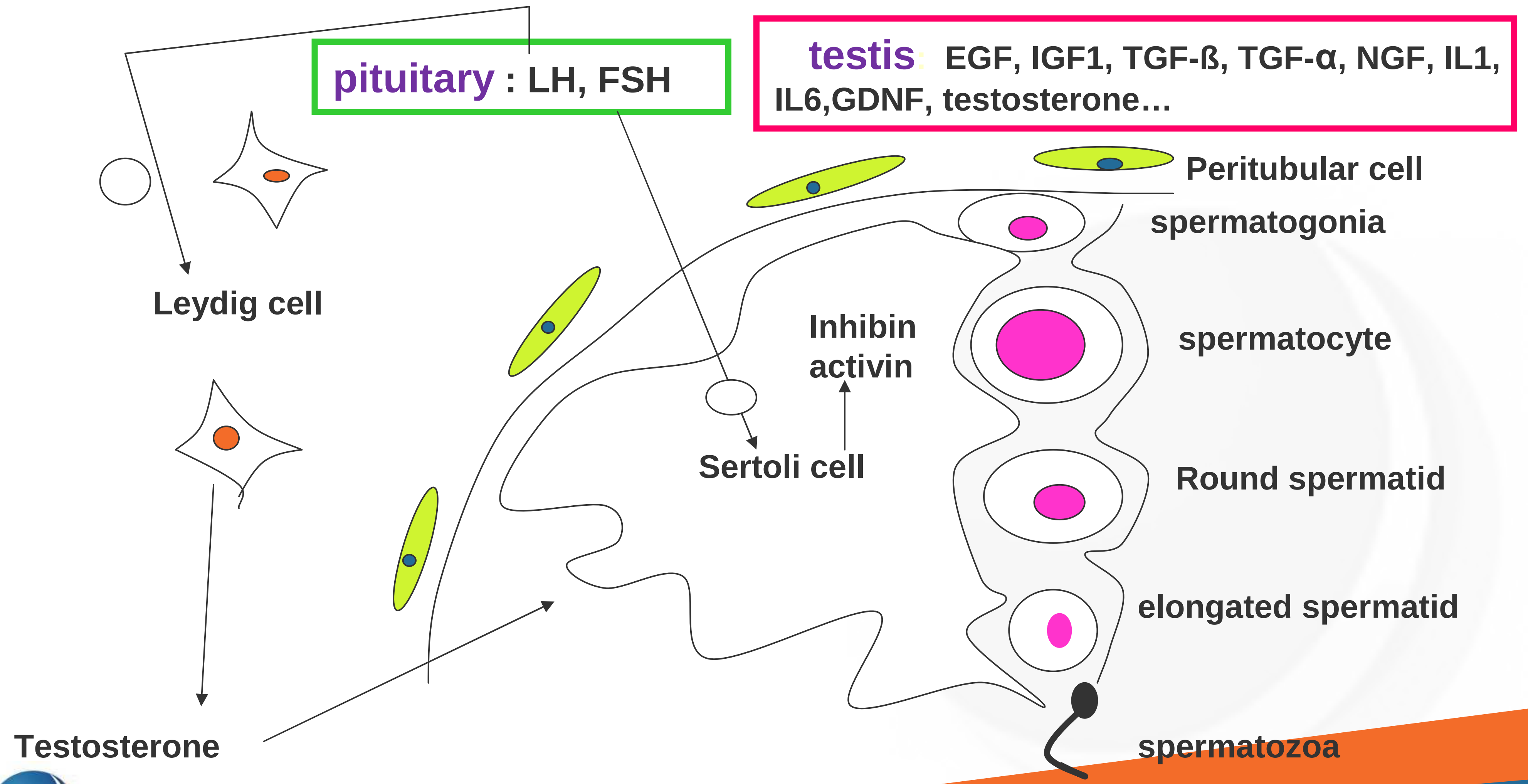
Seminiferous tubules





Russel, L.D., 1990

Regulations of spermatogenesis



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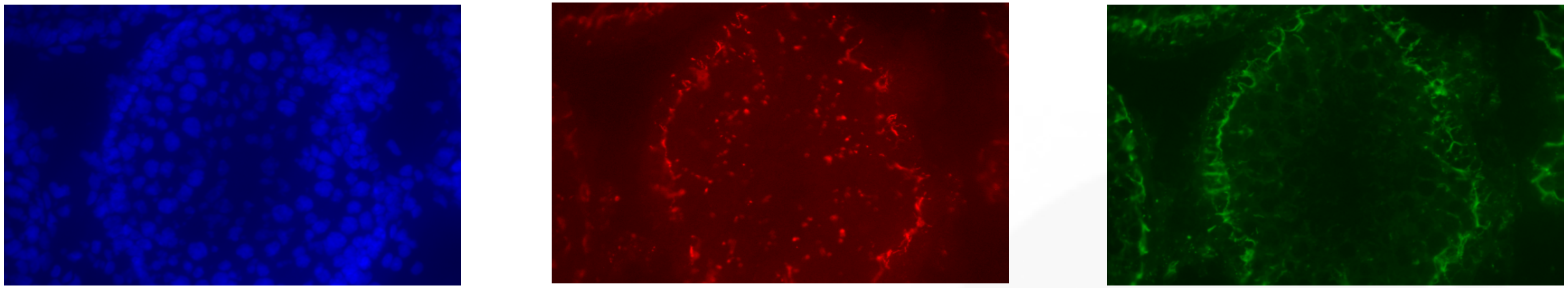
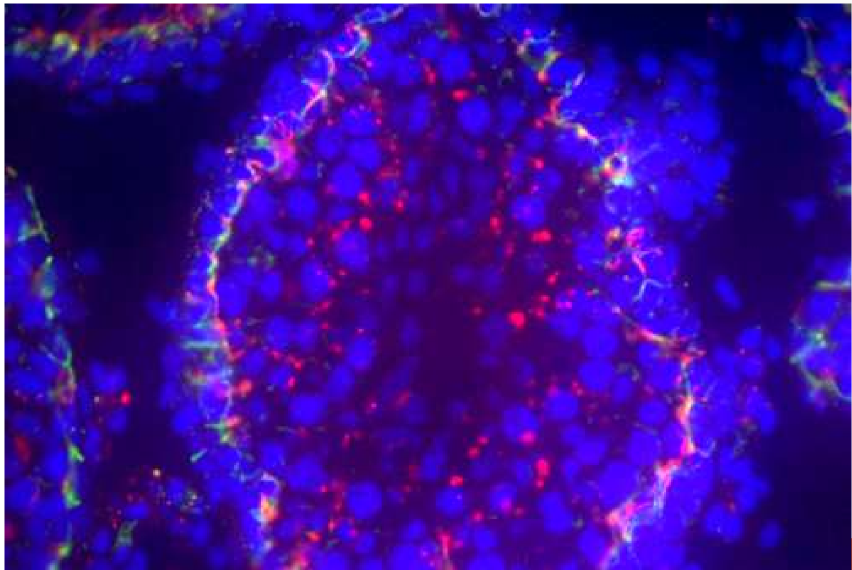
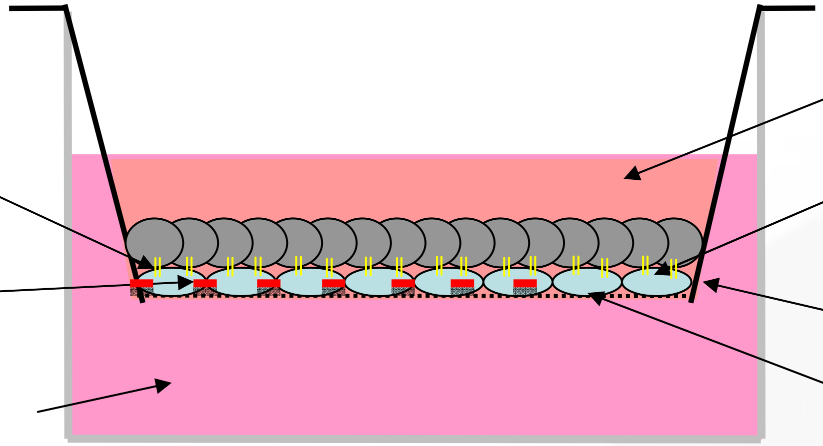
Gap and tight junctions build the « niche » for spermatogenesis





In Vivo 28 dpp (Cx 43/ZO1)

Bio-AlteR Technology®



3D co-culture system in bicameral chambers

- **Cultures of seminiferous tubules: rats** (8/20 days old) **; human)**
- **Maintains the blood-testis barrier**

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Seminiferous tubules « in toto »

Purpose

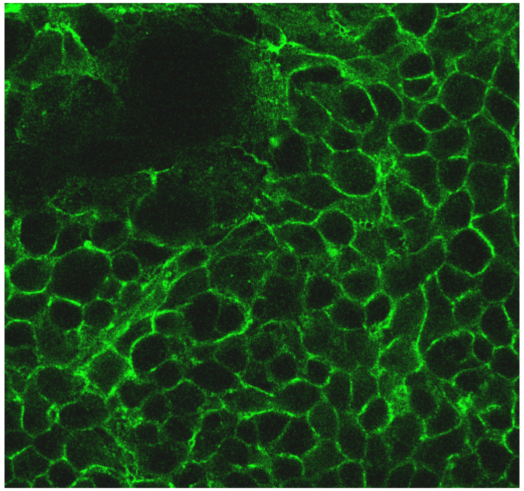
Simultaneous study of all steps of spermatogenesis

Main Characteristics

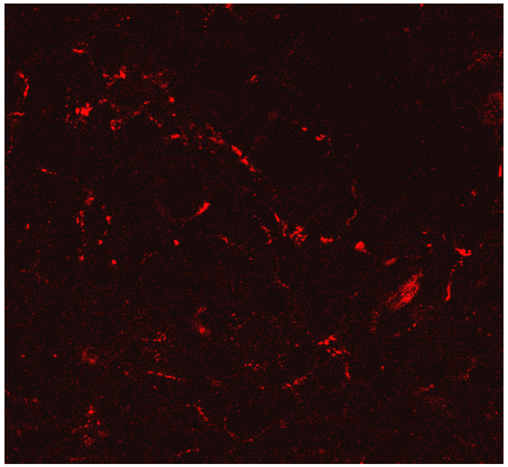
- Culture of all types of germ cells present in the tubes at the time of seeding
- Cell junctions are preserved during seeding
- Germ cells and somatic cells are « corresponding cells ».

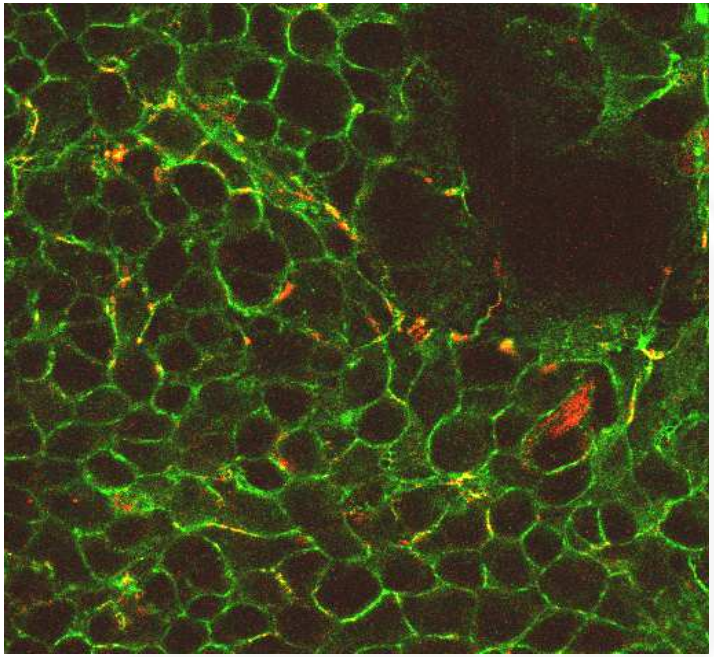
Key of success: Bio-AlteR® keeps BTB intact

Occludin



Cx 43





after 12 days of a culture of testes from 20-day-old rats

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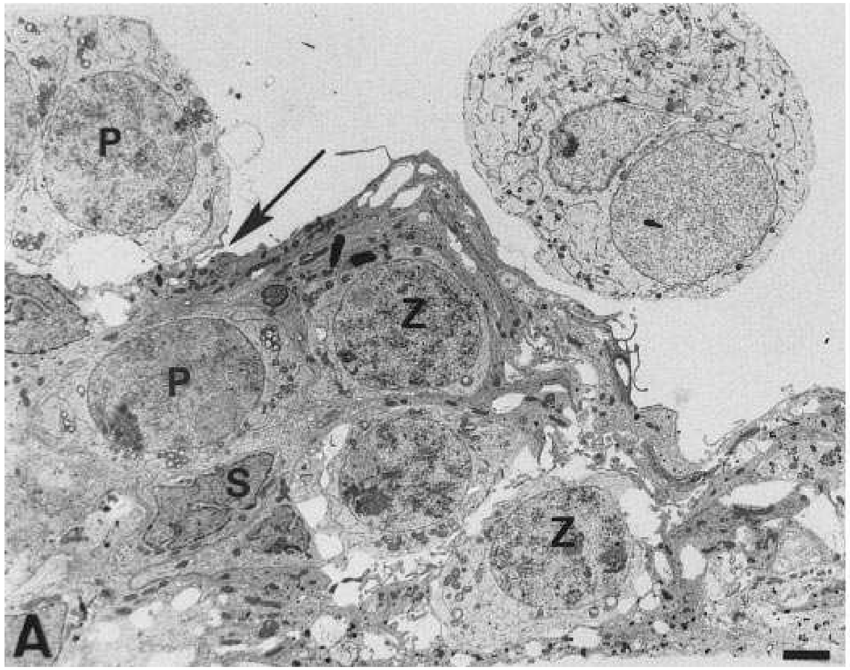
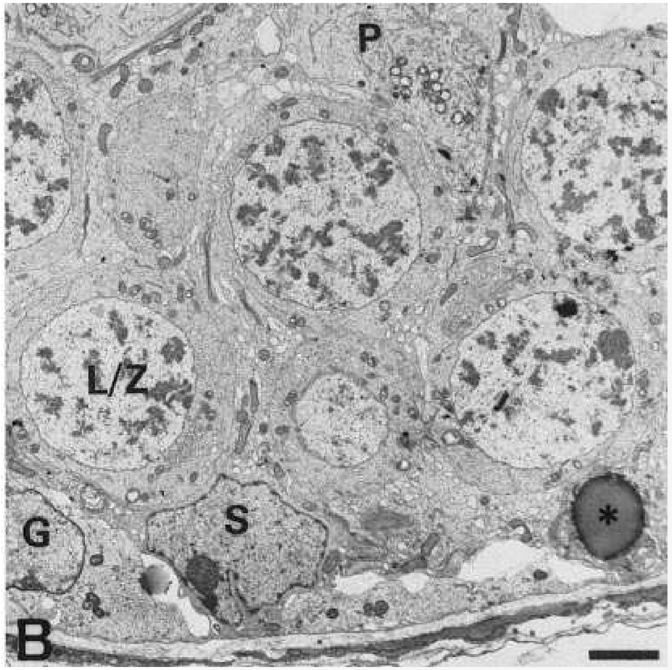
Physiological validation

Maintenance of the BTB throughout the culture period



(A) Cross section of the insert of culture after 7 days of a culture of 20-day-old rats



(B) Cross section of the seminiferous tubule of a 28-day-old rat (Staub & al 2000)

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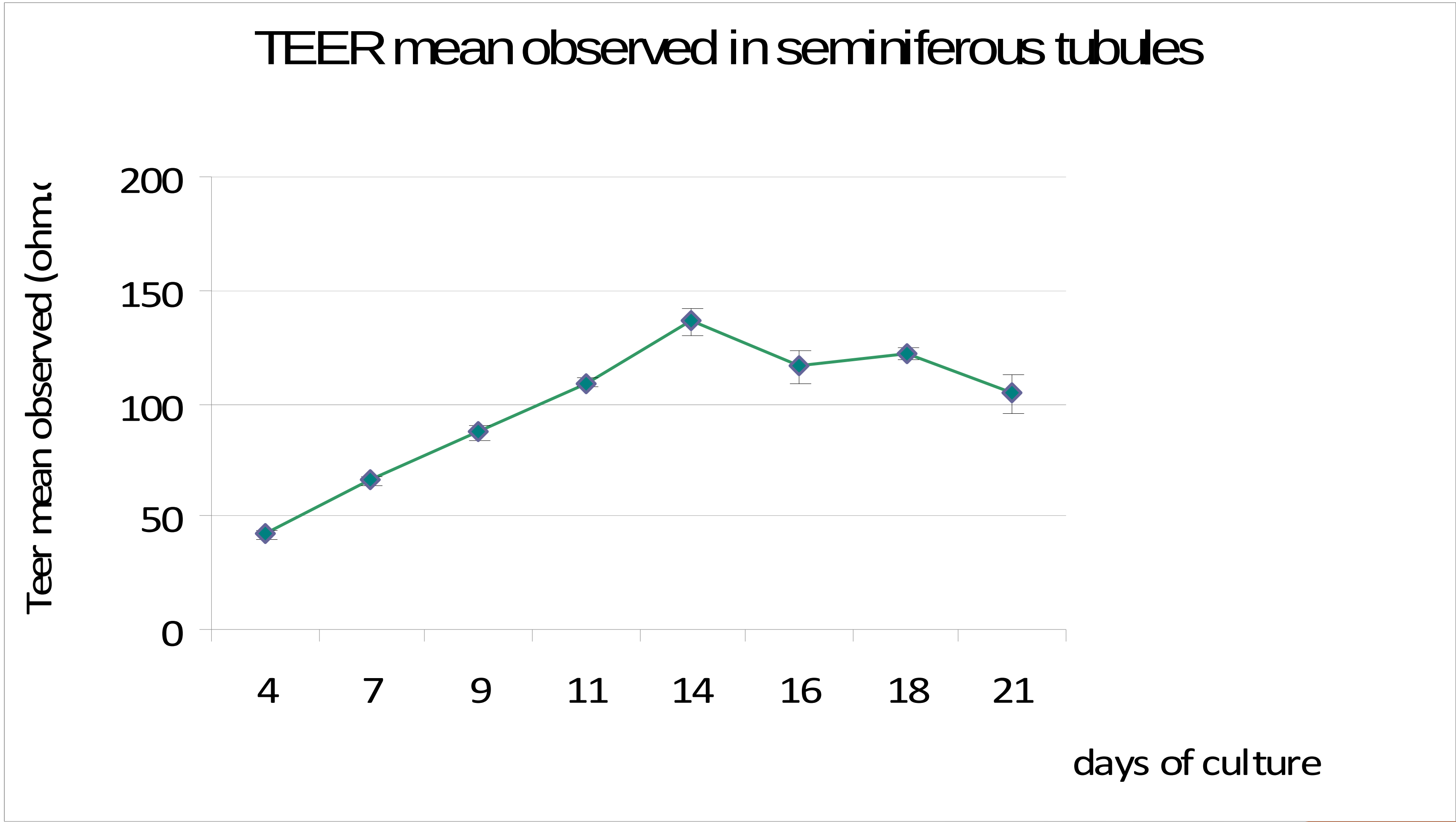
Résistance électrique trans-épithéliale



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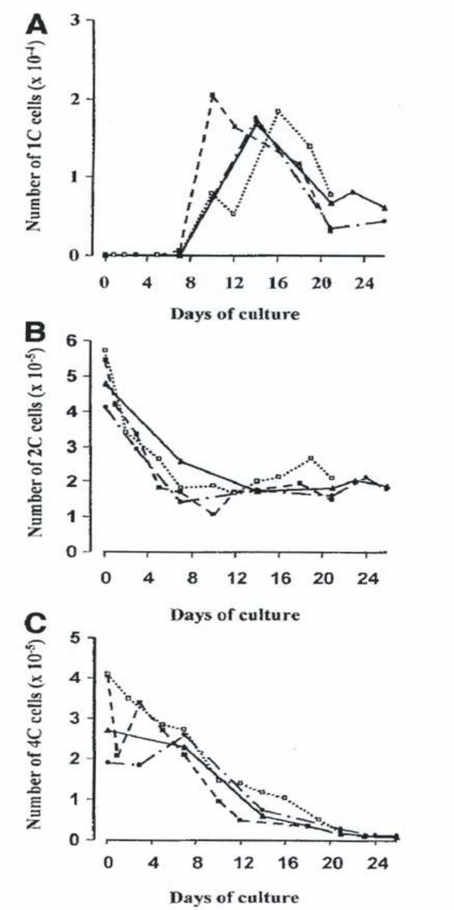
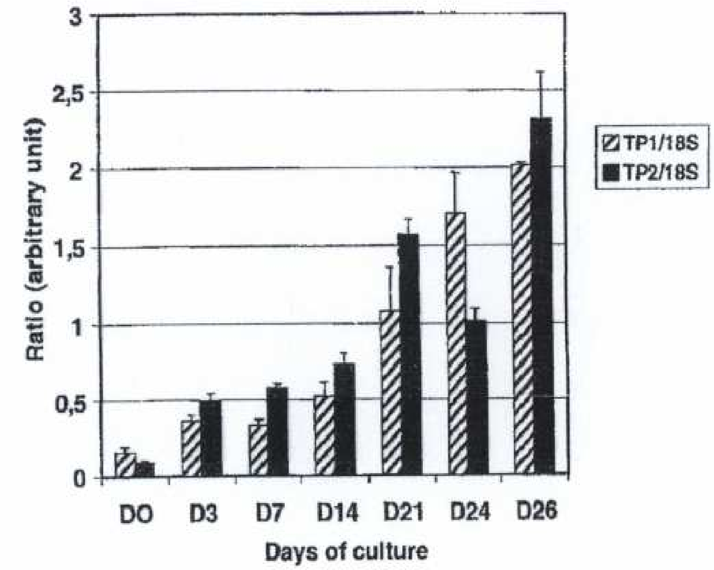
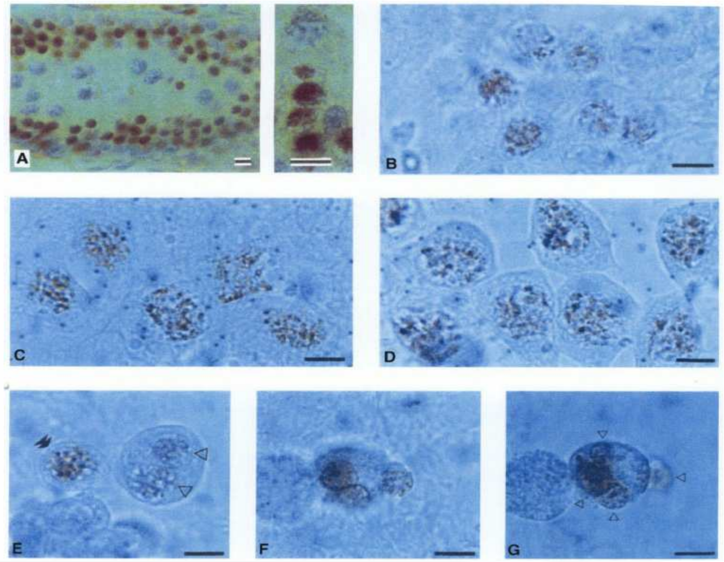
Seminiferous tubule cultures allow to perform the whole meiotic process *ex vivo*







(from Staub et al 2000)

Analysis of cell populations (FACS) Gene expression (RT-PCR) Morphology (cytology/BrdU labeling)

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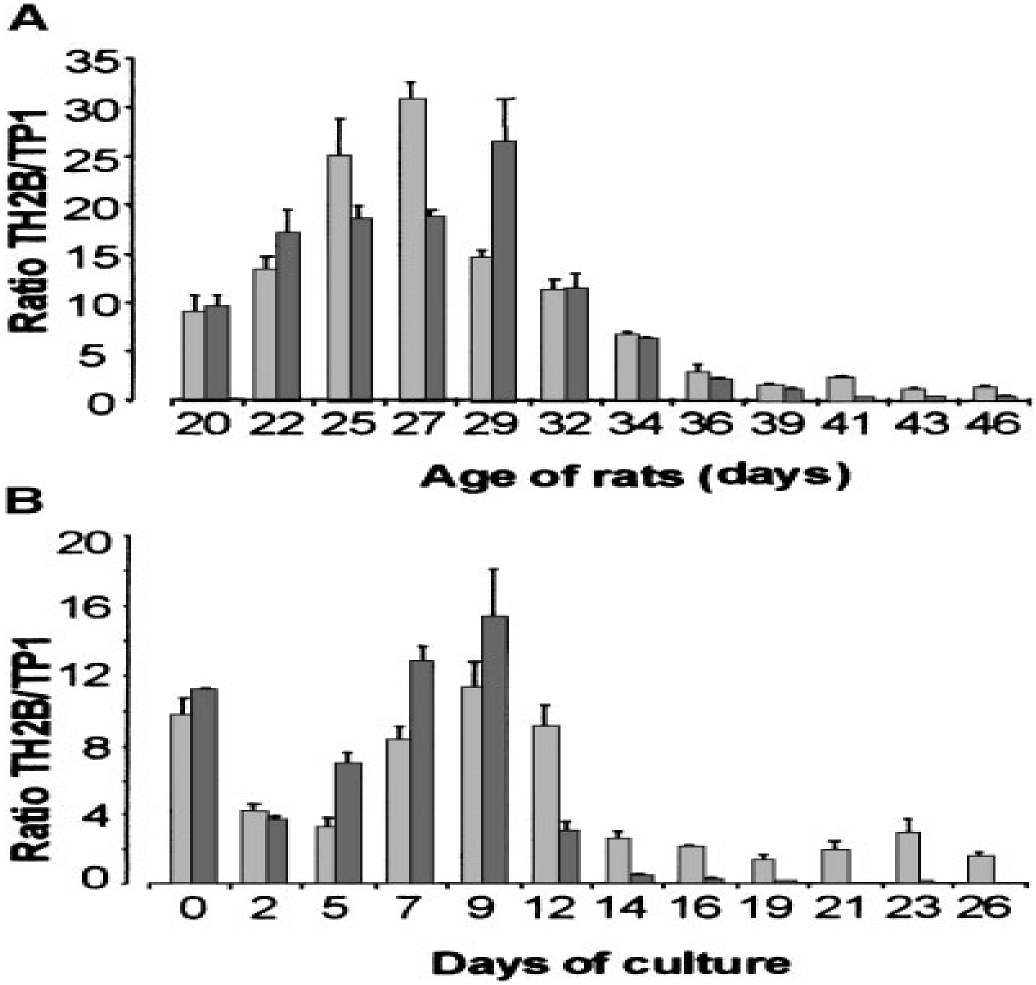
Comparaison des cinétiques d'expression des gènes in vivo in vitro



In vivo

Individual values of two different series of animals

***In vitro* (Bio-Alter®)**

Individual values of two different cultures from 20-days old rats

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(from Perrard *et al* 2003)

Quantitative aspects of the pubertal development of the first meiotic prophase in vivo/ex-vivo

	Age of rats	Leptotene	Zygotene	Pachytene	Diplotene
In vivo	23 days	18%	15.7%	65.1%	1.2%
In vivo	**42 days**	**3.7%**	**12.5%**	**67.8%**	**16%**
In vivo	100 days	2.3%	8.1%	83.1%	6.5%
In vitro (Bio-AlteR®)	**Culture of 23-days old rats (day16)**	**4.3%**	**14%**	**60%**	**21.7%**

The development of the meiotic step in testes of pubertal rats is very similar *in vivo* and *in vitro*

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(Geoffroy-Siraudin et al Tox Sci 2010)

Sertoli cell/germ cell co-cultures

Purpose

Make a « zoom » on a specific step of spermatogenesis

Main characteristics

- Culture of a specific type of germ cells in a large quantity (spgonia/ PL; PS; RS). « zoom » on a particular step
- Destruction of the cell junctions, but re-formation in vitro
- Possibility to grow germ cells with somatic cells from different sources.

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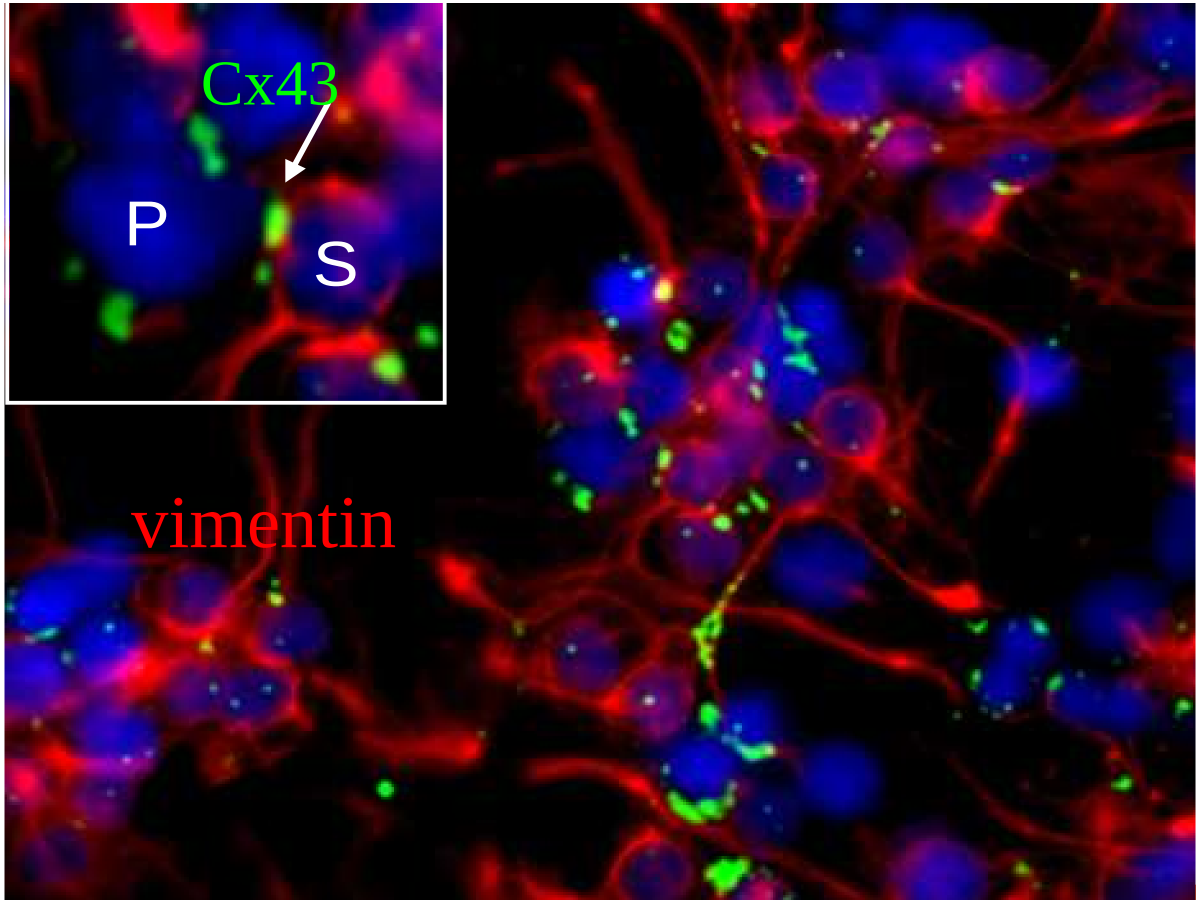
Physiological validation

*1. Re*formation of cell junctions *In vitro* (Bio-AlteR®)



(from Godet et al 2008)

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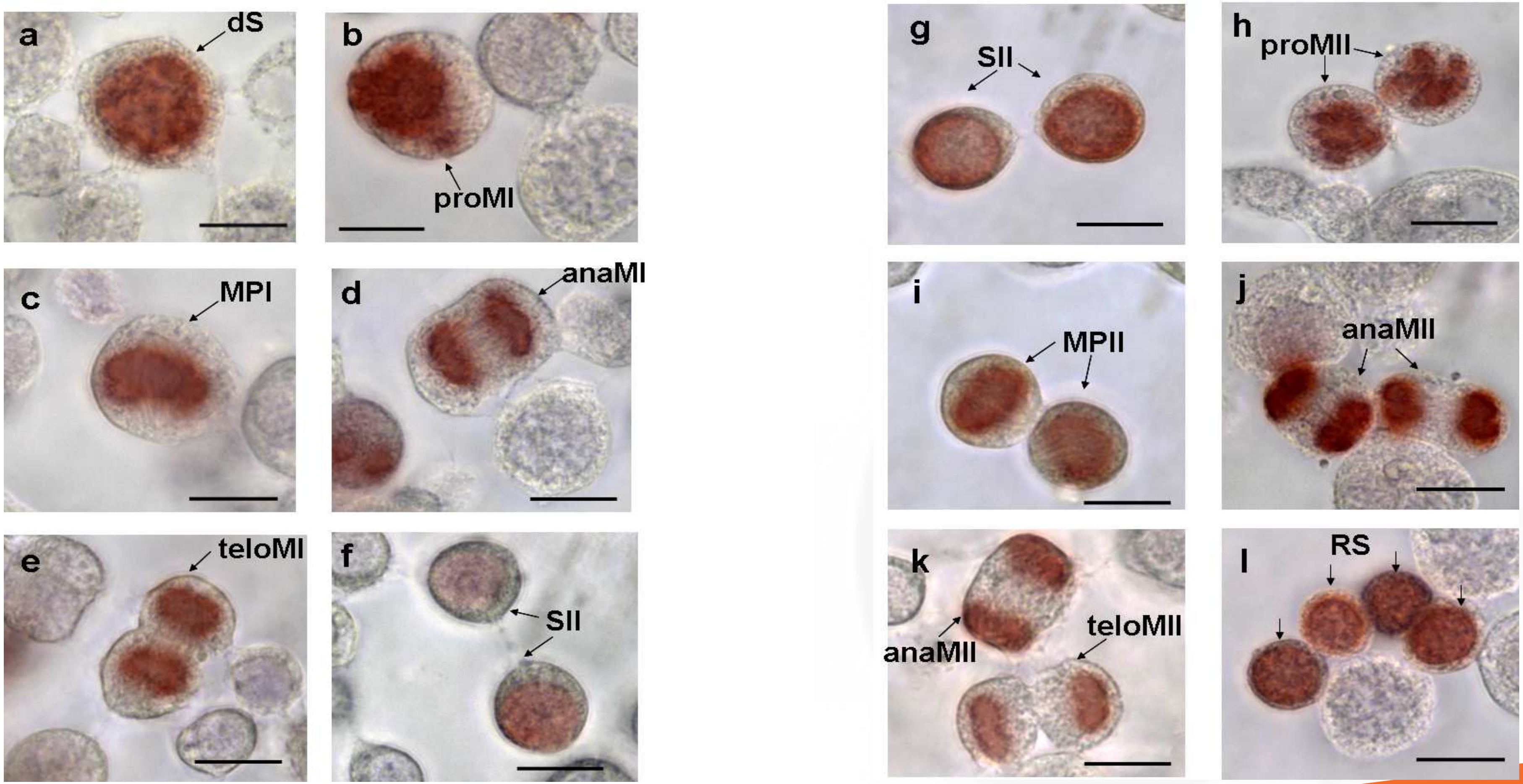
Occurrence of the two meiotic divisions *in vitro* (Bio-AlteR®)



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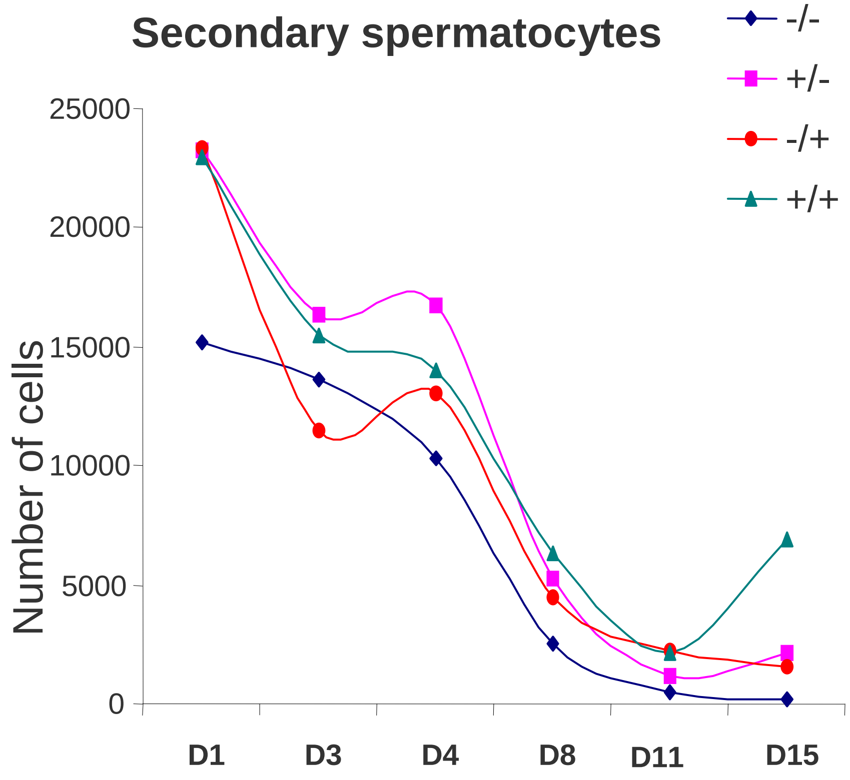
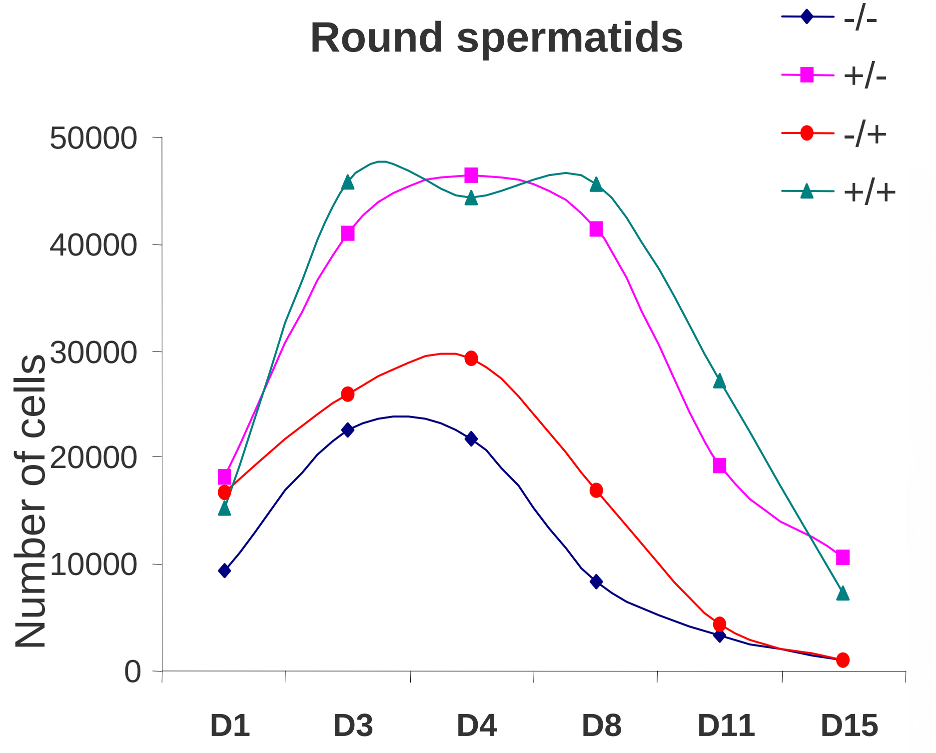
(from Perrard and Durand 2009)

- Effects of FSH and of testostérone on the meiotic divisions *(co-culture of PS with SC)*





-/- control
+/- FSH
-/+ Testosterone
+/+ FSH, testosterone

(from Vigier et al 2004)

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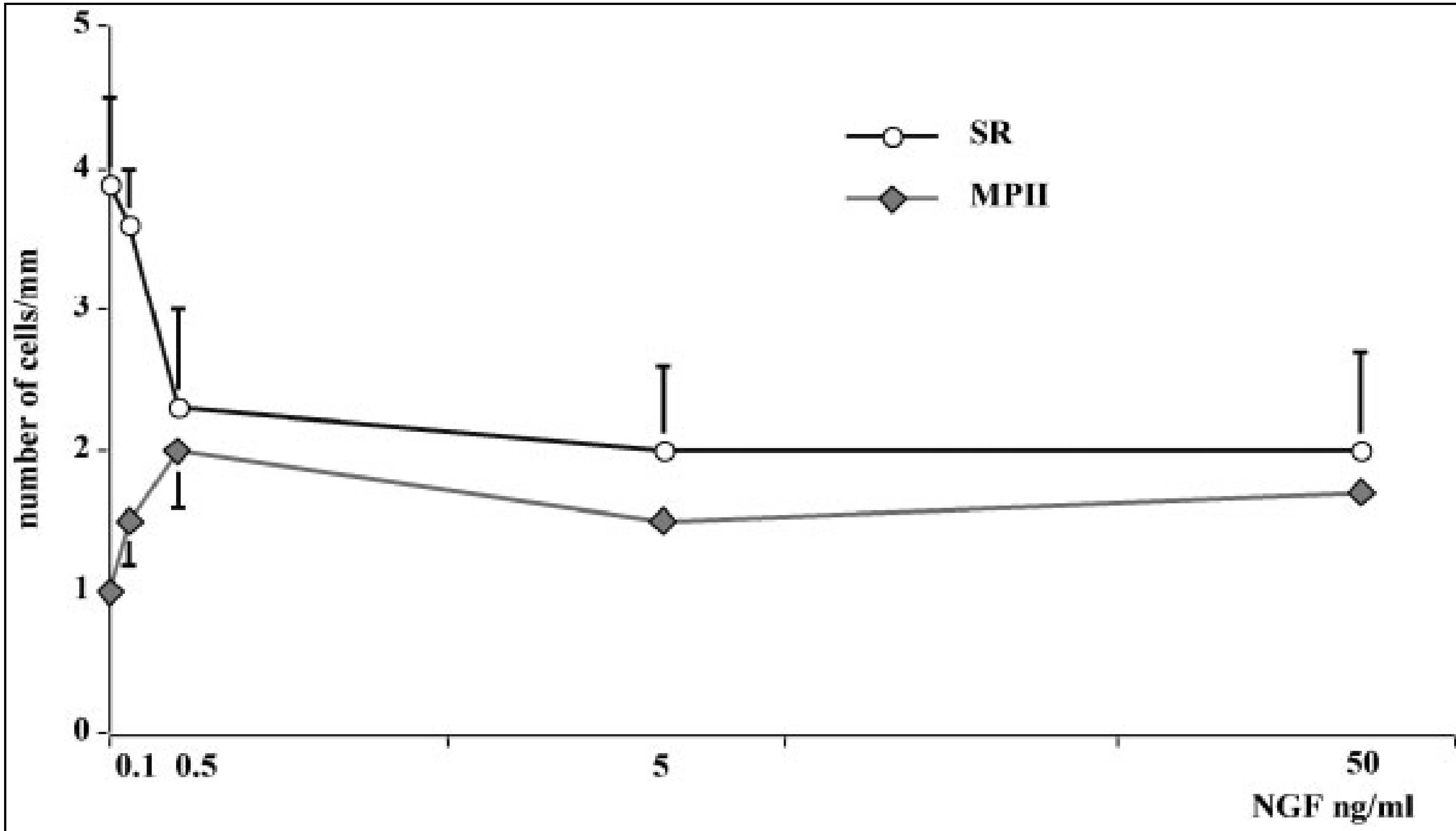
• Effects of exogenous β-NGF on meotic divisions *in vitro*



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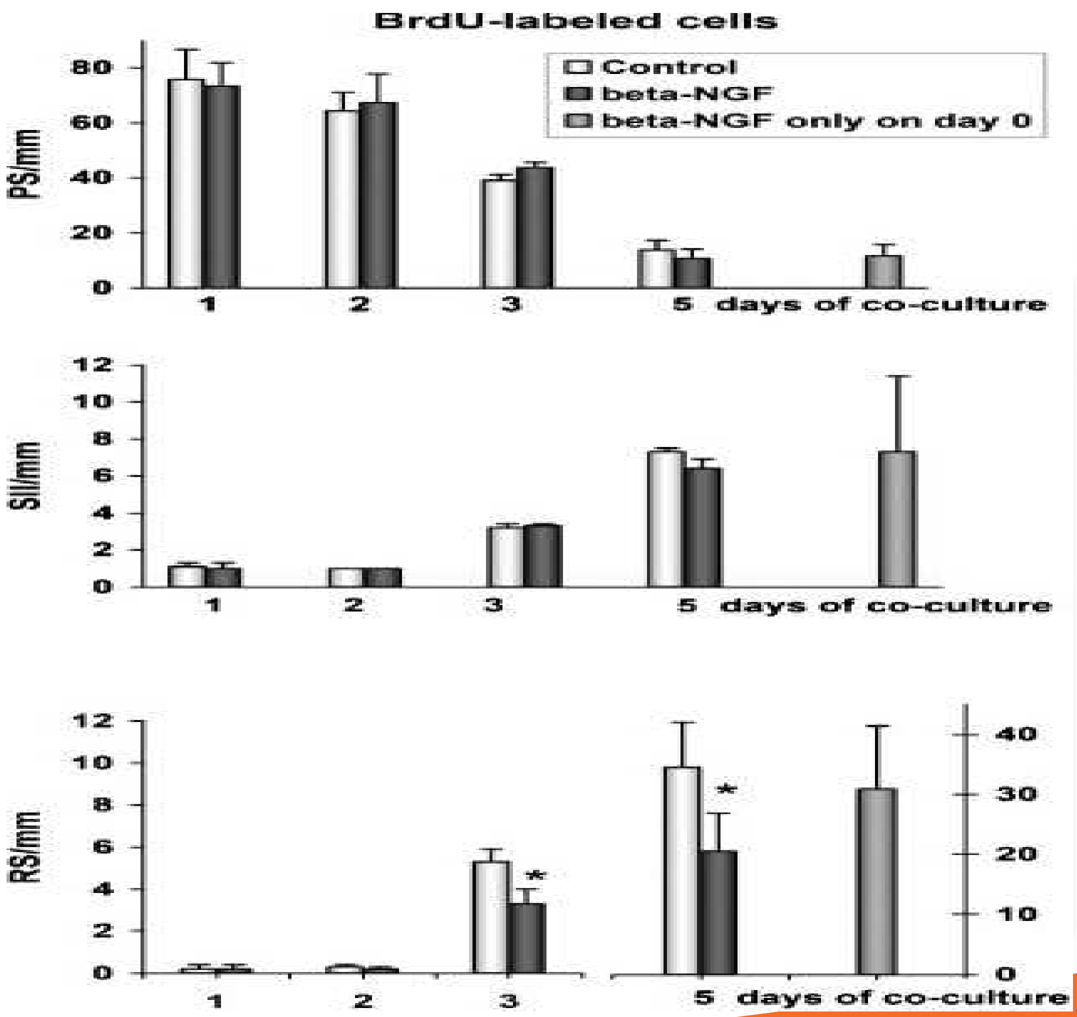
(from Perrard et al 2007)

Reversibility of the NGF effect on the second meiotic division



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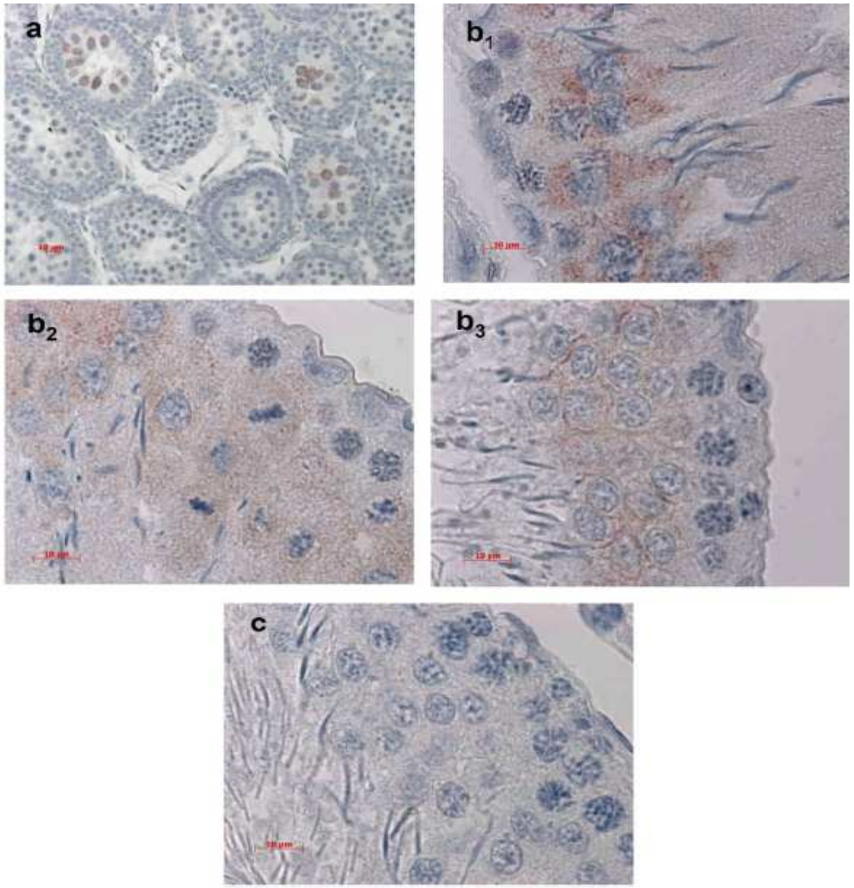
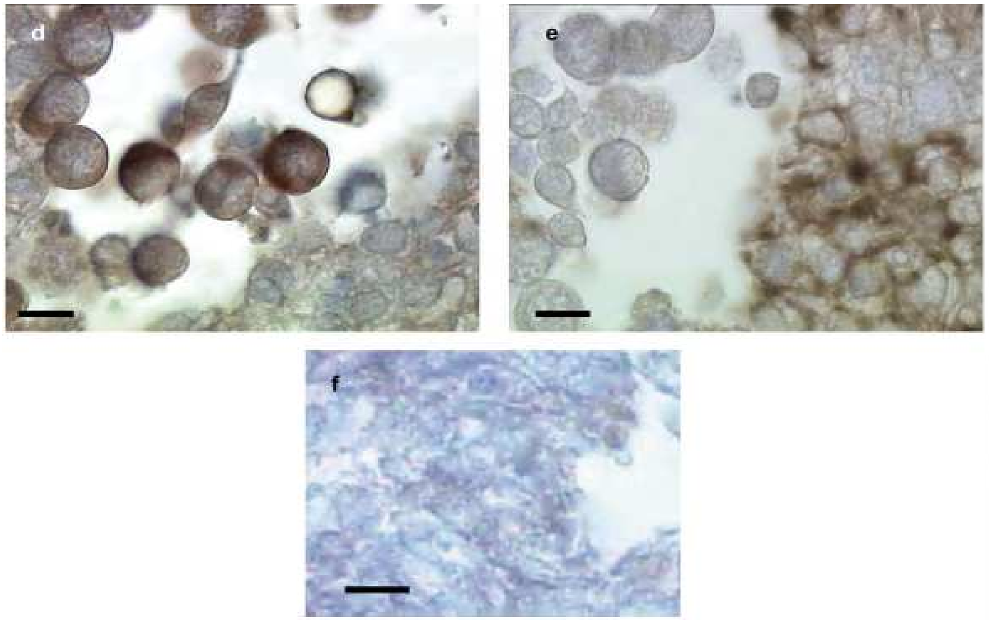
(from Perrard et al 2007)

• Comparaison of NGF production *in vivo/in vitro*

In vivo



In vitro **(Bio-AlteR®)**



Co-culture of PS with SC

NGF is synthesized by **late Pachytene Spermatocytes (PS)**, **secondary spermatocytes (SII)** and **round spermatids (RS)**, both ***in vivo*** **and** ***in vitro***

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(from Perrard et al 2007)

- **Inhibition of endogeneous NGF action**

	PS/mm	SII/mm	RS/mm
Témoin	44+/-7	4.6+/-1.2	5.7+/-1.8
K252a	44+/-5	4.8+/-1.2	6.9+/-1.8*

(TrkA inhibitor)

(n=5 ; *p<0.02 paired t test)

« Endogenous NGF » controls the yield of the second meiotic division

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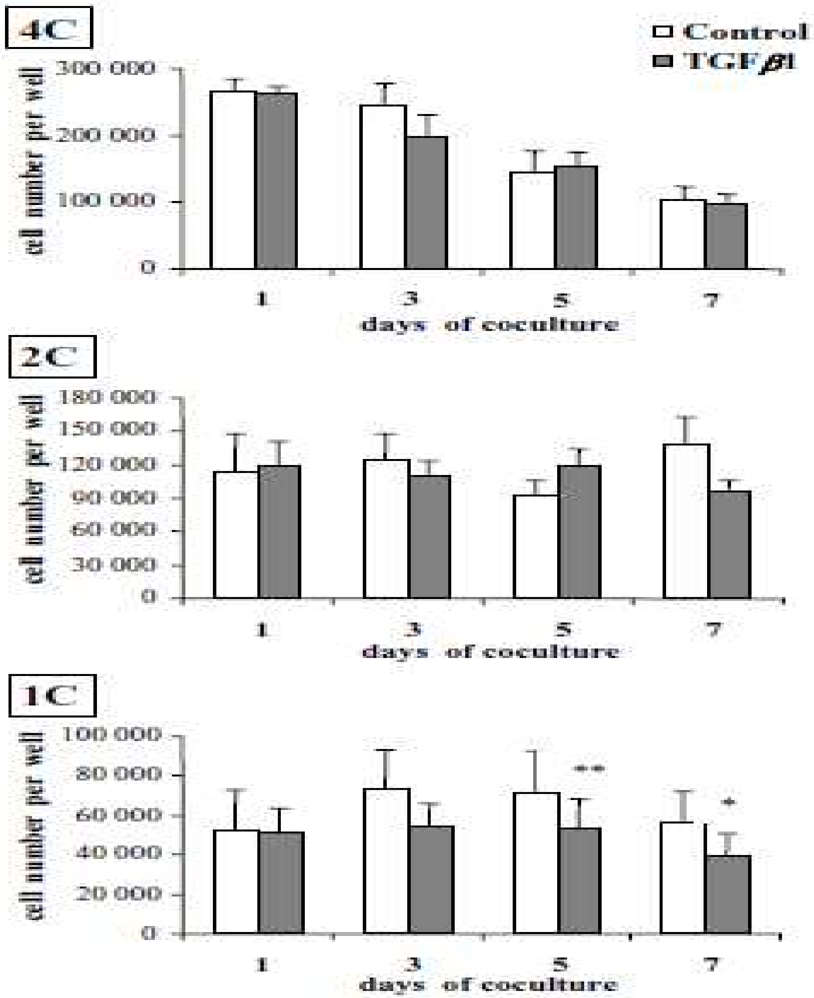
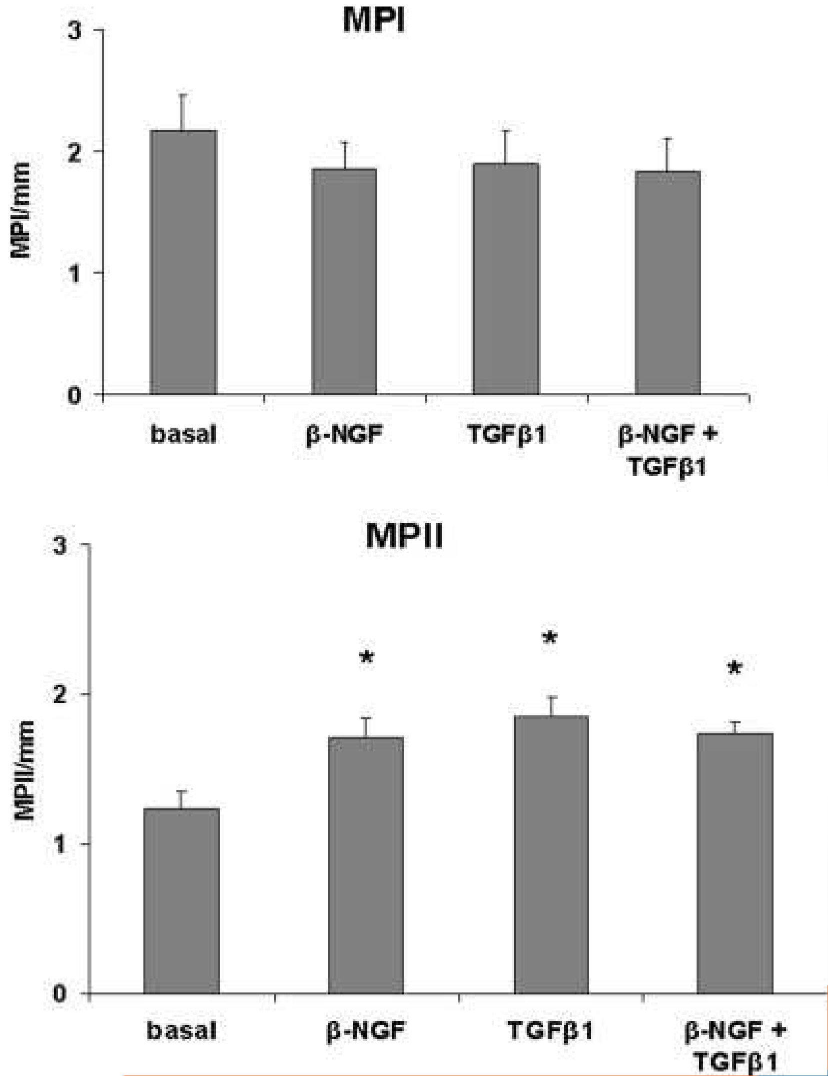
(from Perrard et al 2007)

Effect of TGFβ alone or together with NGF

TGFβ





Damestoy et al 2005

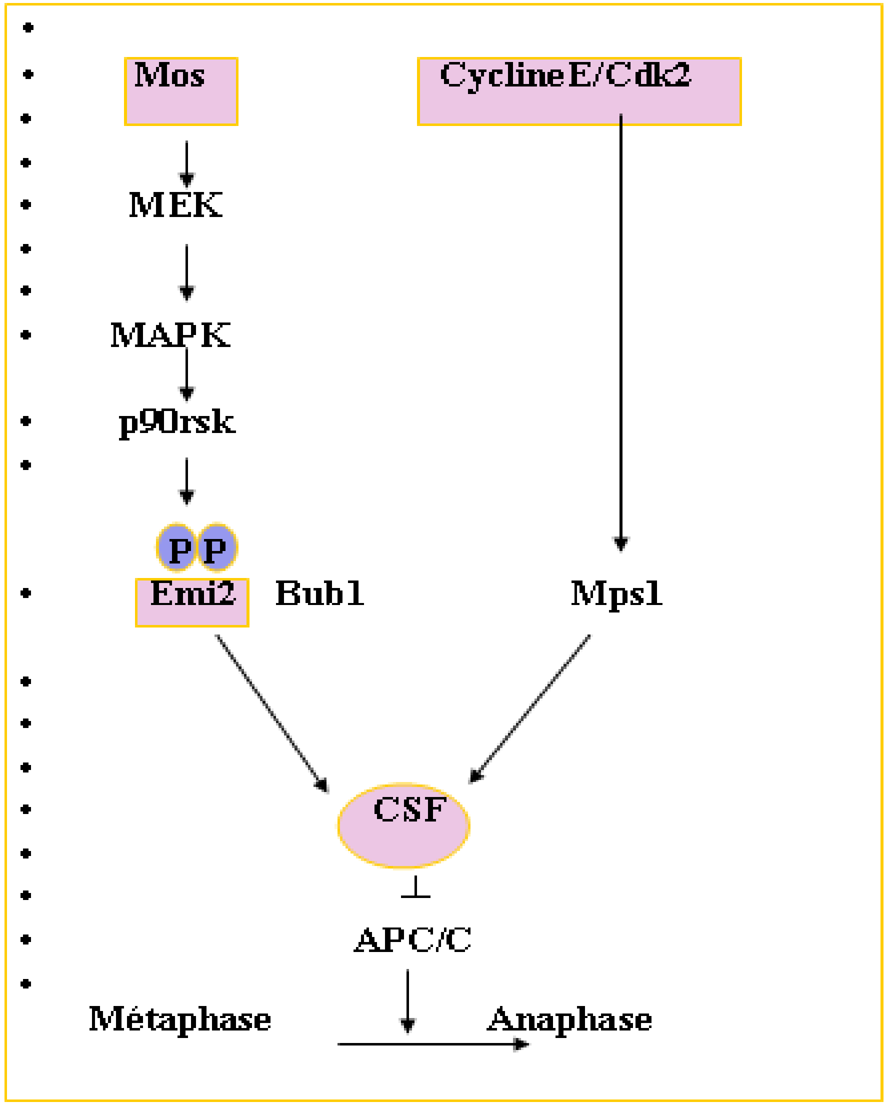
Perrard and Durand 2009

HYPOTHESIS : ANALOGY WITH THE OOCYTE WHICH IS NATURALLY BLOCKED IN MPII

CSF

(Cytostatic Factor)

DOES A CSF ACTIVITY EXIST IN MALE MEIOSIS ?



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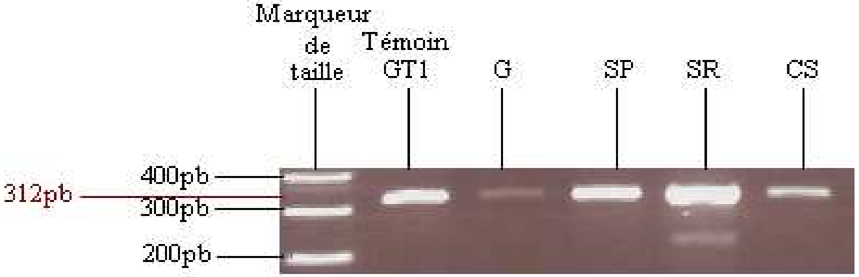
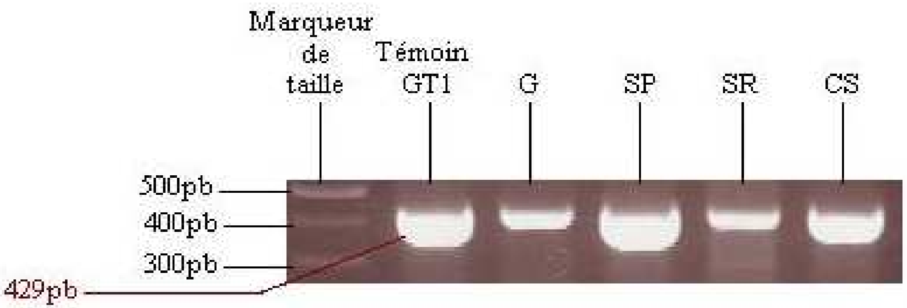
mRNA expressions of Mos, Cyclin E, Cdk2 **and** Emi2 were detected by RT-PCR in 90-day old rat freshly elutriated PS and RS.

Mos

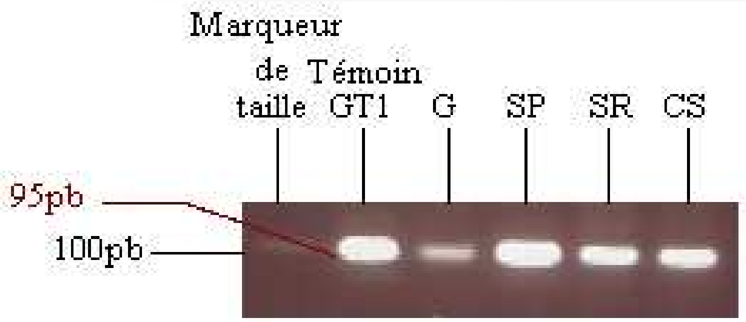


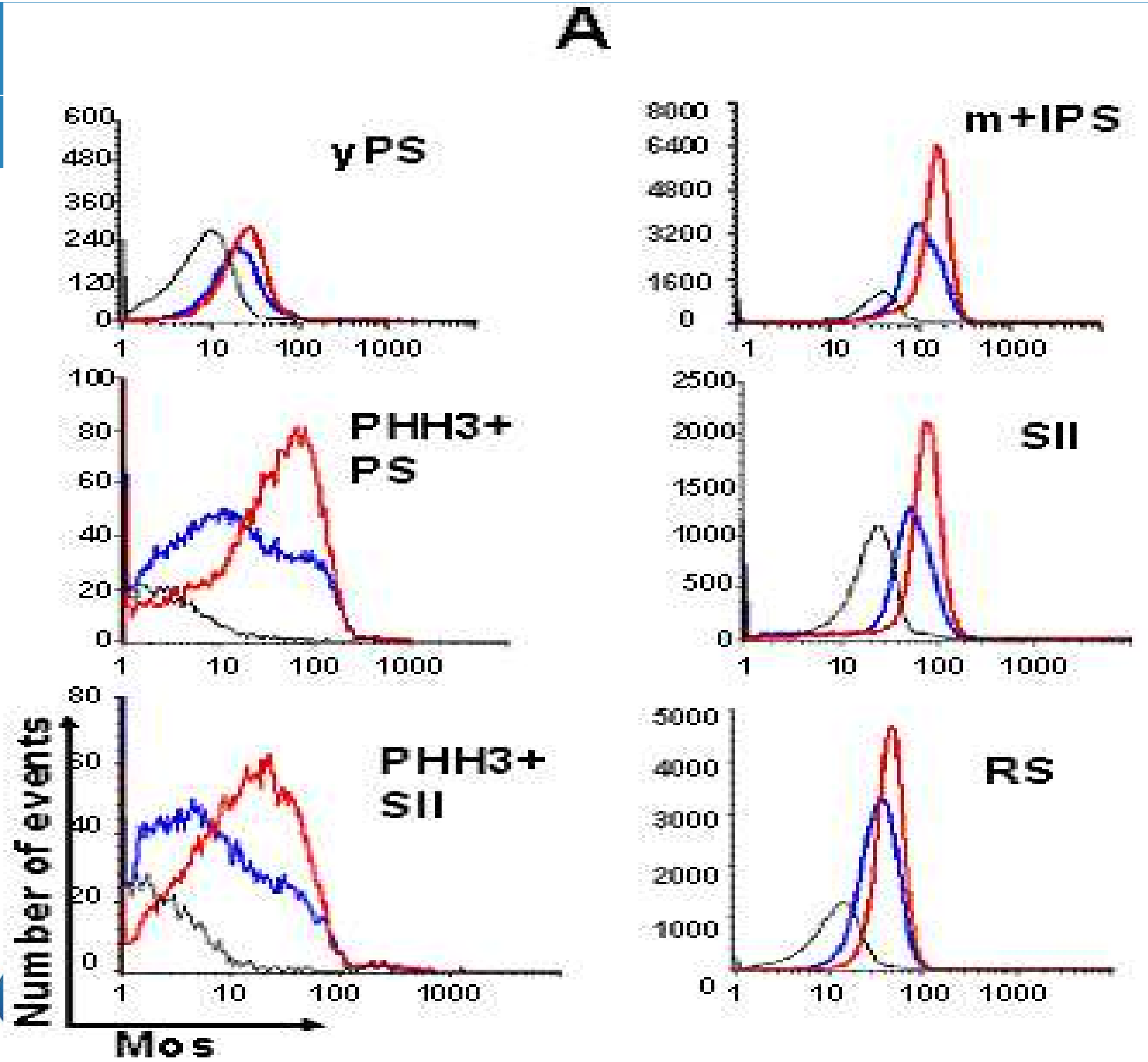
Cycline E

Cdk2



Emi2



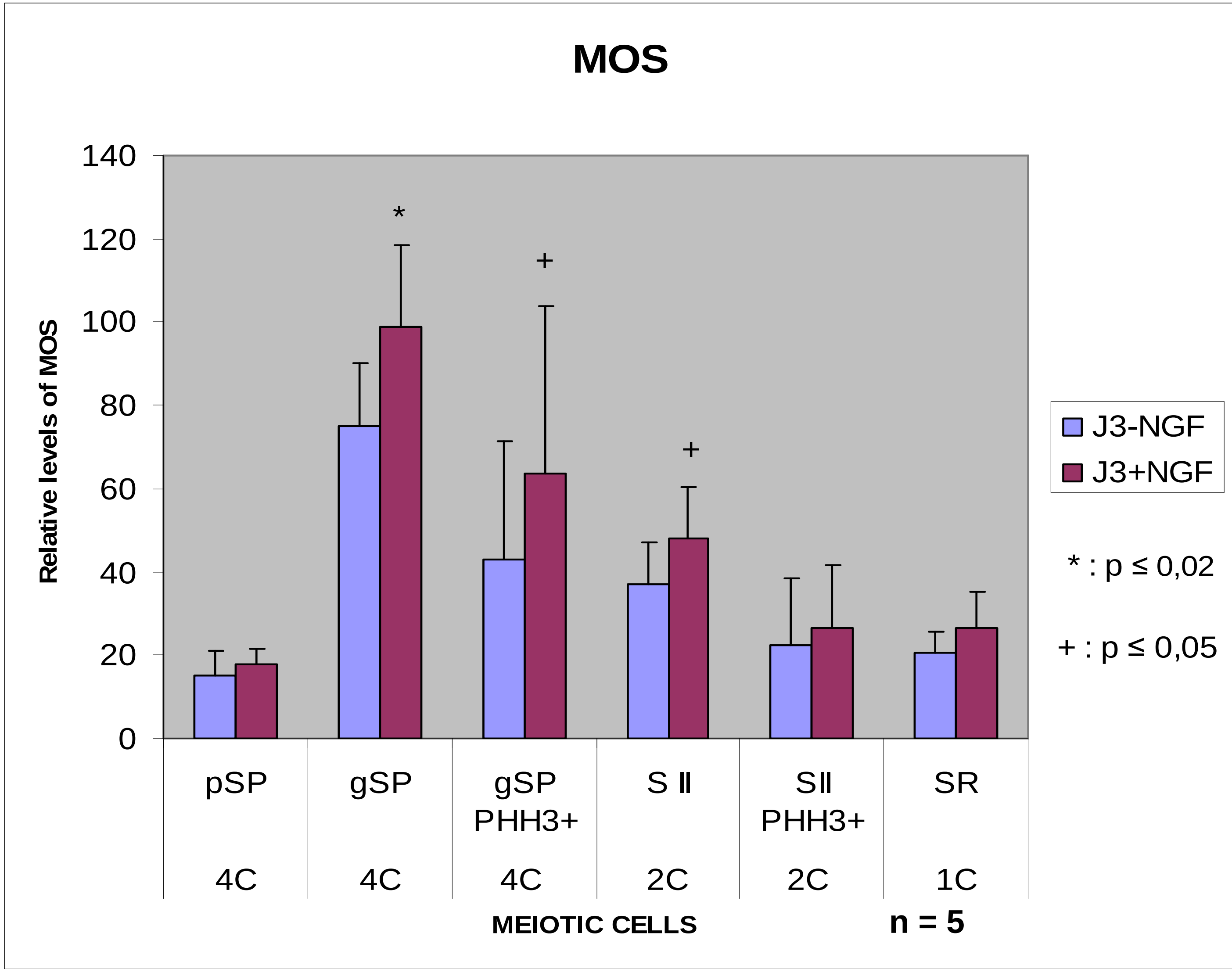


MOS levels

in the absence
or presence of NGF

(from Perrard et al 2009)

Relative levels of Mos **were measured by** Flow cytometry **in** meiotic cells **from** control or β-NGF- treated late PS / SC cocultures on day 3.



Conclusion

β-NGF participates in an auto/paracrine pathway of regulation of the meiotic differentiation of rat PS, by blocking SII in MPII

-> This should adjust the number of round spermatids that can be supported by SC

This would explain why the yield of meiosis in vivo is only 2 RS from 1 PS

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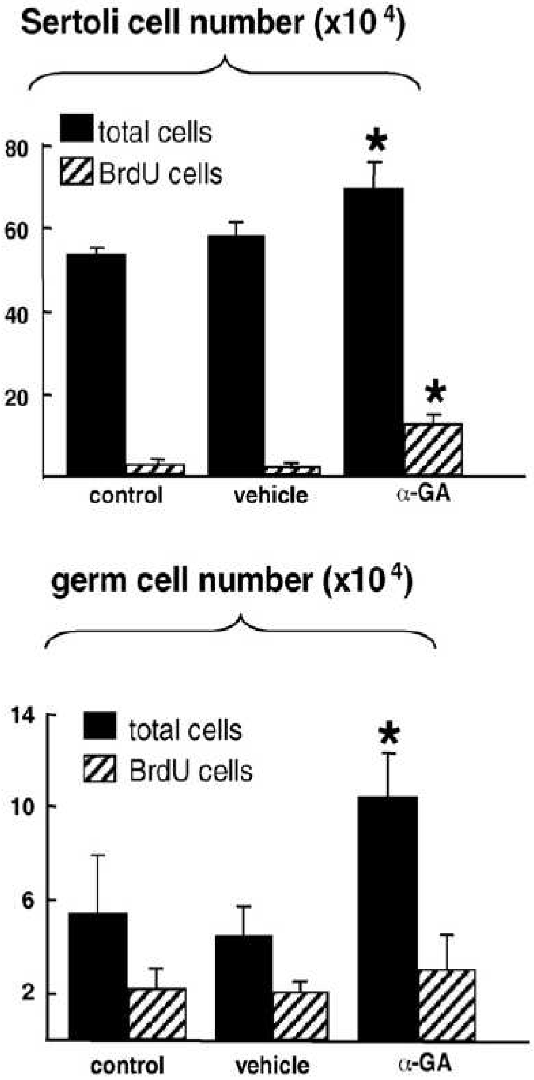
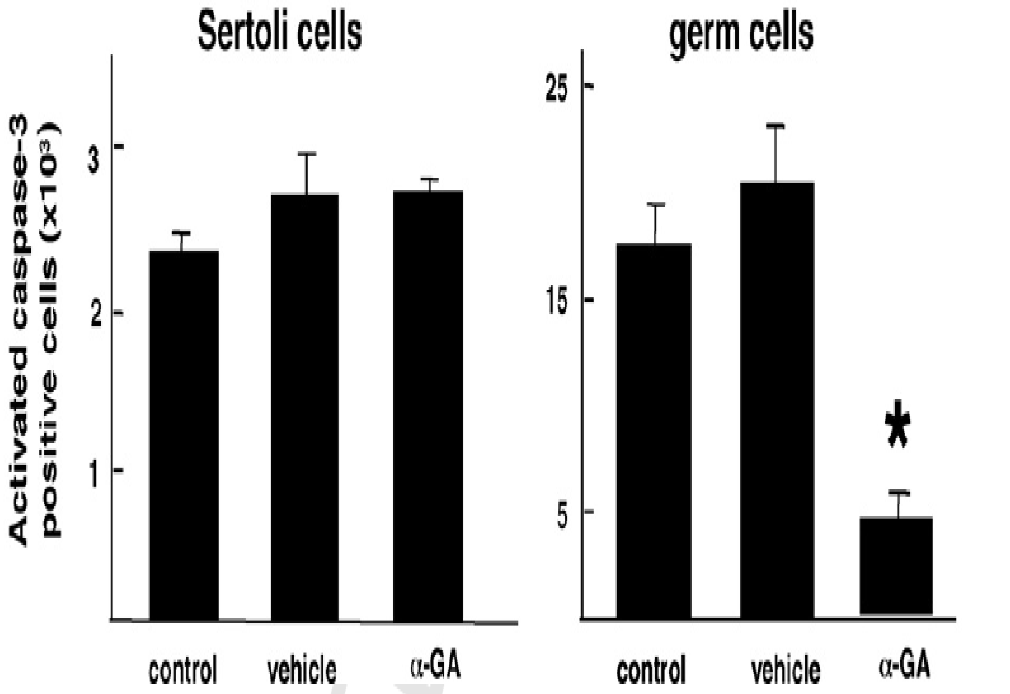
Effect of disruptors of gap communication on sertoli cells and on spermatogonia





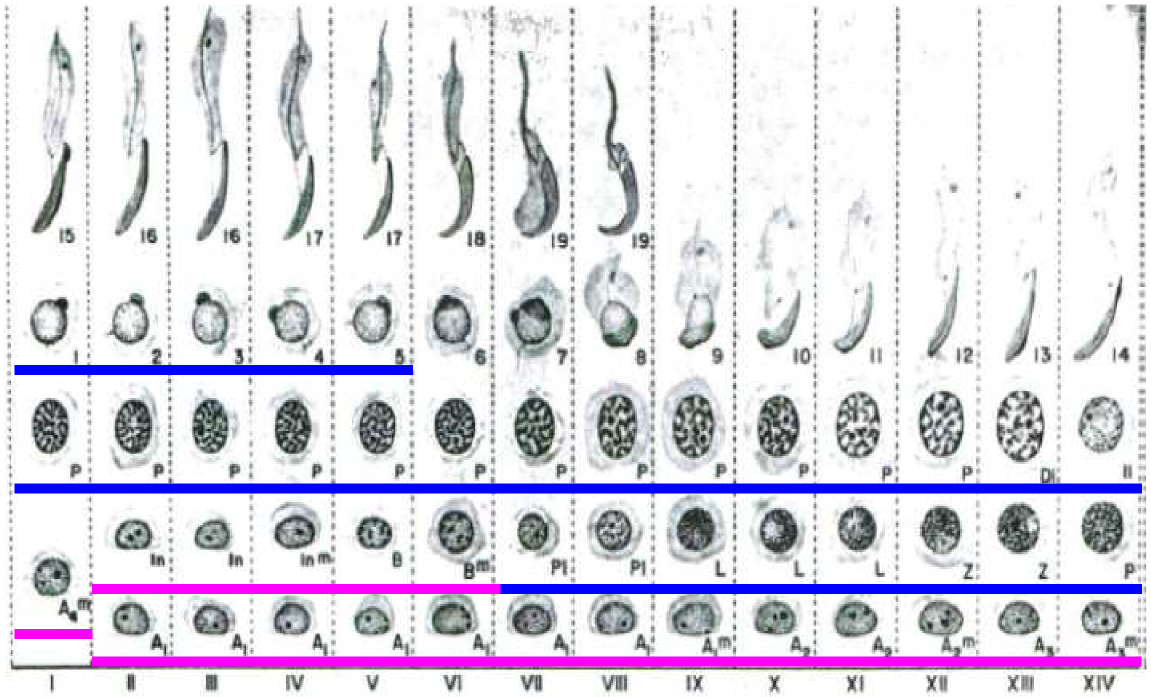
(7-9 days old rats)

(from Gilleron et al 2008)

(from Gilleron et al 2009)

Cycle of the rat seminiferous epithelium

8 days old rat



seeded germ cells

in vitro differenciation

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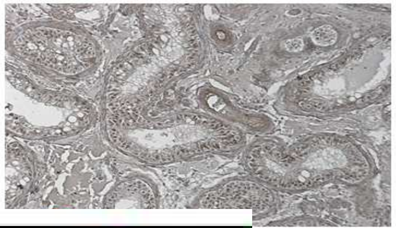
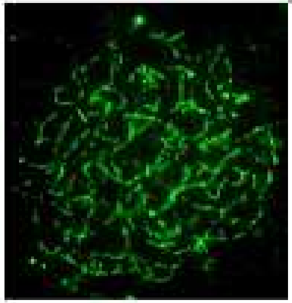
Etude cytologique de la différenciation meïotique homme

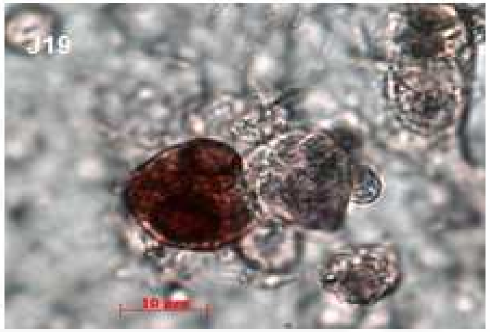


BrdU mis à J2 de la culture



Leptotène

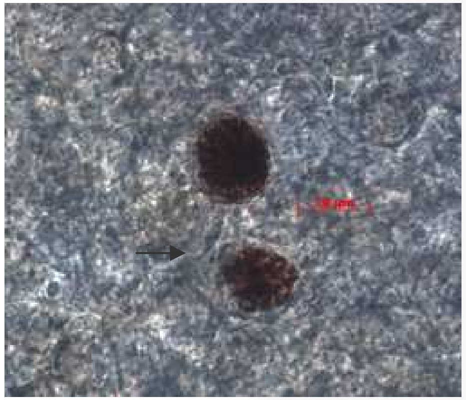
J19

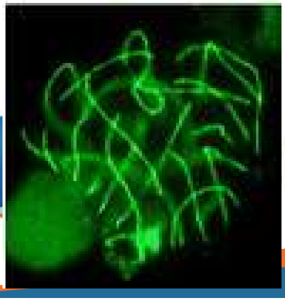


Spermatides Rondes

Zygotène

J22



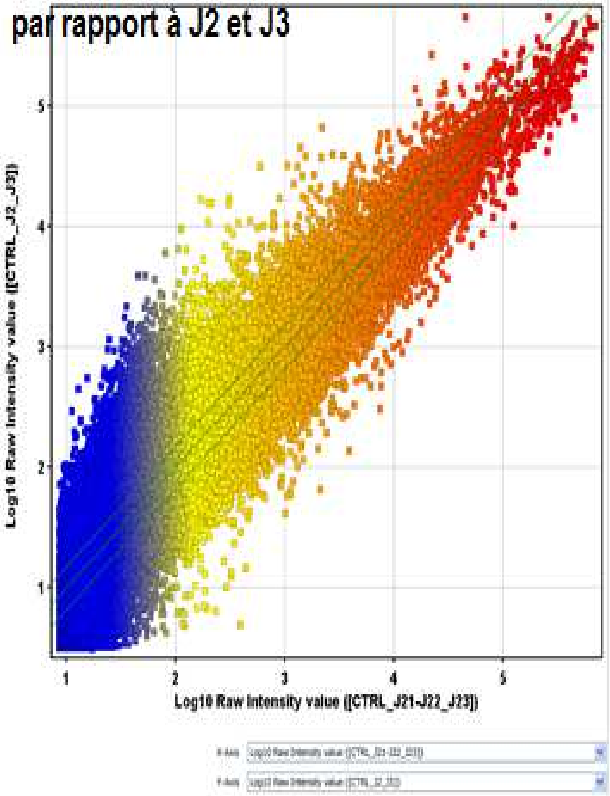


Pachytène

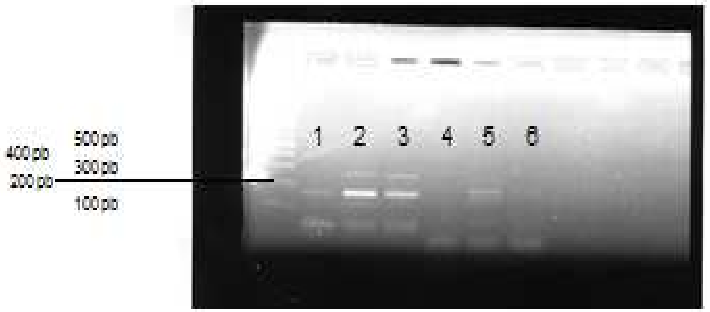
Pachytène

homme

Scatterplot évolution de la culture J21 à J23
par rapport à J2 et J3



TP1 à J13, **J20** et J29
TP2 à **J20**



1 – J13 / TP1	4 – J13 / TP2
2 – J20 / TP1	5 – J20 / TP2
3 – J29 / TP1	6 – J29 / TP2
Amplicon: 161 pb	Amplicon: 244 pb

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Acnowledgements

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- **M-H Perrard, G Montillet**
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  UMR CNRS IMBE 7263, FR 3098 ECCOREV, Université d'Aix-Marseille , France
- **E Christin**
- *Kallistem SAS , Lyon, France*
- **O Prat**
  CEA, Marcoule, France

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Physiological validation

Physiological processes demonstrated in the ex vivo model	References
occurrence of the whole meiotic process	Staub et al 2000
in vitro pubertal development of the meiotic step is close to in vivo	Perrard et al 2003; Geoffroy Siraudin et al 2010
FSH and testosterone have positive effects on meiotic divisions and TP1 expression	Vigier et al 2004
NGF and TGFb block spermatocytes in metaphase II	Perrard et al 2007; Damestoy et al 2005
NGF and TGFβ1 exert a redundant effect on this step	Perrard and Durand 2009
NGF and its receptors are expressed by meiotic cells Sertoli cells express only the NGF receptors	Perrard et al 2007
β-NGF increases metaphases II, while enhancing Mos and Emi2 in spermatocytes	Perrard et al 2009
progression of rat spermatocytes requires MAPkinases and close contacts with Sertoli cells	Godet et al 2008
meiotic divisions are blocked by inhibitors of MPF	Godet et al 2004
GDNF inhibits the S-phase of differentiated A spermatogonia with an enhancing effect on a small population of undifferentiated spermatogonia	Fouchécourt et al 2006
Sertoli cell Cx43 gap junctions control Sertoli cell proliferation and germ cell survival	Gilleron et al 2009

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Validation of Bio-AlteR® with about 20 publications