# Spermatogonial Stem Cells: the Gdnf-Gfra1 pathway regulation is spermatogenetic dependent in trout, and differs from that in mouse

Johanna Bellaïche, Anne-Sophie Goupil, Elisabeth Sambroni, Jean-Jacques Lareyre, Florence Le Gac

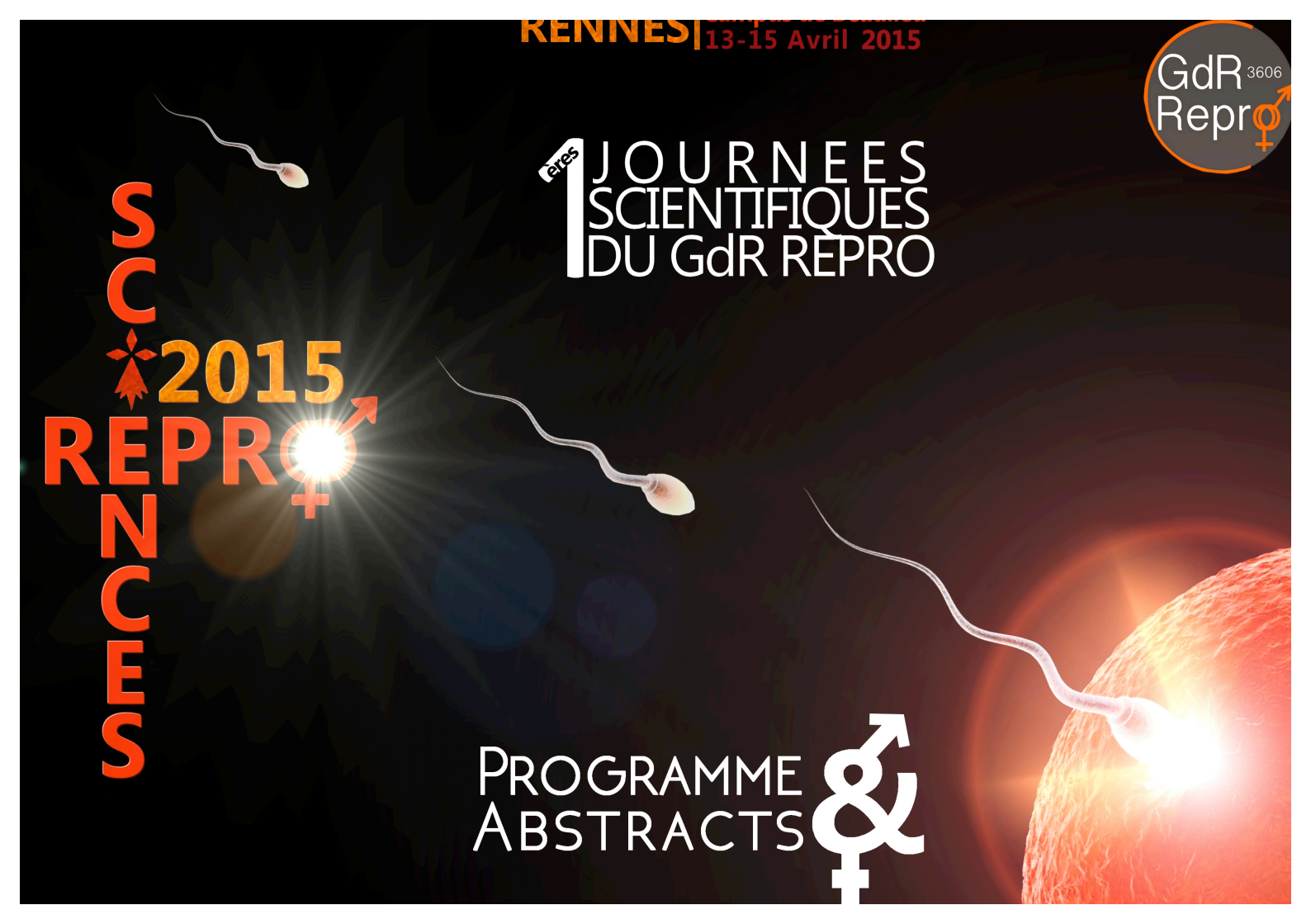
RENNES | 13-15 Avril 2015

GdR 3606 Repro

# 1ères JOURNEES SCIENTIFIQUES DU GdR REPRO

SCIENCES REPRO 2015

## PROGRAMME & ABSTRACTS



## Effect of season and steroid on RFRP3 expression in ewe: a three dimensions analysis of neurons distribution and neurotransmitter markers.

*Julien Bartzen-Sprauer, Hugues Dardente, Karine Anger, Vincent Robert, Caroline Decourt, Massimiliano Beltramo*
*UMR Physiologie de la Reproduction et des Comportements (INRA, UMR85; CNRS, UMR7247; Université François Rabelais Tours; IFCE) F-37380 Nouzilly, France.* jbs67@hotmail.fr

A neuropeptide of the RF-amides family, RFRP3 (RF-amide related peptides-3), has been implicated in the central control of reproduction in mammals. Initially identified by homology to GnIH, that inhibit LH secretion in bird, its physiological functions in mammals appear more complex and variable.
It has been reported that RFRP3 expression is influenced by season and sexual hormones. For example in ewe RFRP3 neurons are less abundant during the breeding season. We investigated the effect of a combination of progesterone analog (flugestone acetate, FA) treatment and season on RFRP3 gene expression by *in situ* hybridization (ISH) on Ile de France ewes (n=5 per group). Different rostrocaudal levels of the hypothalamus were analyzed and neurons labeled by ISH counted on microscope images using a Mercator Software. Labeled neurons were present in the dorso-medial hypothalamus (DMH) and more scattered neurons observed in the nearby hypothalamic regions. Under FA treatment neurons expressing RFRP3 in the DMH were slightly less abundant during breeding season compared to non-breeding season. To define if there is a subpopulation of neurons that is most affected by the reproductive status and FA treatment we perform a tridimensional analysis of neurons distribution. A grid was applied on photomicrographs and RFRP-3 neurons were counted in each case. Our analysis showed a subpopulation in DMH core that account for the seasonal difference observed and is possibly less affected by FA treatment.
At present it is unknown which other neurotransmitters are present in RFRP3 neurons. To assess possible coexpression we performed double ISH using glutamate and GABA neuron markers (*vglut2* and *gad65)*. Preliminary results suggest that GAD65 and Vglut2 are not expressed in RFRP-3 neurons.
Further studies are in progress to establish if RFRP3 neurons contain other neurotransmitters and/or progesterone and corticosteroid receptors.
Funding : ANR Repramide / Bourse region Centre

Rennes du 13 au 15 avril 2015

## Spermatogonial Stem Cells: the Gdnf-Gfra1 pathway regulation is spermatogenetic dependent in trout, and differs from that in mouse.

*Johanna Bellaiche, A.Sophie Goupil, Elisabeth Sambroni, J.Jacques Lareyre, Florence Le Gac*
*Fish Physiology and Genomics INRA, BIOSIT, Biogenouest, Campus de Beaulieu, Rennes.* florence.legac@rennes.inra.fr

We recently characterized putative spermatogonial stem cells (SSCs) in trout spermatozoa [Bellaiche et al 2014 a and 2014b]. What makes these cells self-renew or differentiate to produce spermatozoa is barely understood, in particular in non-mammalian species. Our research explores possible regulations of the spermatogonial stem cell niche in teleost, locally by paracrine factors and peripherally by hormonal regulation. In the present study, we focused on the Gdnf/Gfra1 pathway, known to play a major role in SSC self-renewal in rodents. Using qPCR measurements in purified testicular cell populations, the gdnfb was found expressed in testicular somatic cells and in spermatogonia. In contrast, the transcript of the gdnf receptor, gfra1a, was specifically expressed in a population of undifferentiated-spermatogonia (und-Spg) purified by centrifugal elutriation. Transplantation studies demonstrated that this particular cell population had a high “stemness” potential in terms of gonadal colonization and production of fertile spermatozoa [Bellaiche et al 2014a]. It also preferentially expressed nanos2, a putative SSC marker in trout. Furthermore, by flow cytometry and immunohistochemistry we find that only a sub-fraction of the und-Spg (12%-20%) expressed gfra1a and nanos2.
In trout, spermatogenesis develops along a strict annual cycle. We show that gdnfb and its receptor were expressed in a spermatogenetic activity dependent manner. Interestingly, a dramatic increase of the gdnfb transcript towards the end of the reproductive cycle coincided with the progressive cessation of differentiated spermatogonia proliferation. These results suggest that, in trout, Gdnfb is involved in the repression of und-A-Spg differentiation. In rodents, Fsh was found to up regulate Gdnf. We demonstrate that in trout, in vitro Fsh treatment stimulated the expression of the receptor gfra1a1, but not of its ligand, gdnfb. Fsh treatment also stimulated the proliferation of und-Spg co-cultured with testicular somatic cells. [Bellaiche et al 2014b]
Based on those results we propose that the Gfra1 positive cells correspond to the putative SSCs in rainbow trout and that the balance between SSC self-renewal and differentiation during the trout spermatogenetic cycle is possibly under paracrine regulation by Gdnfb and under peripheral regulation by Fsh via the control of gfra1 expression.
*Bellaiche J., Lareyre J.J., Cauty C., Yano A., Allemand I., Le Gac F. 2014a. Biol Reprod, 90. 79.*
*Bellaïche J., Goupil AS., Sambroni E., Lareyre J.J., Le Gac F. 2014b. Biol Reprod, 91. 94.*
***Supported by*** *EU LIFECYCLE project and CRB-Anim (Infrastructure ANR)*