

BIOGRAPHICAL SKETCH

NAME: Roberto Oleari

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Università degli Studi di Milano, Milan (Italy)	BSc	11/2013	Cardiovascular disease
Università degli Studi di Milano, Milan (Italy)	MSc	09/2015	Developmental neurobiology
Università degli Studi di Milano, Milan (Italy)	PhD	01/2019	Developmental neurobiology
Università degli Studi di Milano, Milan (Italy)	Postdoctoral fellow	01/2025	Developmental neurobiology
Università degli Studi di Milano, Milan (Italy)	Assistant professor	due 01/2031	Cellular and Experimental Biology

A. Personal statement

My research has been always dedicated at identifying novel cellular and molecular mechanisms underlying rare neurodevelopmental genetic disorders related to reproductive function, such as Hypogonadotropic Hypogonadism and Kallmann Syndrome, a disorder resembling CHARGE Syndrome. Specifically, I focused my research on semaphorin signaling genes, which are pivotal for several neurodevelopmental processes. By combining different in silico, in vitro and in vivo models, I consistently contributed to the discovery of novel causative genes of these disorders, studying their biological roles along with neurodevelopmental processes and validating the pathogenicity of human variants in experimental models.

I started my scientific career by graduating in MSc Pharmaceutical Biotechnology (mark: 110/110 cum laude) in 2015 at University of Milan (Italy), with a thesis aimed at dissecting the expression pattern of semaphorins during embryonic development of GnRH neurons in vivo, using both zebrafish and mouse models.

My focus on the relationship between GnRH neuron biology and semaphorin signaling continued with my PhD in Prof. Cariboni lab at Dept. Pharmacological and Biomolecular Sciences, University of Milan (Italy). During this period, I gained solid background in cell biology and mouse genetics, with specific expertise on developmental biology and rare neurodevelopmental syndromes characterized by reproductive defects (see **Contribution to Science**, points 1 and 2). I also spent 4 months in Prof. Ruhrberg lab (Institute of Ophthalmology, UCL, UK), sponsored by Boehringer Ingelheim Foundation, where I strengthened my expertise in the use of transgenic mouse models of semaphorin signaling genes.

After obtaining the PhD in Integrated Biomedical Research (curriculum: Neuroscience) in January 2019 (University of Milan, Italy), I moved to Dr. Basson lab (KCL, UK) as Visiting Researcher sponsored by an EMBO fellowships to work on a rare disease presenting hypogonadism and cerebellar ataxia and caused by variants in transcription factor PRDM13. I continued working on this project as post-doctoral fellow in Prof. Cariboni lab (University of Milan, Italy) and, relevant to this proposal, I was awarded as co-PI with a funding to study the PRDM13-dependent hypothalamus patterning by scRNA-seq (EASI Genomics) (see **Contribution to Science**, point 3). During post-doctoral training in Prof. Cariboni lab (2019-2024), I significantly contributed to several research projects, characterized by international and national collaborations, aimed at dissecting cellular and molecular mechanisms underlying rare neurodevelopmental syndromes (see **Contribution to Science**, point 4 and 5). During this period, I was also awarded with a 1-year post-doctoral fellowship (Fondazione Ghislieri, Italy) and I obtained a ESPE Early Career Developmental Grant as PI, which allowed me to start my track to independence. Overall, these experiences have

been pivotal to strengthen my expertise in cellular and animal models of rare neurodevelopmental syndromes and helped me gain recognition in the field of neurodevelopmental biology, as highlighted by invitations to international meetings, peer-review for international journals and by contributions to influential reviews on neural development. Last, but key to this proposal, I was actively involved different projects based on gene expression studies using microarrays or RNA-sequencing (Busnelli et al., *Arterioscler Thromb Vasc Biol.* 2021 and 2022; Oleari et al., *Dis Model Mech.* 2023; Amoruso et al., *Small* 2025).

Lastly, I successfully applied to an Assistant Professor position in Cellular and Experimental Biology at University of Milan in the Department of Pharmacological and Biomolecular Sciences (Disfeb), which will provide me the possibility to develop my track to independence. In this context, also thanks to the resources provided by this grant, I would like to leverage snRNA-seq datasets generated through EASI Genomics Consortium, the already established collaboration with Dr. Basson and Dr. Howard and my expertise in developmental biology, GnRH and hypothalamus patterning to identify new developmental dynamics involved in neuroendocrine neurons involved in the control of reproduction and novel genetic determinants linked to puberty onset and reproductive disorders.

B. Positions, Scientific Appointments, and Honors

Positions

2025 – present	Assistant Professor in Cellular and Experimental Biology, Dept. Pharmacological and Biomolecular Sciences, University of Milan, Italy
2019 – 2025	Post-doctoral fellow, Dept. Pharmacological and Biomolecular Sciences, University of Milan, Italy
2019	Visiting Research Fellow, Dept. of Dentistry, King's College London, UK
2017	Visiting PhD student, Institute of Ophthalmology, University College London, UK
2015 – 2019	PhD student, Dept. Pharmacological and Biomolecular Sciences, University of Milan, Italy

Honors

2024: Post-doctoral fellowship 2025, Fondazione Umberto Veronesi
 2024: Guido Tarone Award Under35, Associazione Italiana di Biologia e Genetica Generale e Molecolare
 2022: Post-doctoral fellowship, Fondazione Collegio Ghislieri
 2018: EMBO Short Term Fellowship
 2018: IBRO-PERC InEurope Short Stay grant
 2018: Best PhD Student Publication Award, Disfeb, University of Milan
 2018: Best Poster Award, Head Group Meeting Scientific Committee

Invited speaker

2024: Symposium Lecture, 62nd European Society of Paediatric Endocrinology Meeting, Liverpool, UK
 2024: ESN Young Scientist Satellite Conference to FENS Forum 2024, Wien, Austria
 2021: 3rd European Center for Reproductive Endocrinology Meeting, Online
 2016: 4th Workshop NICE, Genova, Italy

Ongoing Research Support

- Research Support Plan – University of Milan (5.000,00 €) 11/1/25-10/31/27
 Title: Studying the role of NPAS2 in GnRH neuron development.
 Role: PI

Completed Research Support

- EASI Genomics Consortium Transnational Call (30.000,00 €) 01/07/21- 30/06/23
 Title: snRNA-seq to decipher the role of PRDM13 on the hypothalamic control of reproduction.
 Role: co-PI

C. Contributions to Science

1) Semaphorins have been linked to the embryonic development of GnRH neurons, neuroendocrine cells that control reproductive functions, and therefore to the pathogenesis of rare genetic reproductive disorders, such as Hypogonadotropic Hypogonadism, Kallmann Syndrome and CHARGE Syndrome. Yet, little is known about the involvement of semaphorin receptors and other class 3 semaphorin members in this context. During my PhD training, I significantly contributed to fill this gap of knowledge as follows:

i) demonstrating the involvement of PLXNA1/A3 in conveying SEMA3A signaling, by analyzing transgenic mice; this work paved the way to the discovery of PLXNA1-3 variants in patients affected by rare fertility disease by other groups.

- **Oleari R**, et al. PLXNA1 and PLXNA3 cooperate to pattern the nasal axons that guide gonadotropin-releasing hormone neurons. **Development**. 2019 Nov 5;146(21):dev176461. doi: 10.1242/dev.176461.

ii) identifying a novel role for endothelial-derived SEMA3G in GnRH neuron biology, highlighting the first gain-of-function semaphorin variant in rare syndromic forms of infertility, combining mouse models and mouse neuronal cell lines.

- **Oleari R***, André V*, Lettieri A*, et al. A Novel SEMA3G Mutation in Two Siblings Affected by Syndromic GnRH Deficiency. **Neuroendocrinology**. 2021;111(5):421-441. doi: 10.1159/000508375. *co-first authors

2) Extracellular matrix components, such as the heparan sulfate proteoglycans, facilitate the formation of soluble factor gradients and stabilize ligand-receptor complexes during development. Variants in the HSPG-modifying enzymes Hs6st1 have been identified in patients with genetic forms of infertility, yet underlying mechanisms, as well as the contribution of other enzymes to the pathogenesis of these disorders, remain unclear. In this context, we demonstrate that single HS6ST1 loss does not affect embryonic GnRH neuron development but leads to delayed pubertal onset in mice and humans. On the other side, the combined loss of HS6ST1 and HS6ST2 disrupts the survival and the migration of GnRH neurons, indicating that HS6ST2 may be a novel gene to be screened in patient with genetic forms of infertility (Oleari et al. submitted).

- Howard SR*, **Oleari R***, et al. HS6ST1 Insufficiency Causes Self-Limited Delayed Puberty in Contrast With Other GnRH Deficiency Genes. **J Clin Endocrinol Metab**. 2018 Sep 1;103(9):3420-3429. doi: 10.1210/jc.2018-00646. *co-first authors

3) To date, up to 50 causative genes of genetic forms of infertility have been identified. Yet, several affected patients do not have a known genetic determinant. In this context, the combination of exome sequencing together with in vivo and in vitro experimental tools has emerged as an effective strategy to identify novel causative genes. By exploiting this approach, we studied the role of transcription factor PRDM13, found mutated in patients with infertility and cerebellar hypoplasia, in the development of cerebellum, hypothalamus and GnRH neurons. Specifically, thanks to a disease mouse model, I confirmed in vivo the effect of PRDM13 loss on neuroendocrine functions, providing new mechanistic insights into embryonic development of GnRH/Kiss1 neurons.

- Whittaker DE*, **Oleari R***, Gregory LC*, et al. A recessive PRDM13 mutation results in congenital hypogonadotropic hypogonadism and cerebellar hypoplasia. **J Clin Invest**. 2021 Dec 15;131(24):e141587. doi: 10.1172/JCI141587. *co-first authors

Linked to this study, I successfully applied to EASI Genomics Transnational Call to perform snRNA-seq on hypothalami from Prdm13 wildtype and knock out embryos at different time points and datasets generated through this project are the subject of this proposal.

4) Beside their role during neurodevelopment, class 3 and 7 semaphorins have emerged as key molecules for the control of brain homeostasis in the median eminence, where GnRH neurons secrete GnRH peptide in a finely regulated fashion. Yet, nothing is known about the role of class 6 semaphorins in GnRH neuron system. We showed a hitherto undescribed role for oligodendrocyte-

derived SEMA6A in the control of brain permeability and GnRH neuron biology, showing that SEMA6A loss is detrimental for puberty onset in mice and humans. Specifically, thanks to a disease mouse model, I confirmed in vivo the effect of SEMA6A loss on GnRH neuron migration and axon extension, puberty onset and vascular brain permeability and I contributed to 2D in vitro permeability assays.

- Lettieri A*, **Oleari R***, van den Munkhof MH*, van Battum EY*, Verhagen MG*, et al. SEMA6A drives GnRH neuron-dependent puberty onset by tuning median eminence vascular permeability. **Nat Commun.** 2023 Dec 7;14(1):8097. doi: 10.1038/s41467-023-43820-z. *co-first authors

5) Research on the development of GnRH neurons has been limited by several challenges due to their limited number, unique origin in the nasal placode, and dispersed presence in the hypothalamus. Consequently, genes involved in GnRH neuron development and associated disorders remain largely unidentified. In this study, we characterized for the first time the transcriptomic profiles of primary embryonic GnRH neurons, isolated from transgenic rat with GFP-tagged GnRH neurons, and immortalized GnRH neuron cell lines (GN11 and GT1-7). By integrating these datasets (available on NCBI GEO: GSE174896 and GSE174902) with existing exome sequencing data from patients, we identified several novel candidate genes. Notably, we discovered that NLGN3, a gene previously linked to autism, is expressed in GnRH neurons and mutated in a syndromic neurodevelopmental disorder that combines hypogonadotropic hypogonadism with autism. I specifically contributed to this work by analyzing transcriptomic profiles and by performing in vitro studies on mouse neuronal cells aimed at validating the pathogenicity of identified human variants.

- **Oleari R***, Lettieri A*, et al. Autism-linked NLGN3 is a key regulator of gonadotropin-releasing hormone deficiency. **Dis Model Mech.** 2023 Mar 1;16(3):dmm049996. doi: 10.1242/dmm.049996. *co-first authors

Relevant publications (10 selected)

1: Amoruso F, Paganoni AJJ, Saraceni A, Magnani A, Brossa A, Merlo GR, Matei C, Willemsen WH, Rezende RC, Jorge AAL, Dal Bello F, Bovolin P, Howard SR[#], **Oleari R[#]**, Cariboni A. Nanoplastics impair GnRH neuron migration and neuroendocrine function: emerging players in the pathogenesis of reproductive disorders. **Small.** Accepted for publication doi: 10.1002/sml.202506171. [#]co-corresponding authors

2: Lettieri A*, **Oleari R***, van den Munkhof MH*, van Battum EY*, Verhagen MG*, Tacconi C, Spreafico M, Paganoni, Azzarelli, Andre' V, Amoruso F, Palazzolo L, Eberini I, Dunkel L, Howard SR, Fantin A, Pasterkamp RJ, Cariboni A. SEMA6A drives GnRH neuron-dependent puberty onset by tuning median eminence vascular permeability. **Nat Commun.** 2023 Dec 7;14(1):8097. doi: 10.1038/s41467-023-43820-z. *co-first authors

3: **Oleari R***, Lettieri A*, Manzini S, Paganoni AJJ, André V, Grazioli P, Busnelli M, Dominuco P, Vitobello A, Phillippe C, Bizaoui V, Storr H, Amoruso F, Memi F, Vezzoli V, Massa V, Scheiffele P, Howard SR, Cariboni A. Autism-linked NLGN3 is a key regulator of gonadotropin-releasing hormone deficiency. **Dis Model Mech.** 2023 Mar 1;16(3):dmm049996. doi: 10.1242/dmm.049996. *co-first authors

4: Whittaker DE*, **Oleari R***, Gregory LC*, Le Quesne-Stabej P, Williams HJ; GOSgene, Torpiano JG, Formosa N, Cachia MJ, Field D, Lettieri A, Ocaka LA, Paganoni AJ, Rajabali SH, Riegman KL, De Martini LB, Chaya T, Robinson IC, Furukawa T, Cariboni A, Basson MA, Dattani MT. A recessive PRDM13 mutation results in congenital hypogonadotropic hypogonadism and cerebellar hypoplasia. **J Clin Invest.** 2021 Dec 15;131(24):e141587. doi: 10.1172/JCI141587. *co-first authors

5: Lettieri A*, **Oleari R***, Paganoni AJJ, Gervasini C, Massa V, Fantin A, Cariboni A. Semaphorin Regulation by the Chromatin Remodeler CHD7: An Emerging Genetic Interaction Shaping Neural Cells and Neural Crest in Development and Cancer. **Front Cell Dev Biol.** 2021 Apr 1;9:638674. doi: 10.3389/fcell.2021.638674. *co-first authors

6: **Oleari R***, André V*, Lettieri A*, Tahir S, Roth L, Paganoni A, Eberini I, Parravicini C, Scagliotti V, Cotellessa L, Bedogni F, De Martini LB, Corridori MV, Gulli S, Augustin HG, Gaston-Massuet C,

Hussain K, Cariboni A. A Novel SEMA3G Mutation in Two Siblings Affected by Syndromic GnRH Deficiency. **Neuroendocrinology**. 2021;111(5):421-441. doi: 10.1159/000508375. *co-first authors

7: Mancini A, Howard SR, Marelli F, Cabrera CP, Barnes MR, Sternberg MJ, Leprovots M, Hadjidemetriou I, Monti E, David A, Wehkalampi K, **Oleari R**, Lettieri A, Vezzoli V, Vassart G, Cariboni A, Bonomi M, Garcia MI, Guasti L, Dunkel L. LGR4 deficiency results in delayed puberty through impaired Wnt/ β -catenin signaling. **JCI Insight**. 2020 Jun 4;5(11):e133434. doi: 10.1172/jci.insight.133434.

8: **Oleari R**, Caramello A, Campinoti S, Lettieri A, Ioannou E, Paganoni A, Fantin A, Cariboni A, Ruhrberg C. PLXNA1 and PLXNA3 cooperate to pattern the nasal axons that guide gonadotropin-releasing hormone neurons. **Development**. 2019 Nov 5;146(21):dev176461. doi: 10.1242/dev.176461.

9: **Oleari R**, Lettieri A, Paganoni A, Zanieri L, Cariboni A. Semaphorin Signaling in GnRH Neurons: From Development to Disease. **Neuroendocrinology**. 2019;109(3):193-199. doi: 10.1159/000495916.

10: Howard SR*, **Oleari R***, Poliandri A, Chantzara V, Fantin A, Ruiz-Babot G, Metherell LA, Cabrera CP, Barnes MR, Wehkalampi K, Guasti L, Ruhrberg C, Cariboni A, Dunkel L. HS6ST1 Insufficiency Causes Self-Limited Delayed Puberty in Contrast With Other GnRH Deficiency Genes. **J Clin Endocrinol Metab**. 2018 Sep 1;103(9):3420-3429. doi: 10.1210/jc.2018-00646. *co-first authors