Dario Brunetti CV

Personal information

Surname: **Brunetti** Name: **Dario**
Date of birth: **11/11/1979**
Place of birth: **Siracusa (SR) – Italy**
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Biosketch

Dr. Brunetti started his scientific career in 2004 working with professor Cesare Galli at the Laboratory of Reproductive Technologies, (now Avantea srl, Cremona, Italy) until 2010, when he received his Ph.D. in Biotechnology at the University of Milan. During this experience, he developed a strong background in the field of embryology, stem cell biology and in the production of large GM animal models.
His research activity carried out in this period improved the techniques to produce GM large animal models becoming a milestone in this research field (Brunetti et al. 2008 Cloning and Stem Cells; Galli et al. 2010 Xenotransplantation 17:397-410 Cozzi, E et al. Xenotransplantation 16 (6), 544-544). GM large animal models generated by Dr. Brunetti were used by different research groups to study:

- ✓ Molecular mechanisms of hyperacute rejection and new immunosuppressive drugs
- ✓ Pathogenesis of genetic and degenerative diseases
- ✓ Experimental surgery where singenic animals are required
- ✓ Development of new cell therapies, using singular animals carrying different marker genes.

From 2010 to 2013, he was a Postdoctoral Research Scientist at the Molecular Neurogenetics Unit of the Neurological Institute C. Besta in Milan. Here, under the supervision of Dr. Valeria Tiranti he worked for a Telethon project (GGP 11088) aimed at investigating the molecular basis of the Neurodegeneration with Brain Iron Accumulation syndrome (omim: #234200) by exploiting a knock-out mouse model for the PANK2 gene, which encodes a mitochondrial enzyme involved in the synthesis of Coenzyme A and whose mutations cause NBIA syndrome in humans. He demonstrated the primary role of the mitochondrial dysfunction in this neurodegenerative disease, elucidating the molecular mechanisms (Brunetti et al 2012 Hum Mol Gen 24:5294-

305), and opening new avenues for the therapeutic treatment based on the use of Panthetine (Brunetti et al. Brain 37:57-68, 2014; Németh AH, Brain 37:8-11, 2014).

In order to improve his skills in the field of Mitochondrial Medicine, in 2013 he moved to the Mitochondrial Medicine Laboratory of the Mitochondrial Biology Unit (MBU) - University of Cambridge (UK), under the supervision of Dr. Massimo Zeviani. At the MBU he worked as Investigator Scientist on two challenging projects. The first was aimed at generating and characterizing a knockout swine model for SURF1, a gene involved in the assembly of respiratory complex IV and responsible for the rare genetic disease called Leigh Syndrome (omim: #25600). This was one of the first animal models created using the CRISPR/Cas9 technology. This study opened new perspectives on the physiological roles Surf1 might play (Quadalti*, Brunetti* et al 2018 Biochim Biophys Acta 1864:2131-2142). The second project involved the study of the molecular mechanisms of a new mitochondrial neurodegeneration syndrome due to mutation in Pitrm1 gene, recently found mutated in two patients affected by progressive ataxia and cognitive impairment. Using cell and animal models, Dr Brunetti made original observations, linking Pitrm1 to Aβ amyloidotic neurodegeneration (Brunetti et al 2016 Embo Mol Med 8(3):176-90; Langer et al 2018 J Med Genet 55(9):599-60). This study opened new perspective on the pathophysiology and designing treatment for adult-onset neurodegenerative conditions (Boczonadi V, Horvath R. EMBO Mol Med. 8:173-5, 2016).
In January 2018 he won the Umberto Veronesi Fellowship and he moved to the Department of Medical Biotechnology and Translational Medicine, University of Milan, to study:

- ✓ the role of Pitrm1 and dysfunctional mitochondrial quality control system in the Alzheimer disease pathogenesis
- ✓ the ability of a nutraceutical intervention with proven mitochondrial rejuvenation properties, to modify AD-related pathology in mice models.

He got the National Qualification as Associate Professor of Applied Biology in 2018.
In January 2019 he started to work as Research Scientist RTDA on different projects aimed to:

- ✓ clarify the role of mitochondrial dysfunction during the aging process in the muscle and in the brain, using murine models with accelerated senescence
- ✓ to explore the therapeutic effect of different molecules targeting mitochondria in primary and secondary mitochondrial disease both in preclinical and clinical trials.

Current position

28/12/2018-present: Research Scientist RTDA (BIO/14) Department of Medical Biotechnology and Translational Medicine, University of Milan
Translational and preclinical research activity:

- ✓ Understanding the role of mitochondrial dysfunction in the pathogenesis of the sarcopenia and of the frailty syndrome in mice and humans.
- ✓ Evaluate the effect of different drugs and nutraceutical compounds in the stimulation of mitochondrial biogenesis as a therapeutic target in different disease (Genetic mitochondrial disease; Sarcopenia and other myophaties; Cognitive decline; Non Alcholic Fatty Liver disease).

Previous positions

01/01/2018-27/12/2018: Visiting Scientist (Umberto Veronesi Foundation Fellowship) Department of Medical Biotechnology and Translational Medicine, University of Milan.
Translational and preclinical research activity:

- ✓ Understanding the role of the mitochondrial protein Pitrm1 in the pathogenesis of the Alzheimer disease

01/07/2013–29/10/2017: Investigator Scientist, Medical Research Council - Mitochondrial Biology Unit, Univ. of Cambridge (UK)
Mentor: Dr. Massimo Zeviani
Translational and preclinical research activity:

- ✓ MitCare. Mitochondrial medicine: developing treatments of OXPHOS-defects in recombinant mammalian models. Duration: 2013-2018, Granting Agency: **ERC (FP7-322424);** 2500 k€. PI: Massimo Zeviani, Role: Co-Investigator and coordination of experiments related to the generation of Surf1 knockout pig model.
- ✓ Mito-ND: Mitochondrial Neurodegeneration **CoEN grant 3038**; PI: Massimo Zeviani, Role: Co-Investigator and coordination of experiments related to understanding the role of the mitochondrial protein Pitrm1 in the pathogenesis of the Alzheimer disease.

2010 – 2013: Postdoctoral Research Scientist

Unit of Molecular Neurogenetics, Neurological Institute C. Besta, Milan Italy

Mentor: Dr. Valeria Tiranti

Translational research activity:

Study of the genetic diseases associated with Brain Iron accumulation (NBIA syndrome) and mitochondrial dysfunction due to mutations in Pank2 and Pla2g6 genes. The study was focused on basic research (understanding the role of the genes involved in the diseases) and translational/preclinical research (negative effect of Ketogenic Diet) and drug therapy (with Panthetine). **Telethon project** (GGP 11088); PI: Valeria Tiranti; Role in the project: Co-Investigator and coordination of experiments on NCSC and animal models.

2007- 2010: PhD Student

Laboratory of Reproductive Technologies - Avantea srl – Cremona, Italy; University of Milan. Mentors: Prof. Cesare Galli & Prof. Fulvio Gandolfi

Research activity:

- ✓ Project "Xenome-Engineering of the porcine genome for xenotransplantation studies in primates: a step towards clinical application" **European Sixth Framework Programme** (LSHB-CT-2006-037377). PI: Cesare Galli; Role in the project: generation of the GM pig models.
- ✓ Generation of GM pig for xenotransplantation. Fondazione Banca Popolare di Cremona, grant N° 060043-2008. PI: Dario Brunetti
- ✓ Production of transgenic pigs for xenotransplantation. Grant N° A0000583 Sovvenzione Globale Ingenio Regione Lombardia-2007. PI: Dario Brunetti

2004- 2007: Research Fellow

Laboratory of Reproductive Technologies - Avantea srl – Cremona, Italy

- ✓ Collaboration to the project "high-tech Network for the generation and use of animal models for gene and cell therapy of human diseases" CARIPLO foundation. PI: Paolo Vezzoni & Cesare Galli; role in the project: Generation of transgenic large animal models.
- ✓ European Science Foundation (EUROCORES Programme, EuroSTELLS) PI: Giovanna Lazzari & Cesare Galli; role in the project: collaborator
- ✓ Generation of PrPc knock-out cattle for the production of safe biomaterials in BSE prevention. CARIPLO foundation. PI: Cesare Galli; Role in the project: collaborator.

Education and qualifications

2018: Advanced course in Clinical Research: Methodology and regulations of Clinical Trials (GCP, GMP, GDP, QC; QA; Pharmacovigilance; Management and accounting of the drug; Monitoring tasks)

2018: National Scientific Qualification to function as Associate Professor of Applied Biology (BIO/13)

2013-2017: Education and research activity in Mitochondrial Medicine - University of Cambridge

2013: Course in Research and Human tissue legislation, MRC Cambridge, UK

2013: Course in Preclinical Research with laboratory animals; University of Cambridge, UK

2010-2013: Education and research activity in Molecular Neurogenetics and Mitochondrial Medicine;-Neurological Institute C. Besta, Milan, Italy

2011: Residential course in Clinical Research, Neurological Institute C. Besta, Milan, Italy

2010: Course in Animal Model for Preclinical Research, IZSLER, Milan, Italy

2007-2010: PhD in Biotechnology, University of Milan, Italy

2007: Course for the management and exploitation of collaborative research results in joint projects between University and Industry. University of Milan, Italy

1998-2004: Animal Science Degree, University of Bologna (score 110/110), Italy

AWARDS

2019: Seal of Excellence, European Commission – Horizon 2020

2015: **MRC Special Award Scheme,** Medical research Council, Cambridge (UK)

2012: Young Scientist Award: NBIA association & The Movement Disorder Society

Funding and fellowship

2019: Telethon Project n° GGP19087 "MITOchondrial THErapeutic Response to a dietary amino-acid formula: a preclinical and clinical trial in primary mitochondrial myopathies" (MITO-THER). Budget requested: 260 k €; Status: Pending

2018: H2020-MSCA-IF-2018 project n°2018 840997 "DIetary supplementation in Sporadic AlzheimeR disease: a Mitochondria targeted approach" DISARM. Budget

requested 183 k €. Status: rejected (score 89.20 / 100; scored as HIGH-Quality Project proposal and awarded with the SEAL OF EXCELLENCE)

2018: Fellowship Umberto Veronesi Foundation. Status: awarded 27K€

2017: H2020-MSCA-IF-2017 project n°794158 "Mitochondrial medicine as a gender-oriented approach to frailty syndrome" MAGNIFY. Budget requested 180 k €. Status: rejected (score 80.60 / 100)

2016: H2020-MSCA-IF-2016 project n° 744033" MITOAPP: Investigating the role of APP overproduction and the mitochondrial dysfunction in Down Syndrome cell models Budget requested 180 k €. Status: rejected (score 80.40 / 100)

2008 Fellowship Banca Popolare di Cremona Foundation grant N° 060043-2008 "Generation of GM pig for xenotransplantation". Budget requested: 24 k €. Status awarded

2006: Grant for young scientists Sovvenzione Globale Ingenio, Regione Lombardia project N° A0000583 -2007. "Production of transgenic pigs for xenotransplantation" Fondazione Banca Popolare di Cremona, Budget requested: 30k €. Status awarded

Supervision of graduate, PhD student and Post Doc

November 2018- present: Thesis Advisor, Department of Medical Biotechnology and Translational Medicine, University of Milan

February - May 2016: Advisor of visiting post doc MRC-MBU Cambridge, UK

January - June 2016: Advisor PhD student MRC-MBU Cambridge, UK

January - June 2011: Thesis Advisor, Unit of Molecular Neurogenetics, Neurological Institute C. Besta, Milan Italy

Invited speaker in national and international conference & seminars

- ✓ **Brunetti D.** "Amyolid β and mitochondrial dysfunction: the role of Pitrm1" 17 Dec 2018 – Neurological Institute C. Besta, Milan.
- ✓ **Brunetti D.** "Defective PITRM1 mitochondrial peptidase is associated with Aβ amyloidotic neurodegeneration", 2 March 2018 Neuroscience Institute CNR Milan.
- ✓ **Brunetti D**. "Genetic modification of animal models: new tools and prospects" – Reproductive Biotechnology Centre, 8 May 2016 Dubai, UAE
- ✓ **Brunetti D.** "Pantothenate kinase-associated neurodegeneration: altered mitochondria membrane potential and defective respiration in Pank2 knock-out mouse model." NBIA association & The Movement Disorder Society - 27 October 2012 Ede, NL
- ✓ **Brunetti D.** "Production of Prion-knock out cattle" European Science

Foundation Exploratory Workshop on Genetic Models of Disease Resistance in Livestock - 1-2 October 2007 Edinburgh, UK

Involvement in the organization of international research groups

In 2007, Dr. Brunetti was involved by Prof. Bruce Whitelaw (Director of the Roslin Institute, UK) in the European Science Foundation Exploratory Workshop for the establishment of a network of European research groups for the development of "Genetic Models of Disease Resistance" in Livestock "- 1-2 October 2007, Edinburgh, UK.

National and International collaborations:

- ✓ Neurogenetics Unit, Neurological Institute C. Besta, Milan, Italy
- ✓ Mitochondrial Biology Unit, University of Cambridge, UK
- ✓ Department of Molecular and Translational Medicine, University of Brescia
- ✓ Advanced Technology Center for Aging Research, INRCA, Ancona, Italy
- ✓ Reproductive Biotechnology Center, Dubai, United Arab Emirates
- ✓ Nestlé Research Institute, Innovation Park, Lausanne, Switzerland

Publications in peer-reviewed scientific journals:

	Scholar	Scopus
H index	10	10
Total cit	461	336

2018

Langer Y, Aran A, Gulsuner S, Abu Libdeh B, Renbaum P,**Brunetti D**, Teixeira PF, Walsh T, Zeligson S, Ruotolo R, Beeri R, Dweikat I, Shahrour M, Weinberg-Shukron A, Zahdeh F, Baruffini E, Glaser E, King MC, Levy-Lahad E, Zeviani M, Segel R. Mitochondrial *PITRM1* peptidase loss-of-function in childhood cerebellar atrophy.
J Med Genet. 2018 May 15.
[2 citations] IF 2017: 5.751

Quadalti C*, **Brunetti D***, Lagutina I, Duchi R, Perota A, Lazzari G, Cerutti R, Di Meo I, Johnson M, Bottani E, Crociara P, Corona C, Grifoni S, Tiranti V, Fernandez-Vizarra E, Robinson AJ, Viscomi C, Casalone C, Zeviani M, Galli C. SURF1 knockout cloned pigs: Early onset of a severe lethal phenotype. *Biochim Biophys Acta*. 2018 Jun;1864(6 Pt A):2131-2142
[1 citations] IF 2017: 5.108 *equal contributors

2016

Brunetti D, Torsvik J, Dallabona C, Teixeira P, Sztromwasser P,Fernandez-Vizarra E, Cerutti R, Reyes A, Preziuso C, D'Amati G, Baruffini E,Goffrini P, Viscomi C, Ferrero I, Boman H, Telstad W, Johansson S, Glaser E, Knappskog PM, Zeviani M, Bindoff LA. Defective PITRM1 mitochondrial peptidase is associated with Aβ amyloidotic neurodegeneration. *EMBO Molecular Medicine* 2016 Mar 1;8(3):176-90
[14 citations] IF: 10.293

2014

Brunetti D, Dusi S, Giordano C, Lamperti C, Morbin M, Fugnanesi V, Marchet S, Fagiolari G, Sibon O, Moggio M, d'Amati G, Tiranti V. Pantethine treatment iseffective in recovering the disease phenotype induced by ketogenic diet in a pantothenate kinase-associated neurodegeneration mouse model. *Brain*. 2014 Jan;137(Pt 1):57-68.
[29 citations] IF: 10.848

2013

Czernik M, Fidanza A, Sardi M, Galli C, **Brunetti D**, Malatesta D, Della Salda L, Matsukawa K, Ptak GE, Loi P. Differentiation potential and GFP labeling of sheep bone marrow-derived mesenchymal stem cells. *Journal of Cell Biochemistry* 2013 Jan;114(1):134-43 **[6 citations] IF: 3.446**

2012

Brunetti D, Dusi S, Morbin M, Uggetti A, Moda F, D'Amato I, Giordano C, d'Amati G, Cozzi A, Levi S, Hayflick S, Tiranti V. Pantothenate kinase-associated neurodegeneration: altered mitochondria membrane potential and defective respiration in Pank2 knock-out mouse model. *Hum Molecular Genetics*. 2012 Dec 15;21(24):5294-305. **[42 citations] IF: 4.902**

Panteghini C, Zorzi G, Venco P, Dusi S, Reale C, **Brunetti D**, Chiapparini L, Zibordi F, Siegel B, Garavaglia B, Simonati A, Bertini E, Nardocci N, Tiranti V. C19orf12 and FA2H mutations are rare in Italian patients with neurodegeneration with brain iron accumulation. *Seminars Pediatric Neurolology* 2012 Jun; 19(2):75-81.
[27 citations] IF: 1.878

2010

Galli C, Perota A, **Brunetti D**, Lagutina I, Lazzari G, Lucchini F. Genetic engineering including superseding microinjection: new ways to make GM pigs. *Xenotransplantation*. 2010 Nov-Dec;17(6):397-410 **[19 citations] IF 4.717**

Lagutina I, Fulka H, Brevini TA, Antonini S, **Brunetti D**, Colleoni S, Gandolfi F, Lazzari G, Fulka J, Galli C. Development, embryonic genome activity and mitochondrial characteristics of bovine-pig inter-family nuclear transfer embryos. *Reproduction*. 2010 Aug;140(2):273-85 **[25 citations] IF: 3.086**

Lazzari G., Colleoni S., Lagutina I., Crotti G., Turini P., Tessaro I., **Brunetti D.**, Duchi R., Galli C. “Short-term and long-term effects of embryo culture in the surrogate sheep

oviduct versus in vitro culture for different domestic species". *Theriogenology*. 1 Apr. 2010, 73(6):748-57 **[35 citations] IF: 2.136**

2008

Brunetti D., Perota A., Lagutina I., Colleoni S., Duchi R., Calabrese F., Seveso M., Cozzi E., Lazzari G., Lucchini F. and Galli C. "Transgene expression of green fluorescent protein and germ line transmission in cloned pigs derived from in vitro transfected adult fibroblasts". *Cloning and Stem Cells*, volume 10 Number 4, Dec, 2008. Pp 409-419 **[42 citations] IF 2.622**

2007

Lagutina I., Lazzari G., Duchi R., Turini P., Tessaro I., **Brunetti D.**, Colleoni S., Crotti G., Galli C. " Comparative aspects of somatic cell nuclear transfer with conventional and zona free method in cattle, horse, pig and sheep". *Theriogenology* volume 67 (2007) pp 90-98 **[53 citations]** ***IF: 2.136***

2006

Lazzari G., Colleoni S., Giannelli S., **Brunetti D**., Colombo E., Lagutina I., Galli C. & Broccoli V. "Direct derivation of Neural Rosettes from Cloned Bovine Blastocysts: A Model of Early Neurulation Events and Neural Crest Specification In Vitro". *Stem Cells*. 2006 Nov; Volume 24 n 11 pp 2514-21 **[41 citations] IF: 5.587**

Peer-reviewed conference proceedings

2015

Zeviani M., Reyes A., Viscomi C., Civiletto G., Cerutti R., Fernandez-Vizarra, **Brunetti D**., Bottani E. "Identification and characterization of new mitochondrial disease genes". 8th Annual Neuromuscular Translational Research Conference 19th and 20th March 2015 - *Neuromuscular Disorders* 25, S1-S3 **IF 2.368**

2013

M Guaraldo, A Cozzi, P Santambrogio, **D Brunetti**, V Tiranti, S Levi. "Pank2 $^{-/-}$ mice tissues show sign of oxidative damage". *American Journal of Hematology* 88 (5), E166-E166 **IF 3.477**

2010

A Perota, **D Brunetti**, I Lagutina, G Lazzari, F Lucchini, C Galli. "Establishment of transgenic PK15 cell clones for human thrombomodulin gene". *Transgenic Research* 19 (2), 347-347 **IF 2.197**

D Brunetti, A Perota, I Lagutina, M Chatelais, B Charreau, R Duchi, Giovanna Lazzari, Franco Lucchini, Cesare Galli. "Production and characterization of Gal-/- minipigs over-expressing hCD55. *Transgenic Research* 19 (2), 325-325 **IF 2.197**

Mn Chieppa, A Perota, **D Brunetti**, C Porcario, M Tortarolo, G Lazzari, C Bendotti, C Corona, Franco Lucchini, C Casalone, C Galli. "Creation of a ubiquitous vector for expression of hSOD1G93A in pig". *Transgenic Research* 19 (2), 326-326 **IF 2.197**

2009

A Perota, **D Brunetti,** B Charreau, M Chatelais, I Lagutina, G Lazzari, I Anegon, DH Sachs, E Cozzi, F Lucchini, C Galli "Generation of cloned CD55-CD39 transgenic

α1,3-galactosyltransferase depleted GAL $^{-/-}$ piglets. *Reproduction, Fertility and Development* 22 (1), 372-373 **IF: 2.105**

E Cozzi, P Simioni, MB Nottle, M Vadori, GM De Benedictis, N Baldan, M Boldrin, SC Robson, F Besenzon, L Cavicchioli, F Calabrese, **D Brunetti**, S Gavasso, B Ekser, C Radu, M Seveso, A Dedja, F Fante, E Salvaris, V Tisato, P Carraro, C Galli, JP Soulillou, G Blancho, AJ d'Apice, E Ancona, PJ Cowan. "Preliminary study in a life supporting pig to primate xenotransplantation model using Gal KO pigs transgenic for human CD39, CD55, CD59 and fucosyltransferase". *Xenotransplantation* 16 (6), 544-544 **[5 citations] IF: 4.717**

Claudia M Radu, Cristiana Bulato, Sabrina Gavasso, **Dario Brunetti**, Andrea Perota, Luca Spiezia, Valeria Ferri, Cesare Galli, Paolo Simioni, Emanuele Cozzi. "Activation of human protein C by alpha 1, 3-galactosyltransferase gene-knockout porcine aortic endothelial cells expressing human thrombomodulin". *Xenotransplantation* 16 (5), 435-436 **IF: 4.717**

Dario Brunetti, Andrea Perota, Irina Lagutina, Mathias Chatelais, Beatrice Charreau, Roberto Duchi, Emanuele Cozzi, Giovanna Lazzari, Franco Lucchini, Ignacio Anegon, Dh Sachs, Cesare Galli. "Double transgenic Gal$^{-/-}$ piglets over-expressing hCD39". *Xenotransplantation* 16 (5), 436-437 **[1 citation] IF: 4.717**

2008

D Brunetti, A Perota, I Lagutina, S Colleoni, F Besenzon, E Cozzi, G Lazzari, F Lucchini, DH Sachs, C Galli. "Isolation and characterization of porcine Gal KO fibroblasts expressing hCD55-hCD39 and hEPCR-hTPA". *Transgenic Research* 17 (5), 1002-1003 **IF 2.197**

2007

D Brunetti, G Rossi, I Lagutina, R Duchi, S Colleoni, M Catania, C Viscomi, D Piga, M Zeviani, G Lazzari, F Tagliavini, C Galli. "Hemizygous prion protein gene (PRNP) knockout in cattle fibroblasts". *Reproduction, Fertility and Development* 20 (1), 230-230 **IF: 2.105**

D Brunetti, A Perota, I Lagutina, G Lazzari, F Lucchini, C Galli. "Expression of Green fluorescent protein in pig blastocysts obtained by somatic cell nuclear transfer". *Xenotransplantation* 14 (5), 466-466 **IF: 4.717**

M Byrne, A Smolic, A Preveolos, C Galli, G Lazzari**, D Brunetti**, G Condorelli, D Kaye. "Cardiac delivery of eGFP transfected ovine fibroblasts using percutaneous recirculation in a large animal model" *Journal of Molecular and Cellular Cardiology* 42(6): PS79 **IF: 5.296**

2005

I Lagutina, **D Brunetti**, G Lazzari, C Galli. "Preliminary data on pig-bovine interspecies nuclear transfer embryo development" *Reproduction, Fertility and Development* 18 (2), 134-135 **[4 citations] IF: 2.105**

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Dario Brunetti, PhD