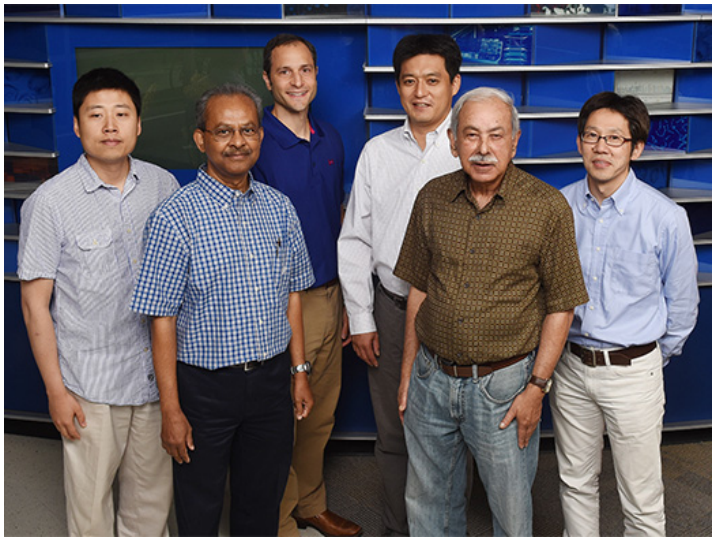
Cincinnati Children's

2015 Research Annual Report

Reproductive Sciences

RESEARCH AND TRAINING DETAILS



Click to view members

Faculty	7
Joint Appointment Faculty	3
Research Fellows	14
Research Students	3
Support Personnel	10
Direct Annual Grant Support	$1,471,466
Peer Reviewed Publications	17

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Research Highlights

Sudhansu K. Dey, PhD

Dr. Dey presented as a Founders' Forum Speaker at the Milestones in Reproductive Biology Seminar at the Gordon Research Conference on Mammalian Reproduction. The conference was held at Colby-Sawyer College, New Hampshire in August, 2014.

Xiaofei Sun, PhD

Dr. Sun gave an oral presentation with Dr. Dey entitled "Ovarian LGR5 is critical for successful pregnancy" at the 3rd State Key Laboratory of Reproductive Biology (SKLRB) Symposium on Reproductive Biology. This conference was held in Beijing, China on October, 2014.

Satoshi Namekawa, PhD

- Dr. Namekawa gave an oral presentation entitled "Xist exon 7 contributes to the stable localization of Xist RNA on the inactive X-chromosome" at the Keystone Symposia (Long Noncoding RNAs: From Evolution to Function). This meeting took place in Cold Spring Harbor, New York, from March 15-20, 2015.
- Dr. Namekawa gave a Poster Presentation on "The Role of exon 7 of Xist in X-Chromosome inactivation" at the 2014 Cold Spring Harbor Meeting (Regulatory and Non-Coding RNAs). This meeting was held August 26-30, 2014, in Cold Spring Harbor, New York.

Tony DeFalco, PhD

- Dr. DeFalco won a Travel Fellowship for the Seventh International Symposium on Vertebrate Sex Determination. At the symposium, he presented "Macrophages and vascular: an unlikely partnership driving fetal testis differentiation." The symposium was held April 13-17, 2015, in Kona, Hawaii.
- Dr. DeFalco was awarded the March of Dimes Basil O'Connor Starter Scholar Award for 2014-2015.
- Dr. DeFalco appeared as an invited speaker at the 48th Annual Meeting of the Society for the Study of Reproduction: "Regulation of gonadal macrophage activity is critical for sexual differentiation and vascular remodeling of the fetal testis." This meeting was held June 2015 in San Juan, Puerto Rico.
- Dr. DeFalco was an invited lecturer on vascular and testis stem cell niches at the Roswell Park Cancer Institute. They event was held May, 2015 in Buffalo, New York.
- Dr. DeFalco travelled to Japan in March of 2015 where he appeared at the University of Tsukuba, the University of Tokyo, the National Institute of Genetics of Japan and the Wakayama Medical University as an invited lecturer to discuss the role of macrophages and mafs in developmental and reproductive biology.

Sanjoy Das, PhD

- Dr. Das appeared as an invited speaker at the March of Dimes Prematurity Research Center Ohio Collaborative meeting held on December 2, 2014.
- Dr. Das appeared as an invited reviewer at The Endocrine Society's 97th Annual Meeting held March 5-8, 2015, in San Diego, California.

Significant Publications

Daikoku T, **Ogawa Y**, Terakawa J, Ogawa A, **DeFalco T**, **Dey SK**. Lactoferrin-iCre: a new mouse line to study uterine epithelial gene function. *Endocrinology.* 2014 July 155(7):2718-24.

In this article, we report the generation of a new iCre knock-in mouse line, in which iCre is expressed from endogenous lactoferrin (Ltf) promoter. Ltf-iCre mice primarily direct recombination in the uterine epithelium in adult females and in immature females after estrogen treatment. These mice will allow for specific interrogation of gene function in the mature uterine epithelium, providing a helpful tool to uncover important aspects of uterine biology.

Cha J, Bartos A, Park C, **Sun X**, Li Y, Cha SW, Ajima R, Ho HY, Yamaguchi TP, **Dey SK**. Appropriate Crypt Formation in the Uterus for Embryo Homing and Implantation Requires Wnt5a-ROR Signaling. *Cell Report.* 2014 Jul 24:8(2):382-92.

The study tested the effects of disrupting Wnt5a-ROR signaling, which resulted in disorderly epithelial projections, crypt formation, embryo spacing, and impaired implantation. These early disturbances under abnormal Wnt5a-ROR signaling were reflected in adverse late pregnancy events, including defective decidualization and placentation, ultimately leading to compromised pregnancy outcomes. This study presents deeper insight regarding the formation of organized epithelial projections for crypt formation and embryo implantation for pregnancy success.

Li Y, **Sun X**, **Dey SK**. Entosis allows timely elimination of the luminal epithelial barrier for embryo implantation. *Cell Rep.* 2015 Apr 21;11(3):358-65.

In this study, we show that LE cells in direct contact with the blastocyst are endocytosed by trophoblast cells by adopting the nonapoptotic cell-in-cell invasion process (entosis) in the absence of caspase 3 activation. Our in vivo observations were reinforced by the results of co-culture experiments with primary uterine epithelial cells with trophoblast stem cells or blastocysts showing internalization of epithelial cells by trophoblasts. We have identified entosis as a mechanism to remove LE cells by trophoblast cells in implantation, conferring a role for entosis in an important physiological process.

Cha J, Burnum-Johnson KE, Bartos A, Li Y, Baker ES, Tilton SC, Webb-Robertson BJ, Piehowski PD, Monroe ME, Jegga AG, Murata S, **Hirota Y**, **Dey SK**. Muscle segment homeobox genes direct embryonic diapause by limiting inflammation in the uterus. *J Biol Chem.* 2015 Jun 12;290(24):15337-49.

Embryonic diapause is a widespread reproductive strategy defined by a temporary arrest in blastocyst growth and metabolic activity within a quiescent uterus without implantation until the environmental and maternal milieu become favorable for pregnancy. We found that uterine Msx expression persists during diapause across species; inactivation in the mouse uterus results in termination of diapause with the development of implantation-like responses ("pseudoimplantation") that ultimately succumbed to resorption. To understand the cause of this failure, we compared proteome profiles between floxed and Msx-deleted uteri. In deleted uteri, several functional networks, including transcription/translation, ubiquitin-proteasome, inflammation, and endoplasmic reticulum stress, were dysregulated. Interestingly, treatment with anti-inflammatory glucocorticoid (dexamethasone) reduced the inflammatory signature with improvement of the diapause phenotype. These findings highlight an unexpected role of uterine Msx in limiting aberrant inflammatory responses to maintain embryonic diapause.

Romero R, **Dey SK**, Fisher SJ. Preterm Labor: one syndrome, many causes. *Science.* 2014 Aug 15;345(6198):760-5.

Preterm birth is associated with 5 to 18% of pregnancies and is a leading cause of infant morbidity and mortality. Spontaneous preterm labor, a syndrome caused by multiple pathologic processes, leads to 70% of preterm births. The prevention and the treatment of preterm labor have been long-standing challenges. We summarize the current understanding of the mechanisms of disease implicated in this condition and review advances relevant to intra-amniotic infection, decidual senescence, and breakdown of maternal-fetal tolerance. The success of progestogen treatment to prevent preterm birth in a subset of patients at risk is a cause for optimism. Solving the mystery of preterm labor, which compromises the health of future generations, is a formidable scientific challenge worthy of investment.

Division Publications

1. Cha J, Bartos A, Park C, Sun X, Li Y, Cha SW, Ajima R, Ho HY, Yamaguchi TP, Dey SK. **Appropriate crypt formation in the uterus for embryo homing and implantation requires Wnt5a-ROR signaling**. *Cell Rep*. 2014; 8:382-92.
2. Cha J, Burnum-Johnson KE, Bartos A, Li Y, Baker ES, Tilton SC, Webb-Robertson BJ, Piehowski PD, Monroe ME, Jegga AG, Murata S, Hirota Y, Dey SK. **Muscle Segment Homeobox Genes Direct Embryonic Diapause by**

Limiting Inflammation in the Uterus. *J Biol Chem*. 2015; 290:15337-49.

3. Cha J, Dey S, Lim H. **Embryo Implantation**. In: TM Plant, AJ Zeleznik, eds. *Knobil and Neill's Physiology of Reproduction*. Amsterdam: Elsevier/Academic Press; 2015:1697-1793.
4. Cha J, Dey SK. **Cadence of procreation: orchestrating embryo-uterine interactions**. *Semin Cell Dev Biol*. 2014; 34:56-64.
5. Chung D, Gao F, Jegga AG, Das SK. **Estrogen mediated epithelial proliferation in the uterus is directed by stromal Fgf10 and Bmp8a**. *Mol Cell Endocrinol*. 2015; 400:48-60.
6. Daikoku T, Ogawa Y, Terakawa J, Ogawa A, DeFalco T, Dey SK. **Lactoferrin-iCre: a new mouse line to study uterine epithelial gene function**. *Endocrinology*. 2014; 155:2718-24.
7. Daikoku T, Terakawa J, Hossain MM, Yoshie M, Cappelletti M, Yang P, Ellenson LH, Dey SK. **Mammalian target of rapamycin complex 1 and cyclooxygenase 2 pathways cooperatively exacerbate endometrial cancer**. *Am J Pathol*. 2014; 184:2390-402.
8. Hasegawa K, Sin HS, Maezawa S, Broering TJ, Kartashov AV, Alavattam KG, Ichijima Y, Zhang F, Bacon WC, Greis KD, Andreassen PR, Barski A, Namekawa SH. **SCML2 establishes the male germline epigenome through regulation of histone H2A ubiquitination**. *Dev Cell*. 2015; 32:574-88.
9. Hu YC, Namekawa SH. **Functional significance of the sex chromosomes during spermatogenesis**. *Reproduction*. 2015; 149:R265-77.
10. Lanekoff I, Burnum-Johnson K, Thomas M, Cha J, Dey SK, Yang P, Prieto Conaway MC, Laskin J. **Three-dimensional imaging of lipids and metabolites in tissues by nanospray desorption electrospray ionization mass spectrometry**. *Anal Bioanal Chem*. 2015; 407:2063-71.
11. Li Y, Sun X, Dey SK. **Entosis allows timely elimination of the luminal epithelial barrier for embryo implantation**. *Cell Rep*. 2015; 11:358-65.
12. Maccarrone M, Bab I, Biro T, Cabral GA, Dey SK, Di Marzo V, Konje JC, Kunos G, Mechoulam R, Pacher P, Sharkey KA, Zimmer A. **Endocannabinoid signaling at the periphery: 50 years after THC**. *Trends Pharmacol Sci*. 2015; 36:277-96.
13. PrabhuDas M, Bonney E, Caron K, Dey S, Erlebacher A, Fazleabas A, Fisher S, Golos T, Matzuk M, McCune JM, Mor G, Schulz L, Soares M, Spencer T, Strominger J, Way SS, Yoshinaga K. **Immune mechanisms at the maternal-fetal interface: perspectives and challenges**. *Nat Immunol*. 2015; 16:328-34.
14. Romero R, Dey SK, Fisher SJ. **Preterm labor: one syndrome, many causes**. *Science*. 2014; 345:760-5.
15. Sun X, Cappelletti M, Li Y, Karp CL, Divanovic S, Dey SK. **Cnr2 deficiency confers resistance to inflammation-induced preterm birth in mice**. *Endocrinology*. 2014; 155:4006-14.
16. Tranguch S, Dey SK. **A lifetime of deciphering complexities of embryo implantation**. *Int J Dev Biol*. 2014; 58:79-86.
17. Yue M, Charles Richard JL, Yamada N, Ogawa A, Ogawa Y. **Quick fluorescent in situ hybridization protocol for Xist RNA combined with immunofluorescence of histone modification in X-chromosome inactivation**. *J Vis Exp*. 2014; :e52053.

Faculty, Staff, and Trainees

Faculty Members

Sudhansu K. Dey, PhD, Professor
Leadership Division Director; Lova Riekert Chair
Research Interests Pregnancy and implantation; reproductive cancers; endocannabinoids

Takiko Daikoku, PhD, Associate Professor
Research Interests Reproductive cancers; blastocyst implantation

Sanjoy Das, PhD, Professor
Research Interests Uterine decidualization; environmental estrogens

Tony DeFalco, PhD, Assistant Professor
Research Interests Genetic and molecular mechanisms of sexual development disorders

Yasushi Hirota, MD, PhD, Adjunct
Research Interests Human reproduction; endometrial biology; implantation (Home Institution: National Institute of Genetics of Japan)

Hyunjung "Jade" Lim, PhD, Adjunct
Research Interests Embryo Implantation (Home Institution: Konkuk University, Korea)

Satoshi Namekawa, PhD, Assistant Professor
Research Interests Epigenetics of germ cells; X chromosome inactivation

Yuya Ogawa, PhD, Assistant Professor
Research Interests Molecular mechanisms of X chromosome inactivation

Xiaofei Sun, PhD, Instructor
Research Interests implantation; endocannabinoid signaling

Joint Appointment Faculty Members

Helen Jones, PhD, Assistant Professor (Pediatric Surgery)
Research Interests Regulation of placental growth and function; fetal growth restriction

Trainees

- **Fenghua Bian, PhD**, State Key Laboratory of Reproductive Biology, Institute of Zoology/Chinese Academy of Sciences, China
- **Hironori Abe, PhD**, Okayama University, Japan
- **Jeeyeon Cha, MD/PhD**, University of Cincinnati Medical School
- **Daesuk Chung, PhD**, University of Colorado
- **Fei Gao, PhD**, Vanderbilt University
- **Sarah Potter, PhD**, Universität Würzburg, Germany
- **Deepti Kumar, PhD**, Miami University, Ohio
- **Yasuko Kato, PhD**, Kyoto Institute of Technology, Japan
- **Jia Yuan, PhD**, College of Life Sciences at WuHan University, China

- **Craig Park, PhD**, McGill University, Canada
- **Wenbo Deng, PhD**, Xiamen University, China
- **Charles-Richard John-Lalith, PhD**, Université de Grenoble, France
- **Kris Alavattam, PhD**, Kettering College, Ohio
- **Norishige Yamada, PhD**, Kagoshima University, Japan
- **Minghui Yue, PhD**, Institute of Genetics and Developmental Biology Chinese Academy of Sciences, China
- **So Maezawa, PhD**, Tokyo University, Japan

Grants, Contracts, and Industry Agreements

Grant and Contract Awards		Annual Direct

Cha, J

Premature Uterine Aging and Preterm Delivery

National Institutes of Health

F30 AG040858	9/16/2011-6/30/2015	$47,676

De Falco, T

Macrophage Regulation of Fetal Testis Vascularization and Morphogenesis

March of Dimes

	2/1/2014-1/31/2016	$68,182

Dey, S

The Role of Bioactive Lipids in Inflammation and Cancer - Project 4

National Institutes of Health (Mayo Clinic)

P01 CA077839	12/1/2012-5/31/2017	$159,030

Endocannabinoid Signaling during Early Pregnancy

National Institutes of Health

R01 DA006668	4/1/2015-3/31/2020	$284,119

Molecular Signaling in Uterine Receptivity to Implantation

National Institutes of Health

R01 HD068524	9/26/2011-6/30/2016	$206,550

March of Dimes Prematurity Research Center Ohio Collaboration Theme 3

March of Dimes

22FY15003	1/1/2015-12/31/2015	$250,000

Namekawa, S

DNA Damage Response Pathways in Mieotic Sex Chromosome Inactivation

National Institutes of Health

R01 GM098605	8/1/2011-7/31/2016	$189,000

Regulatory Mechanism of Active Epigenetic Modifications

March of Dimes

1-FY13-510	6/01/2013-5/31/2016	$90,909

Ogawa, Y

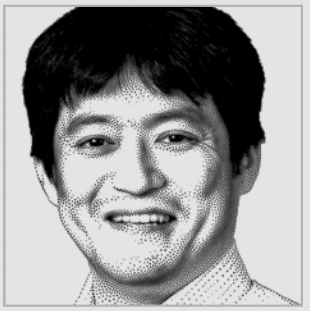
Organization of the Inactive X-Chromosome

National Institutes of Health

R01 GM102184	9/1/2012-8/31/2017	$176,000

	Current Year Direct	**$1,471,466**
	Total	**$1,471,466**

Genomic Research Sheds Light on Possible Causes of Male Infertility



Satoshi Namekawa, PhD

RESEARCH AND TRAINING DETAILS

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Direct Annual Grant Support	$1.4M
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Hasegawa K, Sin HS, Maezawa S, Broering TJ, Kartashov AV, Alavattam KG, Ichijima Y, Zhang F, Bacon WC, Greis KD, Andreassen PR, Barski A, Namekawa SH. SCML2 establishes the male germline epigenome through regulation of histone H2A ubiquitination. *Dev Cell.* 2015;32(5):574-588.

PUBLISHED ONLINE FEB. 19, 2015
Developmental Cell

Research at Cincinnati Children's has identified a critical determinant of the germline epigenome that has important implications for the genomics and evolution of the male germline.

The study in the journal *Developmental Cell* advances research into the possible causes of male infertility, says senior author Satoshi Namekawa, PhD.

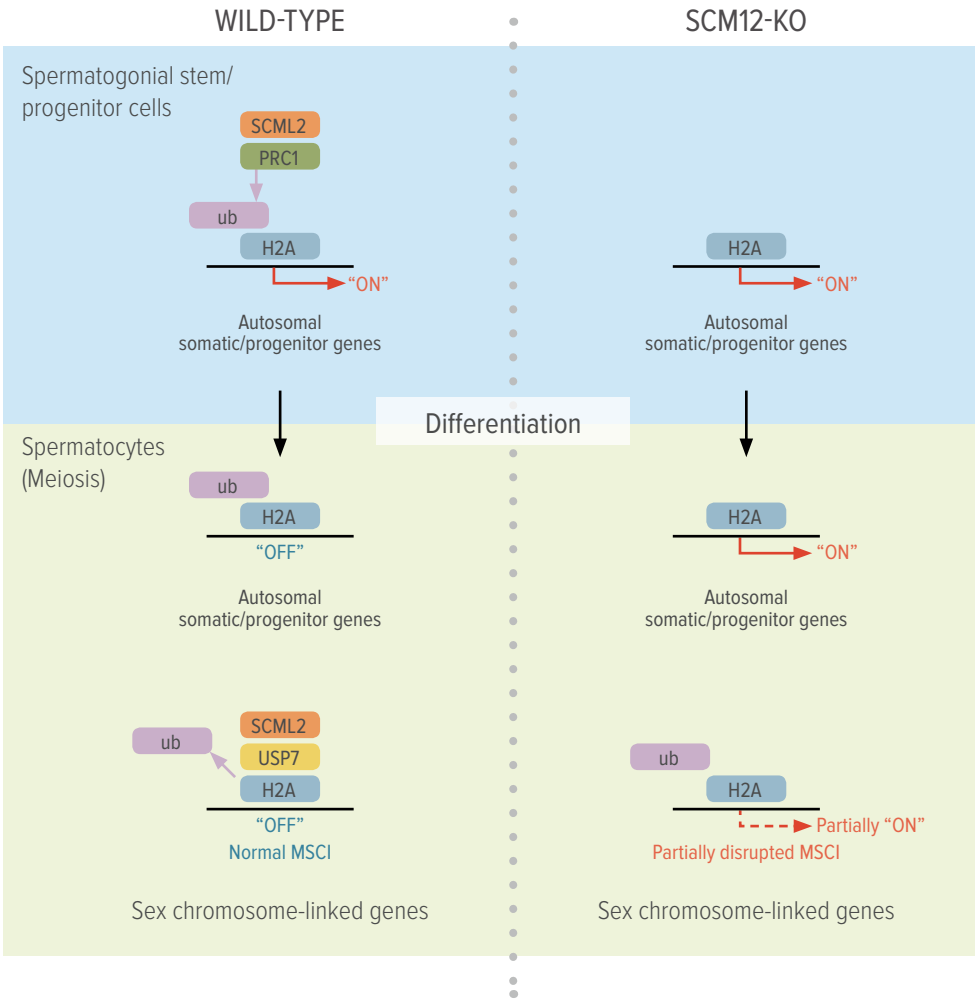
Namekawa and colleagues found that genes commonly expressed in somatic lineages and spermatogenesis-progenitor cells undergo a repression in a genome-wide manner. This repression occurs in the late developmental stages of the male germline, the only lineage that ensures the perpetuation of genetic and epigenetic information across generations.

The research team theorizes that this repression may, in turn, indirectly ensure activation of spermatogenesis-specific genes, an essential step for producing mature spermatozoa. Disruptions to this normal process may explain some forms of male infertility because this mechanism underlies activation of essential genes in spermatogenesis.

The study identifies SCML2, a germline-specific subunit of a polycomb repressive complex 1 (PRC1), as the critical determinant of the germline epigenome. SCML2 establishes the unique epigenome of the germline through two distinct and antithetical mechanisms:

- SCML2 works with PRC1 to promote ubiquitination of histone H2A, a histone modification associated with gene repression, in the stem cell phase of spermatogenesis-progenitor cells. This process appears to mark somatic/progenitor genes on autosomes for repression.
- At the same time, SCML2 also prevents ubiquitination of histone H2A on sex chromosomes during meiosis, thereby enabling unique epigenetic programming of sex chromosomes for male reproduction.

The key finding was revealing essential mechanisms underlying spermatogenesis, which will lead to more focused exploration of how the mechanisms of reproduction operate.



This illustration describes the distinct mechanisms followed by SMCL2 as it regulates histone H2A ubiquitination. By revealing essential mechanisms underlying spermatogenesis, these findings could lead to new understanding of reproduction and reproductive disorders.