# WNT SIGNALLING IN GERM CELLS AND REPRODUCTIVE TRACT DEVELOPMENT

**MANISH KUMAR**

(B.V.Sc & AH, M.V.Sc)

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***"Science is fun. Science is curiosity. We all have natural curiosity. Science is a process of investigating. It's posing questions and coming with a method. It's delving in"***

-Sally Ride

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## DECLARATIONS PART A

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository**, subject to the provisions of the Copyright Act 1968.

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Publications included as part of this thesis

*I the undersigned corresponding author of the following publications:*

Kumar M, Crossingham YJ, Tanwar PS. **Role of Wnt signalling in testicular morphogenesis and carcinogenesis**. Int J Dev Biol, Submitted on November 07, 2011.

Kumar M, Camlin NJ, Holt JE, Teixeira JM, McLaughlin EA, Tanwar PS, **Germ cell specific overactivation of WNT/βcatenin signalling has no effect on folliculogenesis but causes fertility defects due to abnormal foetal development**. Sci Rep, 2016, 6: srep27273.

Kumar M, Atkins J, Cairns M, Ali A, Tanwar PS, **Sustained activation of Wnt signalling in male germ cells perturbs spermatogenesis, possibly through non-coding RNAs**. Oncotarget, 2016, 7: 85709-85727.

Kumar M, Syed SM, Taketo MM, Tanwar PS, **Epithelial Wnt/βcatenin signalling is essential for epididymal coiling**. Dev Biol, 2016, 412: 234-249.

Kumar M, Tanwar PS, **Organ culture and whole mount immunofluorescence staining of mouse Wolffian ducts**. JoVE, 2016, e55134.

*Authorise the inclusion of these works and declare that Research Higher Degree candidate Manish Kumar contributed to these publications. Outlined below are the items that the candidate has contributed towards the fulfilment of the papers:*

- *Conducted and designed most of the experiments*
- *Critically analysed and interpreted the results*
- *Prepared and organised the figures*
- *Contributed in drafting and conceptualising the manuscripts*
- *Contributed in formatting initial and revised versions of the manuscripts*

*Signature: ……* *Date: ……/……/……*
*Pradeep Tanwar* *dd /mm / yyyy*

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## LIST OF PUBLICATIONS INCLUDED AS PART OF THE THESIS

CONTAINED IN:

CHAPTER 1

Kumar M, Crossingham YJ, Tanwar PS. **Role of Wnt signalling in testicular morphogenesis and carcinogenesis**. Int J Dev Biol, Submitted on November 07, 2016.

CHAPTER 2

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CHAPTER 4

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CHAPTER 5

Kumar M, Tanwar PS, **Organ culture and whole mount immunofluorescence staining of mouse Wolffian ducts**. JoVE, 2016, e55134.

ADDENDUM

1. Goad J, Ko YA, Kumar M, Syed SM, Tanwar PS, **Differential Wnt signalling activity limits epithelial gland development to the anti-mesometrial side of the mouse uterus**. Dev Biol, submitted December, 2016.
2. Goad J, Ko YA, Kumar M, Syed SM, Tanwar PS, **Data on the expression of Wnt signaling pathway members in the mouse uterus**. Data in brief, submitted December, 2016.

3. Ghosh A, Syed SM, Goad J, Kumar M, Tanwar PS, **Role of Wnt signalling in self-renewal of the oviductal secretory cells and their conversion to ciliated cells.** Under preparation.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| **A** | |
| Amhr2cre | Anti müllerian hormone receptor 2 cre |
| APC | Adenomatous polyposis coli |
| AR | Androgen receptor |
| ATCC | American type culture collection |
| ANOVA | Analysis of variance |
| | |
| **B** | |
| BSA | Bovine serum albumin |
| BTB | Blood testes barrier |
| | |
| **C** | |
| CFA | Colony formation assay |
| CK8 | Cytokeratin 8 |
| CldU | Chloro-deoxyuridine |
| *CIS* | *Carcinoma in situ* |
| $CO_2$ | Carbon dioxide |
| | |
| **D** | |
| D | Diplotene |
| DAPI | 4′,6-Diamidine-2′-phenylindole dihydrochloride |
| Ddx4 | DEAD (Asp-Glu-Ala-Asp) box protein 4 |
| DHH | Desert hedgehog |
| DKK1 | Dickkopf1 |
| DMEM | Dulbecco’s Modified Eagle Medium |
| DMEM/F12 | Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| dpc | Day post coitum |
| dpn | Day post-natal |
| Dvl | Dishevelled |
| | |
| **E** | |
| EDTA | Ethylenediaminetetraacetic acid |
| EMT | Epithelial-mesenchymal transition |
| Ex3 | Exon3 |
| | |
| **F** | |
| FBS | Fetal bovine serum |
| FGF7 | Fibroblast growth factor 7 |
| Foxo1 | Forkhead box protein O1 |
| FPKM | Fragments per kilobase of transcript per million mapped reads |
| FSH | Follicle stimulating hormone |
| Fzd | Frizzled |

| | |
|---|---|
| **G** | |
| g | Gram |
| GCNA | Germ cell nuclear antigen |
| GFP | Green fluorescent protein |
| GSK3β | Glycogen synthase kinase 3 beta |
| GV | Germinal vesicle |
| GVB | Germinal vesicle breakdown |
| | |
| **H** | |
| HBSS | Hank's balanced salt solution |
| | |
| **I** | |
| IdU | Iodo-deoxyuridine |
| IF | Immunofluorescence |
| IHC | Immunohistochemistry |
| IgG | Immunoglobulin G |
| INSL3 | Insulin-like 3 |
| IVM | *In vitro* maturation |
| | |
| **J** | |
| JNK | c-Jun N-terminal kinases |
| | |
| **K** | |
| kDa | Kilodalton |
| Kg | Kilogram |
| | |
| **L** | |
| LEF1 | Lymphoid enhancer binding factor 1 |
| LH | Luteinizing hormone |
| LiCl | Lithium chloride |
| Lrp5/6 | Low-density lipoprotein receptor-related protein 5/6 |
| L/Z | Leptotene/Zygotene |
| | |
| **M** | |
| mg | Milligram |
| MIS | Müllerian inhibiting substance |
| ml | Millilitre |
| mM | Millimolar |
| mm | Millimetre |
| Mmp7 | Matrix metallopeptidase 7 |
| µg | Microgram |
| µL | Microliter |
| µm | Micrometer |
| | |
| **N** | |
| NaCl | Sodium chloride |
| NBT | 4-nitro blue tetrazolium chloride |
| NIH | National Institutes of Health |

| | |
|---|---|
| NKD1 | Naked cuticle 1 |
| | |
| **O** | |
| OSE | Ovarian surface epithelium |
| | |
| **P** | |
| P | Pachytene |
| PB1 | Polar body 1 |
| PBS | Phosphate buffer saline |
| PCNA | Proliferating cell nuclear antigen |
| PCP | Planar cell polarity |
| PCR | Polymerase chain reaction |
| PFA | Paraformaldehyde |
| PGC | Primordial germ cell |
| PH3 | phospho-Histone3 |
| PI3K | Phosphoinositide-3-kinase |
| PL | Pre-leptotene |
| Plzf | Promyelocytic leukaemia zinc finger protein |
| PMCs | Peritubular myoid cells |
| *prm-cre* | Protamine cre |
| Ptch1 | Patched 1 |
| | |
| **Q** | |
| qRT-PCR | quantitative reverse transcription polymerase chain reaction |
| | |
| R | |
| RIPA | Radioimmunoprecipitation assay buffer |
| RNA | Ribonucleic acid |
| Rspo | R-spondins |
| RT | Room temperature |
| RTA | Real time analysis |
| | |
| **S** | |
| SCT | Sertoli cell tumour |
| SEM | Standard error mean |
| SF-1 | Steroidogenic factor 1 |
| sFRP-1 | Secreted frizzled receptor protein-1 |
| SSCs | Spermatogonial stem cells |
| Stra8 | Stimulated by retinoic acid 8 |
| | |
| **T** | |
| TCF1 | T-cell factor 1 |
| TBS | Tris-buffered saline |
| TGCTs | Testicular germ cell tumours |
| TNAPcre | Tissue non-specific alkaline phosphatase cre |
| TNBT | 5-bromo-4-chloro-3-indoxyl phosphate |
| Tris-HCl | Tris-hydrochloric acid |
| TUNEL | Terminal deoxynucleotidyl transferase |

| | |
|---|---|
| **W** | |
| WD | Wolffian duct |
| Wt1 | Wilm’s tumour 1 |

## ABSTRACT

Development and functioning of the reproductive system is essential for the survival of a species. This system is regulated by multiple signalling pathways including the Wnt pathway. Wnt signalling is crucial for embryonic development of the reproductive system as well as for its proper functioning in post-natal life. However, the precise role of the Wnt pathway in reproductive biology is not fully understood. This thesis aims to shed further light on the role of Wnt signalling in male reproductive tract development and the functioning of male and female germ cells.

To understand the role of Wnt signalling in germ cell biology we developed a mouse model with germ cell-specific overactivation of Wnt signalling. We showed that overactive Wnt signalling in oocytes does not affect oogenesis, however, it does cause embryonic mortality and subfertility through defective germ layer differentiation. In spermatogonial stem cells (SSCs), sustained activity of the Wnt pathway adversely impacts spermatogenesis, as evidenced by progressive germ cell loss and flawed meiotic entry of spermatogonial cells, in an age-dependent manner.

We have also shown that Wnt signalling is essential for the development of the male reproductive tract ductal system as alterations in this pathway results in loss of Wolffian duct (WD) coiling. To prove this, we developed two triple transgenic mouse models and an *in vitro* organ culture system. Overall, our findings demonstrate that Wnt signalling is fundamental for pre-natal development of the male reproductive tract ductal system.

In conclusion, we have provided new understanding on the requirement of Wnt signalling in germ cell biology and development of the male reproductive tract. This study not only fills some of the gaps in the field of Wnt signalling in reproductive biology, but also highlights new areas to be explored.