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Review

# Canonical Wnt signalling in mouse ovarian surface epithelium

Human

M Usongo*

## Abstract

**Introduction**

The gonad arises from the thickening of the coelomic epithelium and then commits into the sex determination process. The Wnts are a family of glycoprotein signalling molecules known mostly for the roles they play in embryonic and ovarian development. Canonical Wnt signalling leads to intracellular accumulation of the multifunctional protein β-catenin which can interact with members of the T-cell factor family to modulate gene transcription. In reviewing the current understanding of ovarian surface epithelium development in the literature, we highlight some previous studies and discuss some of the recent mouse models that have contributed to the understanding of ovarian surface epithelium differentiation.

**Conclusion**

Taking into account the recent emergence of studies examining Wnt signalling in ovary development and ovarian cancer, the current data suggest that mouse ovarian surface epithelium is heterogeneous in Wnt signalling and may contain a population of stem/progenitor cells that are needed to generate the definitive ovarian surface epithelium and allow wound repair of this tissue at ovulation. This hypothesis warrants further investigation and may open up new directions of exploration using the mouse as a model for ovarian surface epithelium development.

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## Introduction

It is well known that sex organs first appear in the embryo as elongated swollen ridges of peritoneum on the ventro–median surfaces of the mesonephros. The future ovarian surface epithelium (OSE) overlies the presumptive gonadal area and by proliferation and differentiation gives rise to part of the gonadal blastema[1]. It differs from the rest of the extraovarian mesothelium during foetal development in that it does not express cancer antigen 125 (CA125), a cell surface glycoprotein expressed by epithelial ovarian tumours as well as by other tissues of Müllerian origin[2]. This difference could be evidence of divergent differentiation between OSE and other mesothelium. Thus part of the coelomic epithelium that gives rise to the OSE does not reach the stage of differentiation where CA125 is expressed as in other coelomic epithelial derivatives. This suggests that the OSE is in a less differentiated state than other mesothelium. The OSE is an inconspicuous monolayer of squamous-to-cuboidal cells covering the mammalian ovary. It is characterised by expression of cytokeratin 8, with some stromal features such as vimentin[1]. It has been suggested that squamous and cuboidal forms of OSE cells represent cell groups that respectively have or have not undergone postovulatory proliferation[3]. In addition to these two cell forms, OSE cells tend to assume columnar shapes, especially within clefts and ovarian inclusion cysts. It is not known whether changes in OSE cell shape are the result of crowding or whether they reflect genetically determined metaplastic changes. OSE is separated from the ovarian stroma by a basement membrane (basal lamina) and differs from all other epithelia by its tenuous attachment to the basement membrane from which it is easily detached by mechanical means such as gentle scraping[1]. The aim of this review is to discuss canonical Wnt signalling in mouse ovarian surface epithelium.

## Discussion

The author has referenced some of his own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

### Ovarian surface epithelium function

Functionally, OSE is implicated in the ovulatory process and is responsible for repair and re-epithelialisation of the ovulatory wound[4,5]. The OSE is believed to actively participate in the ovulatory process. It has been suggested that proteolytic enzymes released from cytoplasmic granules of epithelial cells degrade the tunica albuginea and underlying apical follicular wall, thereby weakening the ovarian surface to the point of rupture[6]. OSE cells located directly over the point of ovulatory rupture undergo apoptosis and are shed from the ovarian surface before ovulation[7]. Thus the wound created at the ovarian surface is repaired by rapid proliferation of OSE cells from the perimeter of the ruptured follicle[8]. During postovulatory repair, the



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OSE undergoes epithelial–mesenchymal conversion as a homeostatic wound healing mechanism, as well as to accommodate OSE cells that become trapped within the ovary at ovulation[1]. The OSE, at the ovulation sites, acquires a flat squamous-like appearance, which is thought to be a metaplastic process in response to injury at ovulation. A repeat of the wounding and re-epithelialisation process provides an opportunity for the accumulation of mutations that may contribute to carcinogenesis. OSE cells express receptors for oestrogens, androgens, progestins, GnRH, FSH, LH[9], and growth factor receptors such as those for EGF and TGFα[1]. The effects of these agents on the physiology and pathology of OSE are not completely defined. Although factors involved in differentiation of the male gonad have been well-studied, few pathways regulating the differentiation of the female gonad have been identified.

**Wnts in ovary differentiation**

Recent studies using gene-specific knockout mice indicate that components of the Wnt signalling pathway are critical during early development of female reproductive tissues[10,11]. Deregulation of Wnt signalling in OSE has been implicated in ovarian tumourigenesis[12]. Several studies describe the spatio–temporal expression of various Wnt signalling components in adult rodent ovaries[13–18]. Some of these components, including *Wnt4*[19] and *Wnt2b*[20], are associated with activation of the canonical Wnt signalling pathway. In addition to *Wnt4*, sex-specific expression within the gonad has been found for *Wnt5a*, *Wnt6*, and *Wnt9a*[21]. The overlapping expression of multiple *Wnt*s suggests functional redundancy, or that these could act synergistically through a common signalling pathway such as the Wnt/β-catenin signalling pathway. Within the ovary, Wnts are primarily restricted to the OSE or stroma. The expression pattern of Wnt signalling pathway components changes throughout various stages of ovarian development[13,16]. While it is clear that Wnt pathway components are expressed in the ovary during development, studies addressing the functional impact of Wnt signalling in the OSE have just begun to emerge.

**Current investigations into Wnt/β-catenin signalling in ovarian surface epithelium**

Members of the Wnt family have been implicated in a number of developmental processes that includes the regulation of cell migration, cell proliferation/apoptosis, cellular differentiation, as well as tumourigenesis. Despite the different roles of Wnts in development, little is known about canonical Wnt/β-catenin activation in OSE development. To this aim, different β-catenin-reporter mouse models have been employed. We recently characterised the Wnt/β-catenin signalling pathway in mouse OSE cells during ovary development using responsive transgenic[22]. These transgenic mice are a reporter strain that contains six copies of the Tcf/Lef response element upstream of the hsp68 minimum promoter driving a β-galactosidase reporter[23]. Our study indicated that Wnt/β-catenin-signalling (β-galactosidase positive) cells are present early in OSE development. Evaluation of ovarian sections during postnatal growth showed β-galactosidase positive cells in the OSE. Approximately 20% of OSE in new-born ovaries were β-galactosidase positive, while only 8% and less than 0.3% were positive in five-day old and 21-day old mice, respectively. The spatio–temporal regulation of Wnt signalling was confirmed by X-gal staining of intact ovaries and flow cytometric analyses (FACS) of isolated OSE cells. Apoptosis was undetected in OSE of neonates and β-catenin/Tcf-signalling cells were proliferative in neonatal mice indicating that neither cell death nor proliferation failure was responsible for the proportion alteration. The maintenance of a constant number of Wnt/β-catenin-signalling cells, accompanied by the increase in appearance of non-signalling cells suggests that the Wnt/β-catenin-signalling cells generate the adult OSE pattern by selective expansion of their non-signalling progeny.

That somatic cells of the indifferent gonad expressed β-galactosidase raise the possibility that β-catenin/Tcf-signalling may be involved in early gonadal differentiation. β-galactosidase expression identified a cell population that overlie the medio–lateral surface of the indifferent gonad (Figure 1). Similar studies using an *Axin2:lacZ* reporter demonstrated that Wnt/β-catenin signalling is activated in the coelomic region of the gonad at E11.5[24]. It is known that coelomic epithelial cells migrate into male and female gonads and contribute to the supporting cell lineage of the gonad[25]. The presence of a β-catenin/Tcf-signalling population on the indifferent gonad raises the possibility that β-catenin/Tcf-signalling may be crucial for proper differentiation of the gonad. *Wnt4*$^{-/-}$; *Rspo1*$^{-/-}$ mice exhibit a defect in proliferation of the coelomic epithelium, impaired testis development and down-regulation of Sox9[24]. Although the disparity in cell proliferation was greater between XY *Wnt4*$^{-/-}$; *Rspo1*$^{-/-}$ and control gonads compared with that of the XX gonads, the results showed that RSPO1 and Wnt4 synergistically regulate early cell proliferation in the coelomic epithelium in both sexes. However, the contribution of Wnt/β-catenin signalling to OSE development awaits the generation of a transgenic mice with restricted β-catenin ablation in the coelomic domain of the gonad.

Evidence indicates sexual dimorphic expression of β-catenin/Tcf signalling in gonads by E12.5 (Figure 2). In mice, *Sry* is expressed first in the central and anterior portions of the genital ridges, with *Sry* down-regulation



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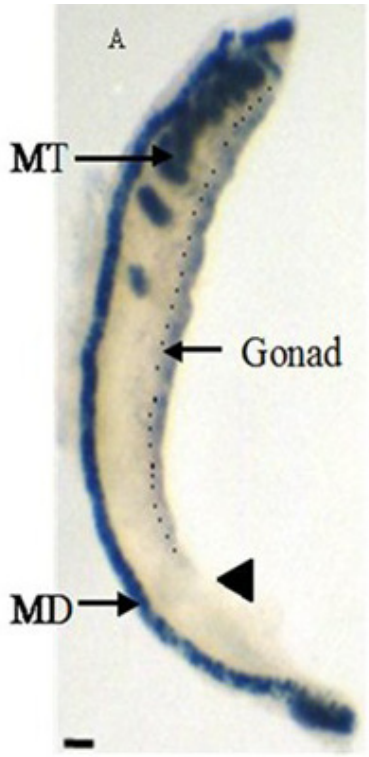


***Figure 1:*** Coelomic epithelium overlying the indifferent gonad displays β-catenin/Tcf-mediated β-galactosidase expression. Whole-mount X-gal staining of E11.5 urogenital ridge. β-galactosidase positive cells overlie the medio–lateral surface of the indifferent gonad (dotted line demarcates the gonad). The mesonephric duct (MD) and mesonephric tubules (MT) also express β-galactosidase. Arrowhead indicates coelomic epithelium extending beyond the gonad is not stained. Scale bar = 10 μm.

subsequently following a similar pattern[26]. The down-regulation of β-galactosidase expression in XY gonads occurs in an anterior-to-posterior wave that begins by E12.5 at the anterior pole. By E13.5, β-galactosidase expression was extinct from the anterior pole but persisted in the posterior pole and ventral surface until E14.5. Previous studies demonstrated that *Sry* mediated inhibition of β-catenin transcription[27]. The fact that *Sry* expression is highest by E12.5 in XY gonad suggests that *Sry* may inhibit β-catenin/Tcf-signalling. However, the male specific down-regulation of β-catenin/Tcf expression by E12.5 is unlikely the result of direct regulation by *Sry*, because *Sry* is not expressed in the coelomic epithelium[28]. Non-cell autonomous paracrine signals such as *Sox9*, *Mis*, and *Dhh* emanating from Sertoli cells[29] may be responsible for down-regulating β-catenin/Tcf expression. The fact that loss of β-catenin/Tcf-signalling begins at the anterior portion of the gonad mimicking Sry expression pattern suggests the anterior–posterior down-regulation of β-catenin/Tcf-signalling in the testis may be related to Sry expression. Canonical Wnt signalling is down-regulated in the testis when SRY/SOX9 expression occurs and Sertoli cells differentiate. As far as the RSPO1/Wnt4 genetic pathway is involved in ovarian differentiation, it is indeed also required for testicular development by stimulating proliferation of the coelomic epithelium of the undifferentiated gonad[24].

Age-dependent decrease in the proportion of stained OSE cells

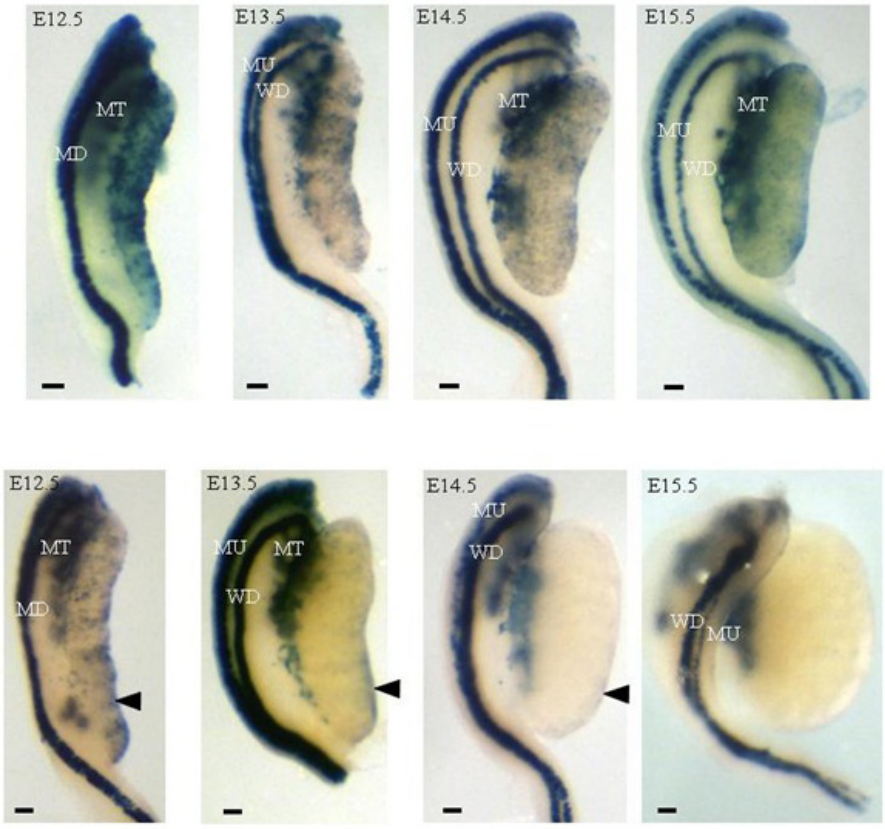


***Figure 2:*** β-catenin/Tcf-mediated β-galactosidase expression is maintained in the embryonic female gonad. Time course of β-catenin/Tcf-mediated transcription in female (upper panels) and male (lower panels) embryonic gonads. Whole-mount β-galactosidase staining demonstrates β-catenin/Tcf expression is sexually dimorphic from E12.5 onwards. Blue staining reflecting β-catenin/Tcf-mediated transcription is observed in the mesonephric tubules (MT), mesonephric duct (MD), Mullerian duct (MU), Wolffian duct (WD). Arrowhead indicates the ventral surface and posterior tip of the male gonad. All gonads are positioned with the anterior region at the top of each panel. Scale bar = 10 μm.



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suggests β-catenin/Tcf-signalling cells generate the adult OSE pattern by selective expansion of their non-signalling progeny. We cannot exclude the possibility of non-signalling cells migrating from the surrounding tissues. While the exact mechanisms of the age-dependent decrease in the proportion of β-galactosidase-stained OSE cells is unknown, RT-PCR survey suggests that it is most likely not due to the absence of Wnt ligands or its frizzled receptor. It is also possible that signals emanating from within the ovarian parenchyma may be responsible for inhibiting β-catenin/Tcf-signalling in OSE cells. The reason(s) for cell-specific β-catenin/Tcf-signalling in mouse OSE are currently not known. However, expression of multiple Wnt ligands within OSE is suggestive of multiple and distinct roles in OSE tissue homeostasis. Based on the fraction of β-galactosidase positive cells and an estimate of the number of OSE cells at each of the ages examined, it appears that the Wnt/β-catenin-signalling cells gave rise to a replacement as well as an expanding population of non-signalling progeny. Nevertheless, further investigation is required to characterise the gene signature profile of the β-catenin/Tcf-signalling cell population.

In a follow-up study, it was determined that non-phosphorylated (active) β-catenin localised predominantly at the plasma membrane of OSE cells[30]. Activation of the canonical Wnt signalling pathway in OSE cells led to stabilisation and nuclear localisation of active β-catenin. Unexpectedly, β-galactosidase reporter activity in OSE cells was not detected following treatment. The deprivation of Ca2+ also failed to induce reporter expression even in the presence of Wnt3A. Nonetheless, stimulation with Wnt3a-conditioned media or lithium chloride increased OSE proliferation. Important insights into the functionality of the canonical Wnt pathway were obtained from ovarian cancer cell lines (HEY, OVCAR3, SKOV3 and SW626) transfected with the TOPFLASH luciferase reporter. The luciferase reporter was activated only in HEY cells following Wnt3a or lithium chloride stimulation suggesting that ovarian cancer cell lines exhibit distinct aberrations of the Wnt signalling pathway. There exists marked similarities between reported ovarian epithelial neoplasm subtypes and Müllerian duct derivatives[1]. The OSE is derived from the coelomic epithelium and thereby has the intrinsic capacity for divergent differentiation along the Müllerian pathways[1]. A side population-enriched and label-retaining cell population in the coelomic epithelium of adult mouse ovary has been identified as possible stem/progenitor cells[5]. Accumulating evidence suggests somatic stem cells may undergo mutagenic transformation into cancer stem cells[31]. Interestingly, both the OSE and Müllerian ducts expressed β-galactosidase signifying active Wnt signalling. Within the context of wound healing and tumourigenesis, these data suggest the OSE is composed of stem cells which, during tumourigenesis, may assume a more differentiated morphology such as that of Müllerian duct derivatives. Whether or not tumorigenic cells assume a more differentiated morphology depends on many factors including cellular environment as well as intrinsic cell programming. Because many of the properties that define somatic stem cells also define cancer stem cells, identification of the β-catenin/Tcf-signalling cell population raises the possibility that endometrioid adenocarcinomas may arise as a result of transformation of this cell population.

## Conclusion

In this review, the possible involvement of the Wnt/β-catenin signalling in OSE development and tumourigenesis has been discussed. Evidence indicates that canonical Wnt signalling is activated in coelomic epithelium of the indifferent mouse gonad and becomes sex specific as the testis differentiates by E12.5. With ovarian differentiation, expression of the β-galactosidase reporter is lost in a majority of the OSE and only approximately 0.2% of adult OSE expresses the reporter transgene. Staining for active β-catenin localised dephosphorylated β-catenin at the plasma membrane. Further, activation of canonical Wnt signalling increased OSE proliferation. These observations place Wnt/β-catenin signalling at the beginning of OSE differentiation and suggest a functional role for its uncontrolled activation in tumourigenesis. It is proposed that the OSE may be populated by stem cells, which under the influence of dysregulated Wnt signalling, may assume a more differentiated morphology. While these are preliminary studies, the experimental data may lay the basis for future studies examining the role of canonical Wnt signalling in OSE biology and carcinogenesis. The precise role of canonical Wnt signalling in OSE differentiation will require the generation of a transgenic mouse line with restricted gene expression in the coelomic domain.

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## Abbreviations list

FACS, flow cytometric analyses; OSE, ovarian surface epithelium.

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