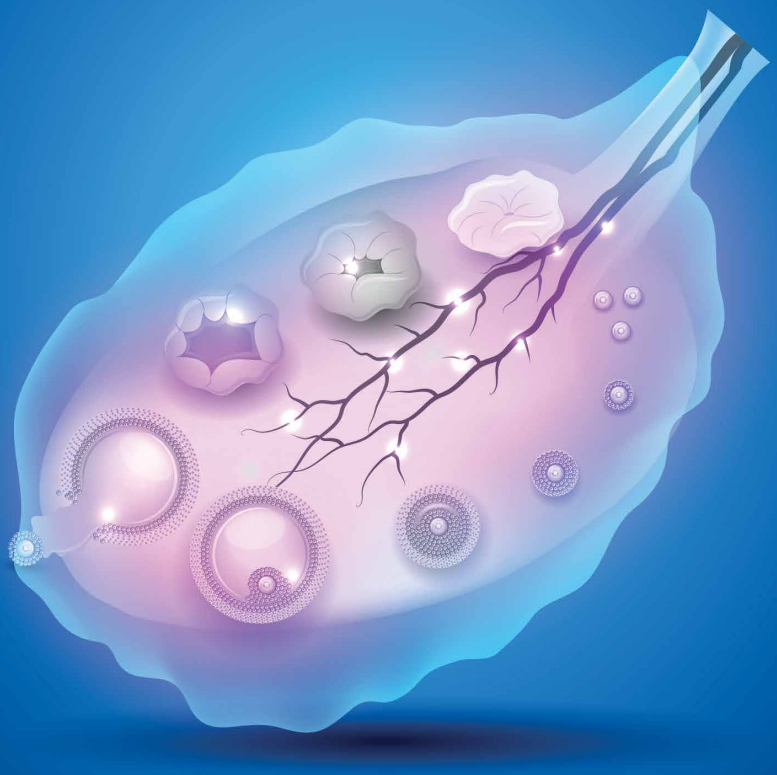
Uncovering New Signalling Pathways in Ovarian Function

Dr Jennifer A. Hernandez Gifford



Scientia

UNCOVERING NEW SIGNALLING PATHWAYS IN OVARIAN FUNCTION

In mammals, the process by which an ovarian follicle, which contains an immature egg, develops to become ready for the egg's release, is highly complex. Termed folliculogenesis, this mechanism relies on the synchronised input of a range of hormones and signalling pathways. **Dr Jennifer Hernandez Gifford** and her team from New Mexico State University have been investigating the role of the 'WNT' family of signalling protein molecules to further elucidate their involvement in follicle development. The team's research provides novel insights that improve our understanding of the pathways involved, with important implications for health, fertility and disease.

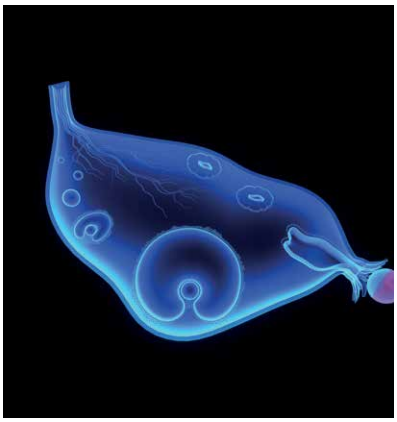
WNT Proteins in Ovarian Function

Women begin puberty with hundreds of thousands of follicles – each with the potential to release an egg at ovulation. During a normal menstrual cycle, one follicle will grow larger until it ruptures at ovulation, releasing the egg. The multifaceted mechanisms behind this process, called folliculogenesis, rely on the synchronised exchange of hormones between the hypothalamus, pituitary, and the ovaries. Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), both members of a group of hormones called 'gonadotropins', are two of the main hormones released by the pituitary.

While the initial stages of follicle development (termed the preantral phase) occur largely in the absence of gonadotropins, the transition from the preantral to preovulatory follicle occurs as a result of increased FSH and LH responsiveness, along with the involvement of numerous other hormones and growth factors. The actions of the gonadotropins are also dependent on other signalling pathways that are active at defined stages of follicular growth.

Around 20 years ago, a group of signalling proteins called the 'WNT' family was first identified as being key to the signalling pathways involved in normal ovarian development, and thus suggesting an important role in ovarian function. Prior to this, abnormal WNT signalling had been linked with certain cancers, but not with ovarian development.

In 1999, researchers found that removing the WNT4 gene, which codes for the WNT4 signalling protein, resulted in partial female to male sex reversal in mice, and a paucity of oocytes at birth. Since this initial discovery of the requirement for WNT signalling proteins in the ovary, further functional studies in the adult mammalian ovary have shown a fundamental need for WNT signalling in normal ovarian function and fertility.



Although our understanding of the importance of WNT signalling in folliculogenesis has grown tremendously in recent years, much remains unknown about the broader physiological involvement of WNT signalling in the adult ovary. With this in mind, Dr Jennifer Hernandez Gifford and her team from New Mexico State University have been working to elucidate the mechanisms, modes of action and importance of the WNT family of signalling molecules and downstream components expressed at specific stages of follicle development.

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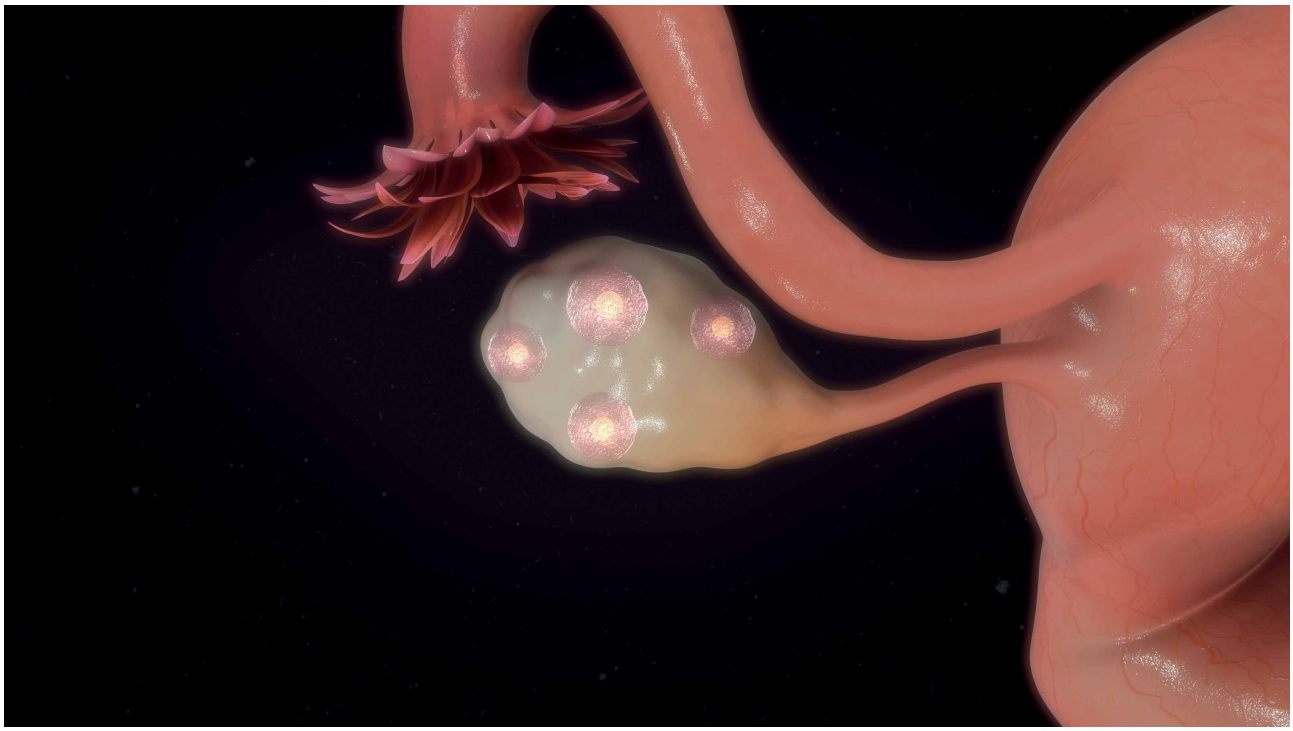


‘To date, our research has provided novel insights into FSH-mediated steroid hormone production and expression of genes important for ovarian maturation,’ explains Dr Hernandez Gifford. ‘Our ongoing research is aimed at further elucidating the signalling pathways involved using large animal models. Improving our understanding of the mechanisms involved in ovarian follicle development and function will allow us to identify targets of cellular pathways affecting oestrogen levels in health and disease.’

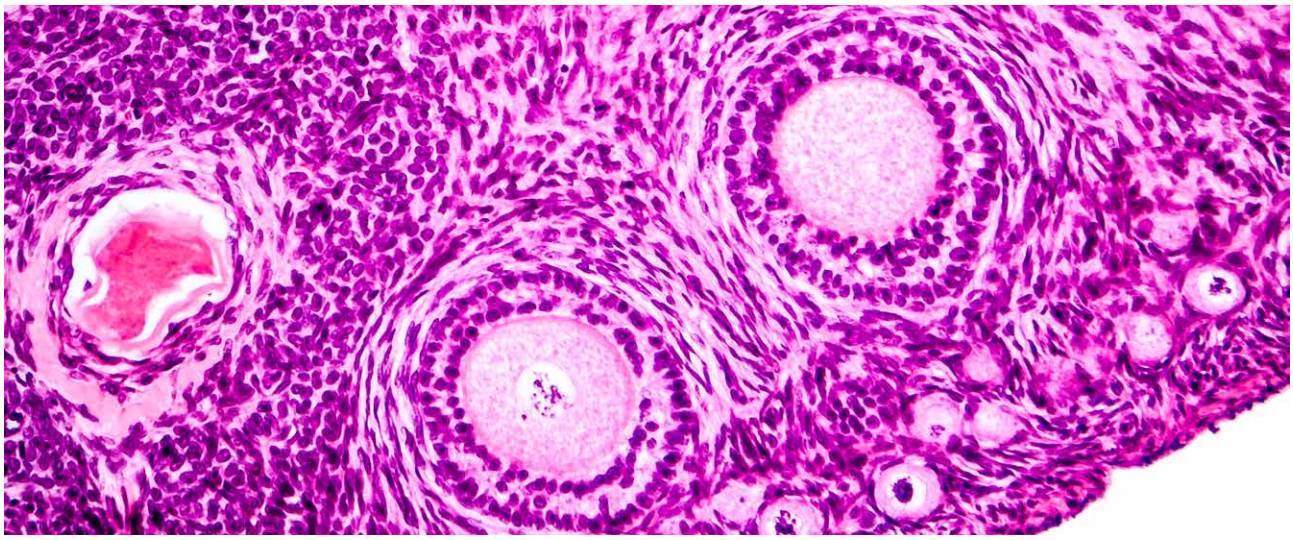
Signalling for Hormone Production

The WNT family of signalling molecules is known to regulate numerous cellular processes, including cell growth, function, differentiation (whereby a cell changes from one type into another) and cell death. Most mammalian genomes comprise 19 WNT genes that code for proteins. The most intensely studied WNT pathway is the ‘canonical WNT signalling cascade’, which regulates levels of an important downstream molecule called beta-catenin (formally designated CTNNB1). Using primary cultures of rat ovarian cells, initial studies by Dr Hernandez Gifford and her team confirmed that CTNNB1 is required for maximal gonadotropin stimulation and ovarian oestrogen production. Although this study, along with several others, began to define a clear role for WNT and CTNNB1 in adult ovarian function, all had been conducted in rodent models. Therefore, Dr Hernandez Gifford and her team were keen to investigate the physiological significance of this signalling pathway in large mammals.

In 2012, the team carried out a study using bovine ovarian cells, which demonstrated for the first time that FSH regulates the CTNNB1 protein and WNT2 gene expression in cattle. This study identified a previously unappreciated role of the WNT signalling pathway in bovine follicular maturation. The team also found that FSH treatment tended to increase the abundance of a protein called AKT, which is known to inactivate members of the CTNNB1 destruction complex.

Based on these results, the team’s next step was to determine whether FSH directly regulates CTNNB1 through the modulation of AKT, or whether it has an indirect effect, by increasing the expression of WNT2, and subsequently activating the canonical WNT pathway.

To investigate the specific contributions of AKT in CTNNB1 accumulation, the team treated bovine ovarian cells with activators of AKT in the presence or absence of FSH. Cells treated with FSH, IGF-1 (Insulin-like Growth Factor-1 – an AKT activator), and both FSH and IGF-1 together exhibited increased CTNNB1 accumulation compared with controls. In contrast, the use of AKT inhibitors suppressed the ability of FSH and the AKT activator to regulate CTNNB1. These findings extended the team’s knowledge regarding how FSH regulates CTNNB1 in bovine ovarian cells, and revealed the importance of AKT-mediated CTNNB1 regulation.



Unexpectedly, this group also demonstrated that canonical WNT signalling actually inhibits FSH stimulation of molecules associated with maturation and differentiation of ovarian follicles. It is therefore likely that FSH regulation of WNT signalling creates a negative feedback loop to ensure that CTNNB1 remains controlled.

Given the temporal expression of FSH, IGF-1 and WNT signalling molecules in the ovary, the team's data suggest that IGF-1 is capable of overriding a negative feedback system set up by WNT signalling on FSH target genes. This ensures that follicle maturation and oestrogen production don't become unregulated, which would have negative effects on fertility.

Although the exact molecular nature of the inhibitory effect remains unclear and requires further examination, this work identifies a new pathway for follicle development through WNT negative feedback. Overall, these results indicate that WNT signalling components not only participate in ovarian hormone production in cattle, but also work in coordination with the pituitary gonadotropin, FSH, and other ovarian molecules such as IGF-1.

Future Benefits for Health and Fertility

The work carried out by Dr Hernandez Gifford and her team paves the way for an improved understanding of the signalling pathways involved in folliculogenesis. The data produced by these studies highlight the importance of WNT molecules in adult ovarian function related to follicle development, hormone production and fertility.

The team's results showing the hormonal regulation of WNT genes at different stages of the oestrus cycle suggest their crucial role in normal ovarian function. Similarly, the finding that FSH requires input from CTNNB1, a lynchpin molecule in canonical WNT signalling, further implicates CTNNB1 in the regulation of follicle maturation.

These findings have important practical applications for our understanding of the molecular processes that may help or hinder fertility. As such, they hold great potential for developing new approaches to improve the success of assisted reproduction.

Furthermore, the team's most recent work highlights even more potentially interesting developments. 'New data have revealed ovarian pathways by which bacterial infection has the potential to alter oestrogen production and subsequent fertility,' says Dr Hernandez Gifford.

A greater understanding of the functions of WNT proteins in folliculogenesis could also help to provide a clearer picture of how this complex family of molecules plays roles in the development of various diseases. In fact, recent research now implicates WNT proteins in an extensive array of health problems in humans, including diabetes, osteoporosis and heart disease, as well as certain cancers.

Inspiring the Next Generation

In addition to her research, Dr Hernandez Gifford is also passionate about inspiring and training the next generation of scientists. 'An exciting additional achievement has come through my involvement in working with both graduate and undergraduate students,' she says.

She makes a point of always involving undergraduate students in her projects, as such experience is vital for ensuring their successful integration into the world of research after they graduate. 'All of the undergraduate students that have worked in my lab have successfully entered schools of medicine, veterinary medicine, dentistry or graduate school,' she says. 'Graduate students from my lab have pursued careers as Extension agents, have been selected for prestigious post-doctoral fellowships, moved to medical facilities to work as research associates, and have accepted faculty positions at community colleges.'

Meet the researcher

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Dr Jennifer Hernandez Gifford is an Associate Professor in the Department of Animal and Range Sciences at New Mexico State University. After completing a MS at New Mexico State University, and a PhD at Washington State University in Animal Science, she conducted postdoctoral research at Washington State University in the School of Molecular Biosciences. In 2009, she joined the faculty at Oklahoma State University and was involved in teaching undergraduate and graduate courses in physiology, endocrinology and biotechnology. Here, she also established a strong research program in the area of ovarian follicle development and steroidogenesis. In 2016, Dr Hernandez Gifford returned to her Alma Mater, New Mexico State University, where she continues her teaching and research program. The long-term goal of her lab's research is to provide fundamental knowledge about the physiological role and mechanism of action of ovarian signalling molecules involved in follicular development, which impact health and disease. Dr Hernandez Gifford is also extremely passionate about mentoring and working with students, and was awarded the prestigious NACTA Educator Award for her work in this area.

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FURTHER READING

BI Gomez, BH Aloqaily, CA Gifford, DM Hallford, JA Hernandez Gifford, ASAS-SSR Triennial Reproduction Symposium: Looking back and moving forward—how reproductive physiology has evolved: WNTs role in bovine folliculogenesis and oestrogen production, Journal of Animal Science, 2018, 96, 2977–2986.

JA Hernandez Gifford, The role of WNT signalling in adult ovarian folliculogenesis, Reproduction, 2015, 150, 137–148.

BI Gómez, CA Gifford, DM Hallford, JA Hernandez Gifford, Protein kinase B is required for follicle-stimulating hormone mediated beta-catenin accumulation and estradiol production in granulosa cells of cattle, Animal Reproduction Science, 2015, 163, 97–104.

AD Stapp, BI Gomez, CA Gifford, DM Hallford, JA Hernandez Gifford, Canonical WNT signalling inhibits follicle stimulating hormone mediated steroidogenesis in primary cultures of rat granulosa cells, PLoS ONE, 2014, 9, e86432.

BI Castañon, AD Stapp, CA Gifford, LJ Spicer, DM Hallford, JA Hernandez Gifford, Follicle-stimulating hormone regulation of estradiol production: Possible involvement of WNT2 and β-catenin in bovine granulosa cells, Journal of Animal Science, 2012, 90, 3789–3797.