

FSH–FSHR3–stem cells in ovary surface epithelium: basis for adult ovarian biology, failure, aging, and cancer

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Abstract

Despite extensive research, genetic basis of premature ovarian failure (POF) and ovarian cancer still remains elusive. It is indeed paradoxical that scientists searched for mutations in FSH receptor (*FSHR*) expressed on granulosa cells, whereas more than 90% of cancers arise in ovary surface epithelium (OSE). Two distinct populations of stem cells including very small embryonic-like stem cells (VSELs) and ovarian stem cells (OSCs) exist in OSE, are responsible for neo-oogenesis and primordial follicle assembly in adult life, and are modulated by FSH via its alternatively spliced receptor variant *FSHR3* (growth factor type 1 receptor acting via calcium signaling and the ERK/MAPK pathway). Any defect in FSH–*FSHR3*–stem cell interaction in OSE may affect folliculogenesis and thus result in POF. Ovarian aging is associated with a compromised microenvironment that does not support stem cell differentiation into oocytes and further folliculogenesis. FSH exerts a mitogenic effect on OSE and elevated FSH levels associated with advanced age may provide a continuous trigger for stem cells to proliferate resulting in cancer, thus supporting gonadotropin theory for ovarian cancer. Present review is an attempt to put adult ovarian biology, POF, aging, and cancer in the perspective of FSH–*FSHR3*–stem cell network that functions in OSE. This hypothesis is further supported by the recent understanding that: i) cancer is a stem cell disease and OSE is the niche for ovarian cancer stem cells; ii) ovarian OCT4-positive stem cells are regulated by FSH; and iii) OCT4 along with LIN28 and BMP4 are highly expressed in ovarian cancers.

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Introduction

Gonadotropin hormones including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and human chorionic gonadotropin (hCG) hormone are the central players of reproduction. A recent review (Mertens-Walker *et al.* 2012) has elegantly discussed the available literature suggesting that FSH in particular may be the main hormone responsible for ovarian cancers. High levels of FSH and FSH receptor (*FSHR*) expression in epithelial ovarian cancers (EOCs) are associated with a poor prognosis. FSH affects various aspects of ovarian cancer metastasis, such as it suppresses apoptosis, supports tumor growth by inducing increased expression of VEGFA (VEGF), facilitates blood vessel growth, and may alter certain oncogenic pathways that facilitate proliferative and invasive phenotype. Indeed, studies using nanoparticles conjugated to the β subunit of FSH showed a lot of promise both *in vitro* and *in vivo* to increase the specificity and delivery of chemotherapeutic drugs (Zhang *et al.* 2009).

Hypothesis of ‘incessant ovulation’ being responsible for ovarian cancers was proposed more than 40 years ago (Fathalla 1971). This hypothesis indicates that

ovarian cancer in women may occur due to frequent ovulation that causes repeated wounding and post-ovulation repair. This may result in accumulation of mutations in the epithelial cells and ultimately the formation of tumors. The other hypothesis termed ‘gonadotropin theory’ of cancer initiation (Stadel 1975) is supported by several circumstantial evidences including: i) increased incidence of ovarian cancers with advanced age when gonadotropin levels are elevated; ii) high FSH levels in ovarian cyst and peritoneal fluid; iii) protective effect of breast feeding and multi-parity that help suppress gonadotropin levels; and iv) increased risk of developing ovarian cancer in women with polycystic ovarian syndrome (PCOS) and those undergoing hyper-stimulation of ovaries with FSH. But the gonadotropin hypothesis for development of ovarian cancers is still not well accepted. Studies carried out on genetically modified mice support the gonadotropin hypothesis. Sterile, germ-cell-deficient homozygous *Wv* mice having <1% of normal number of oocytes at birth develop epithelial morphological changes including surface invaginations, inclusion cysts, and tumors associated with elevated gonadotropin levels similar to aged women (Murphy & Beamer 1973). Interestingly,

when the FSH levels in these mice were suppressed by Gonadotropin-releasing hormone agonist (Zoladex, 3.6 mg slow-release goserelin depot injection every 28 days from the age of 7 days up to 245 days), no tumor developed (Blaakaer *et al.* 1995). Furthermore, these mice being sterile never ovulate and thus the concept of incessant ovulation does not explain the origin of ovarian cancer in these mice. Similarly, Chen *et al.* (2007) questioned the very basis for incessant ovulation to result in ovarian cancer as more than 90% of FORKO mice develop ovarian epithelial tumors by 12 months of age, which are otherwise sterile and never ovulate (Danilovich *et al.* 2001). Smith & Xu (2008) have postulated that depletion of follicles and germ cells might underlie the etiology of ovarian cancers.

One needs to remember that FSH levels are increased in all aged, menopausal women but incidence of ovarian cancers is not very high. Thus, more than FSH, FSHRs may have a more crucial role in ovarian cancers. Besides ovarian cancers, elevated levels of FSH are also associated with premature ovarian failure (POF), resistant ovarian syndrome (ROS), and PCOS, and intense efforts have been undertaken to detect naturally occurring mutations in *FSHR* associated with these clinical conditions. Natural mutations detected in *FSHR* are relatively low compared with LH and thyroid-stimulating

hormone (TSH) receptors (Themmen & Huhtaniemi 2000, Meduri *et al.* 2003, Themmen 2005, Huhtaniemi & Themmen 2005). Mutations in *FSHR* appear to be a very rare cause of PCOS, POF, and primary to early secondary amenorrhea combined with arrested follicular maturation and anovulatory infertility. As shown in Table 1, mutations reported in exons 7 and 10 are always associated with primordial follicles (PFs) up to pre-antral stage. A large number of studies have been undertaken to search for mutations in *FSHR* in women having POF or ROS with negative results in New Zealand (Woad *et al.* 2013), India (Achrekar *et al.* 2010), Argentina (Sundblad *et al.* 2004), Japan (Takakura *et al.* 2001), Mexico (de la Chesnaye *et al.* 2001), the UK (Conway *et al.* 1999), and Brazil (Vilodre *et al.* 2008). First, reported Finnish mutation in *FSHR* by Aittomaki *et al.* (1995) was not found in 35 American women with ovarian failure (Layman *et al.* 1998) and was found in only one woman out of a total of 2840 women screened from Switzerland, Denmark, and Singapore (Jiang *et al.* 1998). Similarly, Bas *et al.* (2009) did not find any activating mutations in exon 10 of *FSHR* in four children with granulosa cell tumors. Besides *FSHR*, other sites have also been searched to find associations such as LHCGR/LH receptor (*LHR*), *CYP17*, *inhibin*, *CYP19*, *NR5A1*, and several other autosomal genes that result in non-syndromic POF such

Table 1 Mutations in *FSHR* and associated status of ovarian follicles (Siegel *et al.* 2013).

References	Exons where mutation is detected	Effects on ovary
Aittomaki <i>et al.</i> (1995)	7	First, reported mutation in the Finnish population in women with primary amenorrhea. They were <20 years of age, had elevated gonadotropin levels and normal karyotype. Ultrasound of ovaries showed presence of follicles of varying sizes. On histological evaluation, only primordial follicles were observed in some patients, but in others, pre-antral or antral follicles were identified. In one patient, even a mature follicle was identified. Corpora lutea were not reported indicating the failure of ovulation
Beau <i>et al.</i> (1998)	6 and 10	An Armenian woman with secondary amenorrhea and hypergonadotropic hypogonadism, elevated gonadotropin levels, and normal karyotype. On transvaginal ultrasound, the ovaries were normal in size and contained numerous, small (3–5 mm in diameter) antral follicles. Impaired cAMP production. <i>FSHR</i> function not abolished completely, FSH triggered cAMP response (24 + 4%) and FSH stimulation resulted in an increase in the size of follicles from 5 to 8.3 mm
Touraine <i>et al.</i> (1999)	3 and 10	A 19-year-old female with primary amenorrhea and elevated FSH levels. Ovary had many follicles up to 3 mm in size. No binding with FSH was observed. No change in follicular size after FSH stimulation, primordial, primary, secondary follicles and three antral follicles were observed
Doherty <i>et al.</i> (2002)	10	A young female with primary amenorrhea. Hypergonadotropic ovarian failure was found to be a compound heterozygote for two inactivating <i>FSHR</i> mutations: previously identified Finnish founder mutation 566C→T novel 1255G→A mutation in the other allele. These mutations, resulting in Ala189Val and Ala419Thr substitutions. Located in extracellular and transmembrane domain. The patient had primary amenorrhea and normal female secondary sexual characteristics. Small ovaries. No histological study of the ovaries performed
Allen <i>et al.</i> (2003)	10	The patient presented with primary amenorrhea at 17 years of age Streak ovaries MRI confirmed the presence of follicles
Meduri <i>et al.</i> (2003)	10	Normal-sized ovaries in women with high serum levels of FSH. Secondary or primary amenorrhea with normal puberty. Ovaries showed a normal follicular development up to the small antral stage and a disruption at further stages. Primordial and intermediary follicles (few primary follicles) were observed
Kuechler <i>et al.</i> (2010)	Minimum deletion in exons 9 and 10 and maximum deletion in exons 7–10	A 17-year-old female with primary amenorrhea, hypergonadotropic hypogonadism, and disturbed folliculogenesis. Many primordial follicles, but no secondary or tertiary follicles

as *FOXL2*, *NOBOX*, *LHX8*, *FOX10A*, *FOX03A*, *FIGLA*, *POU5F1*, *NANOS3*, *RASGRP3*, *KIT*, *GDF9*, *BMPs*, *TGFB1* (*TGFβ*), etc., but with no significant correlation (reviewed by Prof. Simpson 2008, Simpson during ASRM Meeting 2013). Genome-wide studies have recently revealed 8q22.3 as a possible regulatory region (Qin *et al.* 2012), which is not related to the *FSHR* gene located on chromosome 2.

Existing paradigm in ovarian biology suggests that FSHR are expressed on the granulosa cells in ovaries and on the Sertoli cells in testes (Renner *et al.* 2013, Siegel *et al.* 2013). This implies that research efforts till date to search for a genetic basis of POF and cancer have remained focused on FSHR coupling to G protein to activate cAMP on granulosa cells although both ovarian cancer and POF do not involve granulosa cells. It is well known that majority of ovarian cancers arise from the ovary surface epithelium (OSE) and POF implies absent follicles rather than follicles with defective granulosa cells. Stem cells present in the OSE have recently been shown to be involved in neo-oogenesis and PF assembly in adult mouse ovary (Bhartiya *et al.* 2012). Thus, changes in the OSE appear to be more crucial rather than granulosa cells of growing follicles for POF and cancer. It is likely that the world-wide research efforts to search for mutations in *FSHR* responsible for POF and ovarian cancer have been off the track. This happened basically because of the ease of availability of granulosa cells for research compared with the OSE cells. We need to acknowledge the large body of the available literature (Table 2) at both protein and transcript levels suggesting the presence of FSHR on OSE in normal and cancerous states (Bose 2008), 20-fold higher in oocytes compared with granulosa cells (Meduri *et al.* 2002), in blood vessels of normal gonads (Vu Hai

et al. 2004) as well associated with various metastatic tumors (Siraj *et al.* 2013; Radu *et al.* 2010).

Thus, the present review goes beyond the existing paradigm of FSH-FSHR action on granulosa cells in the field of ovarian biology and examines closely the alternately spliced FSHR isoforms (Sairam & Babu 2007) and their action on OSE. These variants may be implicated in normal ovarian function and also any aberration in them may result in menopause and be responsible for pathologies such as POF and ovarian cancer. A compromised somatic niche, for the stem cells lodged in the OSE, with age may not be able to provide the crucial growth factors/cytokines and thus result in suppressed stem cell differentiation into oocytes leading to senescence/menopause as has been suggested previously (Niikura *et al.* 2009, Massasa *et al.* 2010). Cancer is considered to be a stem cell disease and 90% of ovarian cancers arise in OSE. Thus, FSH-FSHR biology with a stem cell perspective may explain POF (altered stem cell biology may lead to failure of postnatal oogenesis and PF assembly resulting in POF), ovarian aging resulting in senescence/menopause (suppressed differentiation of stem cells into oocytes), and cancer (uncontrolled proliferation of stem cells) and is the focus of the present review (Fig. 1).

FSH and FSHR isoforms

FSH is a pituitary glycoprotein hormone composed of α and β subunits of which α subunit is common to FSH, LH, hCG, and TSH, whereas the β subunit confers hormone specificity. Only the mature FSH dimer binds to FSHR. Various mutations in FSH and its effect on fertility have been recently reviewed by Siegel *et al.* (2013).

Table 2 FSH receptor (FSHR) expression in ovary surface epithelium (OSE).

References	Salient findings
Zheng <i>et al.</i> (1996)	Expression of FSHR was studied in human OSE and fallopian tube by RTPCR and <i>in situ</i> hybridization (ISH). ISH studies demonstrated the strongest <i>FSHR</i> mRNA expression in Graafian follicles, uniform and moderate in primary follicles and OSE, weak in thecal cells, and absent in corpus luteum and corpus albicans. Abundant expression in Fallopian tube mucosal epithelium. FSHR expression results were further confirmed by RT-PCR and sequencing of the amplified products
Davies <i>et al.</i> (1999)	Administration of gonadotropins to sexually mature mice induced proliferation of OSE concurrently with the process of folliculogenesis
Parrott <i>et al.</i> (2001)	The study demonstrated the effect of FSH and LH on bovine ovaries/OSE and human ovarian cancer cell lines (OCC1 and SKOV3) using RTPCR, qRTPCR, and northern blot techniques. Northern blot analysis demonstrated expression of <i>FSHR</i> transcripts of variable sizes (1–7 kb) among different cell types ((four in bovine granulosa cells – 7, 3, 2, and 1.5 kb), two in human OCC1 – 3 and 2 kb and one in bovine OSE – 1 kb and no transcript in SKOV 3)
Meduri <i>et al.</i> (2002)	Presence of FSHR (and not LHR) detected in oocytes (from primary through preovulatory stage) using high-affinity MAB. <i>FSHR</i> mRNA (reverse transcribed by random hexamer and PCR for exons 7 and 10) of denuded human oocytes and porcine oocytes revealed a 20-fold higher level than granulosa cells
Wang <i>et al.</i> (2003)	Quantitative expression of FSHR in different types of ovarian epithelial tumors (OETs) presumed precursor lesion and peritoneal implants were studied. Variable expression levels (highest in OSE and contralateral ovaries with and without OETs; lowest in antral follicles, SBTs, and peritoneal implants associated with SBTs, increasing parallel to proliferation activity of OETs and decreasing level with advancing carcinoma grade) were observed
Stewart <i>et al.</i> (2004)	Both gonadotropins and hormones increased OSE proliferation in rats
Tan & Fleming (2004)	Performed PCNA staining in normal mouse OSE and found PCNA-positive cells at sites distant from the site of follicle rupture
Burdette <i>et al.</i> (2006)	Both PMSG and hCG increased OSE proliferation that could occur before and also after ovulation. No signs of apoptosis were evident in OSE post-ovulation in CD1 mice
Bhartiya <i>et al.</i> (2012)	PMSG treatment results in increased FSHR expression in adult mouse OSE
Patel <i>et al.</i> (2013)	FSHR expressed on the stem cells and germ cell nests in sheep OSE smears and the surrounding epithelial cells are negative

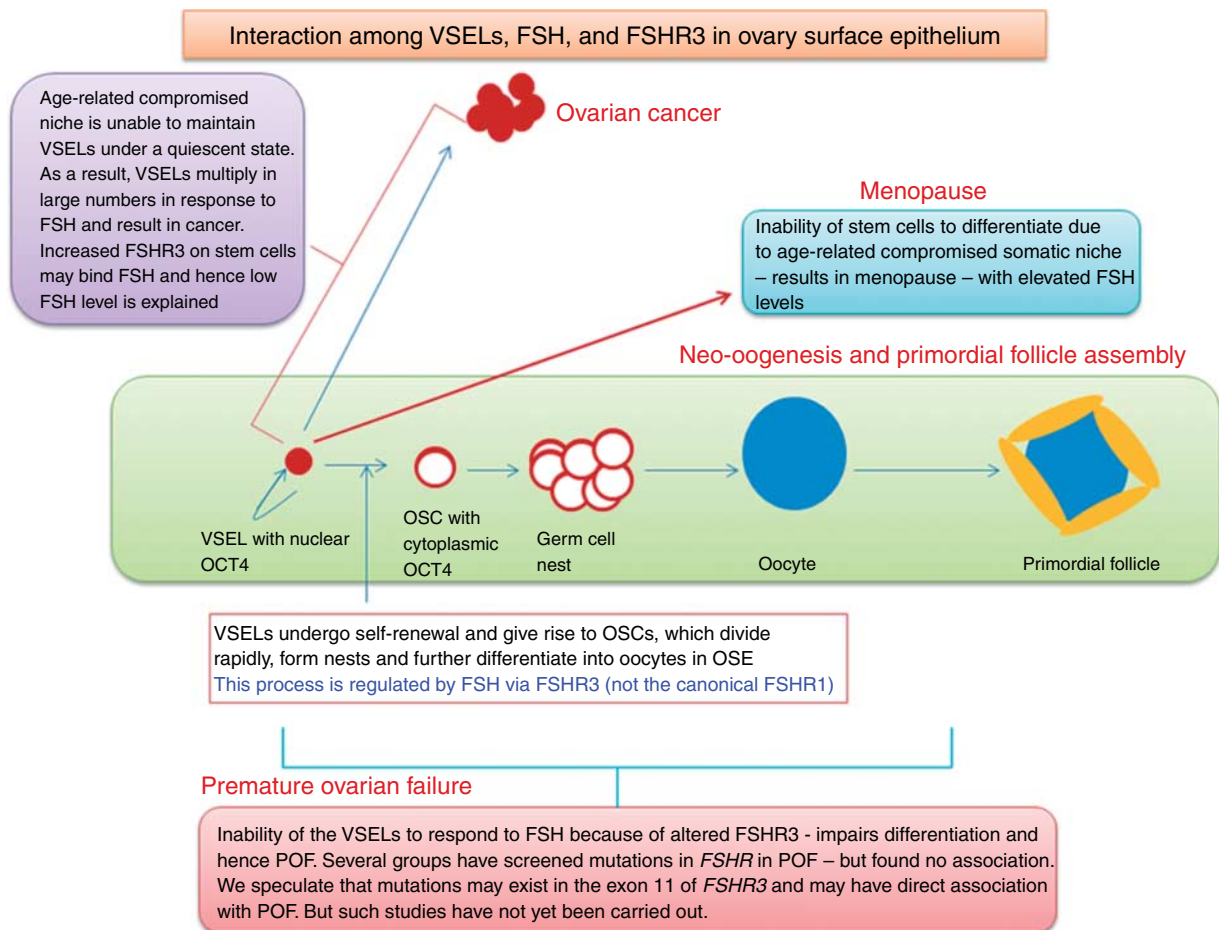


Figure 1 Schematic of the hypothesis put forth in the review. Two distinct populations of stem cells exist in OSE including the VSELS with nuclear OCT4 and the immediate descendant OSCs with cytoplasmic OCT4 (Parte *et al.* 2011, 2013, Bhartiya *et al.* 2012, 2013a,b). VSELS undergo self-renewal and also give rise to the OSCs, which rapidly divide, form germ cell nests, and further differentiate into the oocytes. Oocytes get surrounded by granulosa cells, which possibly arise by epithelial–mesenchymal transition of the epithelial cells to assemble as a primordial follicle. This process of neo-oogenesis and PF assembly in adult ovary is regulated by FSH via FSHR3 (Patel *et al.* 2013). Any alteration in the FSH–FSHR–stem cell interaction will result in POF. Mutations in *FSHR3* (specifically in exon 11) may result in POF and has not yet been studied. With advanced age, the somatic microenvironment (niche) is compromised and thus oocytes fail to differentiate resulting in senescence (menopause in women) and under certain conditions the stem cells may undergo uncontrolled proliferation resulting in cancer. OSE, ovary surface epithelium; VSELS, very small embryonic-like stem cells; OSCs, ovary stem cells, OCT4, transcription factor, nuclear OCT4 is crucial to maintain stem cells in the pluripotent state and, once cells initiate differentiation, OCT4 is expressed in cytoplasm and is eventually lost as cells differentiate further. FSH, follicle-stimulating hormone; *FSHR3*, alternatively spliced transcript of FSH receptor; POF, premature ovarian failure.

The *FSHR* gene is located on chromosome 2 and covers 54 kb in the humans and consists of ten exons and nine introns (Fig. 2). Sairam's group has made seminal contribution to the field of ovarian biology and has shown that FSHR exists as four alternatively spliced isoforms (R1, R2, R3, and R4) of which FSHR1 and FSHR3 have known biological functions and may help explain multiple actions of FSH including cellular growth, proliferation, differentiation, and steroidogenesis (Sairam & Babu 2007, Table 3). Exons 1 through 9 encode the extracellular domain while the exon 10 encodes the C-terminal part of extracellular domain, the transmembrane domain, and intracellular domain. In addition, a putative intracellular exon 11 is also described. Canonical *FSHR1* transcript comprises ten exons. *FSHR2* transcript has an additional

intracellular exon 11 on the C-terminal end joining with the truncated exon 10. *FSHR3* consists of exons 1–8 spliced with exon 11, while exons 9 and 10 are spliced out. *FSHR4* comprises only exons 1–4. *FSHR4* is considered as a soluble receptor isoform, whereas *FSHR2* is considered to be a dominant negative receptor isoform (Yarney *et al.* 1997a).

Various studies conducted by Sairam's group that led to the identification of *FSHR* isoforms in sheep and some functional characterization of putative *Fshr3* isoform in mice are reviewed in Table 3. They also compared the putative peptide sequences of human FSHR3 and bovine FSHR3 with ovine FSHR3. FSHR1 is a G protein-coupled receptor that acts via the cAMP signaling pathway, whereas FSHR3 is a growth factor type I receptor that

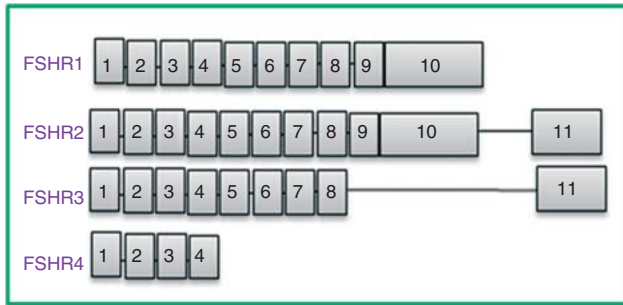


Figure 2 Exon structure of FSH receptor transcripts (splice variants) reported and characterized by Sairam's group (Table 2). The canonical FSHR1 comprises ten exons with the tenth exon being the largest. FSHR2 has a shorter exon 10 and an additional exon 11 at the 5'-end. FSHR3 comprises eight exons similar to canonical FSHR1 and additional exon 11 at the 5'-end. FSHR4 is the smallest comprising only four exons. Of the four transcripts, biological activity has been assigned to only *FSHR1* and *FSHR3*. FSHR1 is a G protein-coupled receptor that acts via the cAMP pathway and is expressed on granulosa cells in ovary and Sertoli cells in testis. FSHR3 functions as a growth factor receptor and acts via calcium signaling and the MAPK/ERK pathway. It is expressed on ovary surface epithelial cells (Li *et al.* 2007) specifically on the stem cells present in the OSE (Patel *et al.* 2013) and also in follicles at different stages of development (Patel *et al.* 2013, Sullivan *et al.* 2013).

acts through calcium signaling and the MAPK/ERK pathway. FSHR1 is expressed on granulosa cells of growing follicles, whereas the smaller transcript (*FSHR3*) is predominant in ovarian surface epithelial cells and cancer cells when compared with granulosa cells (Li *et al.* 2007). Targeted disruption of FSHR in FORKO mice resulted in abnormal gametogenesis and hormonal imbalance (Dierich *et al.* 1998). As shown in Table 4, two studies clearly showed that FSH/pregnant mare serum gonadotropin (PMSG) treatment results in selective upregulation of *Fshr3* compared with *Fshr1* in mouse granulosa cells (Babu *et al.* 2001) and in sheep OSE cells (Patel *et al.* 2013). Sullivan *et al.* (2013) studied *FSHR* transcripts by qPCR in sheep follicles segregated based on the size into small, medium, and large. *FSHR3* expression was higher than *FSHR1* and *FSHR2* in small- and medium-sized follicles irrespective of estrus. They conclude that alternately spliced *FSHR* transcripts are involved in follicular dynamics and *Fshr3* appears to be the most predominant form in sheep ovaries. But these are only a handful of studies, whereas the huge body of the literature on FSHR analysis in ovarian tissues and tumors fails to discriminate between FSHR splice variants as the antibodies/primers used are common for all the variants.

Exon 10 of canonical *FSHR1* has remained the main target to search for mutations leading to POF and cancer. As mentioned earlier, till date, only few naturally occurring mutations have been reported for *FSHR* compared with almost 30 described for *LHR* (Themmen & Huhtaniemi 2000, Themmen 2005), although FSH is a key player controlling these processes. Siegel *et al.* (2013) provided an exhaustive review on the molecular basis of FSH action,

including an update on mutations reported on *FSHR*. As shown in Table 1, mutations in the extracellular and transmembrane domains of protein region (corresponding to exons 6, 9, and 10 of the *FSHR* transcript) do not result in complete absence of follicles as follicles up to pre-antral stage were observed in these patients.

To conclude from this section, pleiotropic action of FSH on the ovary via alternatively spliced FSHR isoforms has not yet been well studied. Exon 11, which is specific to *FSHR3*, has not yet been screened for mutations in cases of POF and cancer. Although exon 11 of *FSHR3* is largely intracellular, rather than FSH binding – it is plausible that cell signaling gets altered resulting in POF and cancer and is worth requiring further studies. But what is more exasperating is that gene and protein sequences for FSHR3 are not yet reported for mouse and human in the NCBI database. Only information available is about sheep FSHR3 in the UNIPROT database.

OSE, stem cells, and FSH

The ovary is surrounded by a whitish layer of OSE, which comprises simple squamous to cuboidal to low pseudo-stratified columnar epithelial cells. Embryologically OSE originates from the coelomic epithelium, which also gives rise to the epithelia lining the Fallopian tube, endometrium, cervix, and upper part of vagina. It has recently been advocated that besides OSE, epithelial neoplasms may also arise from the distal part of Fallopian tubes including fimbria (Karst *et al.* 2011) and later get deposited onto the OSE. This should not be of any concern as both OSE and oviductal epithelium have a common origin in coelomic epithelium (Auersperg 2013). OSE cells show a change in shape from cuboidal, flat to columnar and undergo a distinct cyclic change comprising the proliferative and quiescent phase. Gaytan *et al.* (2005) showed that two out of three of total proliferative activities in rat ovaries are associated with ovulation repair, whereas one out of three of proliferative activities occurs in areas distant from the site of ovulation. Singavarapu *et al.* (2010) carried out immunocytochemistry of whole mouse ovary and concluded that proliferation in OSE is more prominent during antral follicle growth rather than ovulatory wound repair. Wright *et al.* (2002) studied rhesus macaque OSE and found no signs of proliferation or apoptosis in the vicinity of follicle rupture. Thus, proliferation in OSE is an important event and does not occur only in response to post-ovulation repair. Recently, we have reported that treatment with the FSH analog PMSG results in extensive proliferation of OSE on day 2 with no associated sign of ovulation and we implicated it to neo-oogenesis from the stem cells lodged in the OSE (Bhartiya *et al.* 2012). Several groups have reported *FSHR* in normal OSE (Table 2) as well as in cancerous tissues. Ji *et al.* (2004) reported that *FSHR* mRNA was expressed in higher

Table 3 Compilation of contributions made by Sairam's group on FSH receptor transcripts.

References	Salient findings
Yarney <i>et al.</i> (1993)	Sheep testicular cDNA library constructed in pcDNA1 vector was screened using a 1.6 kb probe of rat LHR under low stringency. A 2085 nucleotide open reading frame was found to encode a 678 aa FSHR protein. RNA blot showed two major transcripts of 2.6 and 6.7 kb and a small 1 kb transcript in sheep testis. Transfection of HEK 293 cells with cloned oFSH revealed cAMP accumulation. Thus, cloned receptor revealed properties similar to the WT FSHR
Khan <i>et al.</i> (1993)	Cloned and characterized two alternately spliced forms of sheep testicular <i>FSHR</i> mRNA. A smaller receptor cDNA of 727 bp (from exon 1 to exon 4) codes for 134 amino acids and 1.1 kb cDNA clone (extending up to the 8th exon) codes for a mature protein of 259 amino acids
Yarney <i>et al.</i> (1997a)	By PCR, northern analysis, cloning, and transfection studies on HEK 293 cells, a 2.4 kb transcript with absent cAMP-induced functions was reported. It consisted of nucleotides –121 to 1924 of FSHR and a novel 3' region comprising nucleotides 1925–2307 that encoded a protein of 670 amino acids containing the entire extracellular domain and an altered C-terminus
Yarney <i>et al.</i> (1997b)	Reported five distinct mRNA transcripts in male lambs of two different sheep genotypes. Of these, the 1.1 kb transcript (151A1) comprising the first four exons of the <i>FSHR</i> gene was most abundant from 11 to 22 weeks of age coinciding with increased steroidogenic and spermatogenic function
Sairam <i>et al.</i> (1997)	Studied the expression of a transcript (that encoded exons 1–8 and continued as a single transmembrane domain) in HEK 293 cells. This transcript was found to be expressed on the cell surface, showed high binding affinity with FSH, and no competition with LH. They detected this transcript in both sheep ovary and testis. Results showed that the seven transmembrane domains of the WT receptor are not crucial for cell surface expression of FSHR and for hormone binding
Sairam & Subbarayan (1997)	This study was an attempt to clone the sheep <i>FSHR</i> gene. Obtained 14 clones and they reported exon 11 for the first time beyond the exon 10, which results in two different motifs including a dominant negative receptor and a growth factor type 1. The 2.1 kb 5'-upstream region showed regulatory elements like ERE, CRE, an orphan receptor, and potential germ cell-specific and methylation sites
Babu <i>et al.</i> (1999)	Documented for the first time that alternate splicing of G protein-coupled FSHR1 results in a transcript that comprises first eight exons of FSHR1 spliced to another DNA segment resulting in a novel carboxyl terminus with a single transmembrane domain. It was assigned to growth factor type 1 receptor family. It showed binding to FSH via calcium signaling in both sheep ovary and testis
Babu <i>et al.</i> (2001)	Examined the expression of FSHR1 in adult mouse ovary and after PMSG treatment in immature mouse ovary – using sheep-specific primers. FSHR3 was highly expressed in PMSG-treated ovary as confirmed by both RT-PCR and western analysis
Sairam & Babu (2007)	The work done on sheep and mouse gonadal tissues was reviewed. A single large gene of ~250 kb is a modular structure whose pre-mRNA undergoes alternative splicing creating several subtypes (<i>FSHR1–FSHR4</i>). These isoforms have identical N-terminus segments and different topographies are generated by differing carboxyl termini. Alignment of FSHR2 and FSHR3 with human and bovine genome suggests similarity at both genome level in the organization of exon 11 and peptide sequence (almost 64% identity in the carboxyl terminus)

amounts in invasive tumors followed by low malignant tumors and least in normal OSE. Choi *et al.* (2004) reported that over-expression of FSHR in OSE cells led to an increase in EGFR, c-myc, and HER2/neu proteins and activated the ERK1/2 MAPK pathway. This suggests a pivotal role of FSHR expressed in the OSE during ovarian cancer development.

FSH treatment results in proliferation of OSE and it appears to be a direct effect via FSHR expressed on OSE cells. OSE proliferation after PMSG treatment has been reported earlier in mice (Davies *et al.* 1999, Burdette *et al.* 2006). Similar to PMSG-induced proliferation in OSE (Bhartiya *et al.* 2012), recombinant FSH treatment in mice (5 IU for 48 h) also results in marked changes in OSE (Fig. 3). Besides the observed effect on normal ovaries, FSH treatment is also reported to activate epithelial cell proliferation and oncogenesis in several human OSE immortalized normal, neoplastic (IOSE-29, IOSE-29E, and HOSE), and ovarian cancer cell lines (OVCAR-3 and OVCA) (Schiffenbauer *et al.* 1997, Zheng *et al.* 2000, Syed *et al.* 2001, Choi *et al.* 2004, Ji *et al.* 2004, Abd-Elaziz *et al.* 2005). Interestingly, this effect of FSH is associated with activation of MAPK/ERK rather than via the cAMP pathway (Choi *et al.* 2002, 2005). Li *et al.* (2007) showed that FSH acts on tumorigenic mouse ovary surface epithelium cell line ID8 via the

growth factor-type receptor isoform (FSHR3) to bring about proliferation via calcium signaling and the ERK pathway – distinct from the canonical cAMP pathway associated with G protein-coupled FSHR1 isoform. Mertens-Walker *et al.* (2010) reported that gonadotropins activate the MAPK/ERK1/2 pathway in EOC cell lines (OV207 and OVCAR) responsible for increased proliferation and migration. Hilliard *et al.* (2013) have recently demonstrated that gonadotropins induce proliferation of normal mouse OSE and growth in soft agar and also suppress apoptosis.

To conclude this section, FSH exerts direct action through FSHR expressed on OSE cells through the MAPK/ERK pathway (suggesting involvement of FSHR3) in addition to its well-studied effect through FSHR expressed on granulosa cells via the cAMP pathway (via canonical FSHR1) in growing follicles. This action of FSH mediated through FSHR3 on the OSE may be implicated in ovarian biology as well as pathology.

FSH–FSHR3–stem cell interaction is responsible for neo-oogenesis and PF assembly in adult mammalian ovary

It has recently been shown that stem cells are localized in the adult mammalian OSE. The first paper challenging

Table 4 Studies enumerating biological effects of FSHR transcripts

Reference	Summary
Yarney <i>et al.</i> (1997)	This study was conducted to determine variations in the expression of alternate FSH receptor mRNA transcripts in relation to changes in FSH release, hormone binding activity and testicular function during pubertal development of ram lambs from two genotypes of sheep Five distinct species of mRNA transcripts of approximately 1.1, 1.5, 2.0, 2.5 and 6.5 Kb were highly expressed from 11 to 22 weeks of age in sheep Amongst these transcripts, the 1.1 Kb molecular species was the most abundant The results indicated that contrasting changes in the production of specific alternatively spliced mRNA transcripts may mediate changes in FSH receptor expression The novel truncated transcript, which predictably encodes a soluble protein of either intra- or extracellular fate, could be physiologically relevant
Sairam <i>et al.</i> (1997)	¹²⁵ I-h-FSH binding studies were done in HEK 293 cells (both crude membrane preparations and surface of intact cells) stably transfected with reconstructed HK18 cDNA clone and with full length ovine FSHR cDNA Expression of altered FSHR transcript was studied in freshly collected adult sheep ovary and testicular tissues and stably transfected HEK 293 cells Expression of altered FSHR on cell surface was further confirmed by immunohistochemistry Comparative hydropathy analysis of the HK18 and full length receptor revealed putative single transmembrane domain in putative protein of altered FSHR which was further confirmed by hormone binding studies Postulated a revised model for FSHR gene implicating the existence of an additional exonic sequence in FSHR gene
Babu <i>et al.</i> (1999) and Toyuz <i>et al.</i> (2000)	HEK 293 cells were transfected with FSHR isoforms R1, R2 and R3 Only R3 induced rise in intracellular calcium in response to FSH treatment LH or individual alpha or beta subunits of FSH showed no effect Response was because of calcium influx and not due to intracellular mobilization of calcium This was further confirmed since use of calcium channel blocker suppressed the response FSH response via FSHR3 was seen in both ovary and testis
Babu <i>et al.</i> (2000)	Immortalized granulosa cells lacking endogenous FSHR were used for transfection with R1 and R3 FSH associated proliferation was studied by BrdU/ tritiated thymidine uptake Transfected cells showed rapid and transient activation of ERK1 and ERK2 pathways Pretreatment with MAPK/ERK and PKC inhibitors prior to FSH stimulation blocked ERK1 and ERK2 activation suggesting that FSH acts via MAPK/ERK pathway to induce proliferation
Babu <i>et al.</i> (2001)	Investigated hormonal regulation of FSHR3 after PMSG treatment in immature mice FSHR3 levels were significantly up-regulated by RTPCR compared to FSHR1 after PMSG FSHR3 were localized in granulosa cells membrane
Li <i>et al.</i> (2007)	Tested the hypothesis whether FSH can stimulate ovarian cancer cell proliferation by acting on FSHR3, using the tumorigenic mouse OSE cell line ID8 FSH enhanced ID8 proliferation in a concentration-dependent fashion Signalling pathway included cAMP-independent activation of ERK downstream of an SNX482 sensitive component likely to be the Cav2.3 calcium channel Northern analysis using probes specific for exons 7 and 11 of FSHR identified consistently only one 1.9 kb transcript Immunoblot analysis confirmed expression of FSHR3 but not FSHR1 in ID8 Data suggest that signalling promotes proliferation of ovarian cancer cells
Patel <i>et al.</i> (2013)	Studied sheep ovary surface epithelium cells <i>in vitro</i> Stem cells of two different sizes (VSEs and OGSCs), few blood cells and epithelial cells were visualized in OSE smears Stem cells expressed FSHR whereas the epithelial cells were negative Upon FSH treatment, stem cells underwent rapid proliferation within 15 h <i>in vitro</i> as visualized by presence of germ cell nests <i>In situ</i> hybridization studies revealed selective activation of FSHR3 after FSH treatment RNA was extracted from the cultured cells after 3 and 15 h and studied for FSHR1 and FSHR3 transcripts. Only FSHR3 mRNA levels were up regulated at 3 h and return to basal level by 15 h Results show that FSH via FSHR3 stimulates the ovarian stem cells to undergo proliferation, clonal expansion and further differentiation
Sullivan <i>et al.</i> (2013)	Studied relative expression of FSHR1, FSHR2 and FSHR3 in sheep ovarian follicles from normal estrus cycle Follicular fluid was aspirated from small (2.0 mm), medium (2.1–4.0 mm), large (4.1–6.0 mm) and pre-ovulatory (more than 6.1 mm) follicles. Cells were pelleted and used for RNA extraction whereas the fluid was used for measuring estradiol and progesterone (P) levels P levels were maximum in pre-ovulatory follicles along with a slight increase in estradiol level FSHR1 and FSHR3 transcripts were studied by qRT PCR FSHR3 expression was maximum in medium sized follicles compared to FSHR1 and R2 It was also more than LHR which were maximally expressed in pre-ovulatory follicles Thus in addition to canonical G protein coupled receptor, FSHR3 is also involved in follicular dynamics

the concept of a fixed number of follicles in the adult ovary (Johnson *et al.* 2004) reported the presence of stem cells in the adult mouse OSE. Szotek *et al.* (2008) using BrdU incorporation and doxycycline inducible histone 2B–green fluorescent protein pulse–chase techniques

identified a label-retaining stem/progenitor population in the coelomic epithelium of the adult mouse ovary. These cells were observed to exhibit quiescence with asymmetric label retention and functional response to estrous cycling *in vivo* by proliferation, showed *in vitro*

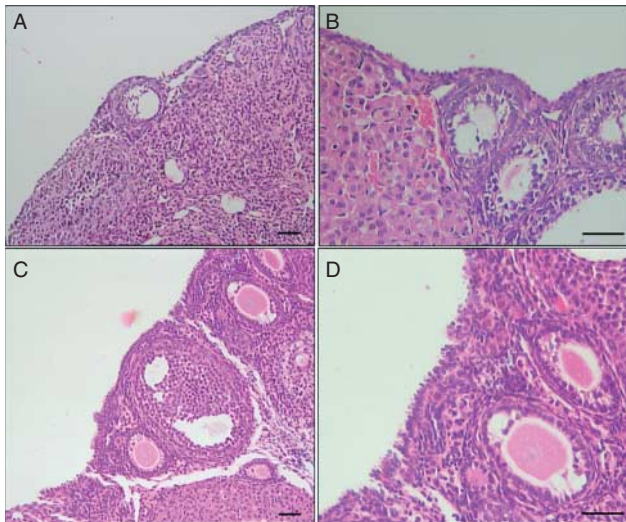


Figure 3 Effect of recombinant FSH treatment on mouse ovary surface epithelium. (A and B) H&E stained sections of the adult mouse ovary. The presence of a non-discrete layer of ovary surface epithelium comprising small, flat to cuboidal epithelial cells is observed. (C and D) Effect of recombinant FSH treatment (5 IU, 48 h) on OSE cells. The prominent, multilayered ovary surface epithelium comprising columnar epithelial cells is observed. These results are similar to the effect observed after PMSG treatment by our group (Bhartiya *et al.* 2012). Scale bar represents 20 μ m.

colony formation, and were enriched as side population. Although the concept of stem cells in adult ovaries is still not well accepted – the conflict appears to be more technical (Bhartiya 2012, Bhartiya *et al.* 2013a, Woods *et al.* 2013) and hopefully will be resolved in due course. In addition to the seminal contributions made by Jonathan Tilly's group in the field, recently equating ovarian stem cells (OSCs) to spermatogonial stem cells (SSCs) in testis (Woods *et al.* 2013), and their protocols to isolate OSCs (Woods & Tilly 2013), our group has reported an additional population of stem cells (VSELs) in both ovary and testis (Bhartiya *et al.* 2010, 2013b, Parte *et al.* 2011), in addition to the OSCs/OGSCs.

A gentle scraping of OSE from mouse, rabbit, sheep, monkey, and human ovaries shows the presence of two population of stem cells including nuclear OCT4-positive very small embryonic-like stem cells (VSELs) and slightly bigger progenitors with cytoplasmic OCT4 (OGSCs/OSCs), which exist singly or as a cluster termed the germ cell nest (Bhartiya *et al.* 2013a,b, Parte *et al.* 2014). Similarly, Virant-Klun's *et al.* (2013) has reported that OSE harbors stem cells, which spontaneously differentiate into oocyte-like structures during *in vitro* culture. Our group also observed spontaneous differentiation of OSE cells into oocyte-like structures and that the epithelial cells undergo epithelial–mesenchymal transition to form granulosa-like cells that surround the developing germ cells resulting in follicle assembly (Parte *et al.* 2011). Further studies in marmosets and humans have revealed that ovarian cortical tissue slices,

besides being a good source of PFs (for cancer patients to achieve biological parenthood), are also an excellent source of stem cells (Parte *et al.* 2013). We observed that FSH treatment results in increased proliferation of OSE and increased number of stem cells as reflected by increased expression of pluripotent transcripts (*OCT4A* and *NANOG*), even in a sample collected from a 60-year-old woman. The stem cells from aged ovaries have the ability to differentiate into oocyte-like structures during *in vitro* culture (because the inhibitory factors *in situ* were overcome *in vitro*). Similarly, others have also described the presence of stem cells in post-menopausal women OSE, which differentiate into oocytes and parthenote embryos in culture (Virant-Klun & Skutella 2010). Various events such as formation of germ cell nests, Balbiani body-like structures, and cytoplasmic streaming, which are extensively studied in fetal ovary, are recapitulated during *in vitro* oogenesis in adult OSE cultures along with characteristic expression of stem/germ cell/oocyte markers (Parte *et al.* 2014).

VSELs are spherical cells with an intensely hematoxylin-stained nucleus surrounded by a thin rim of cytoplasm and a high nucleo-cytoplasmic ratio. These stem cells are easily visualized in gently scraped OSE smears (Parte *et al.* 2011) and express various pluripotent markers including nuclear *OCT4* and undergo clonal expansion in response to PMSG treatment (Bhartiya *et al.* 2012). Patel *et al.* (2013) have shown that sheep OSCs express FSHR whereas the epithelial cells are distinctly negative. *In situ* hybridization results further showed the presence of both *FSHR1* and *FSHR3* mRNAs in the stem cells; however, only *FSHR3* showed active transcription after FSH treatment of OSE cells *in vitro*. Quantitative RT PCR studies on *FSHR* transcripts R1 and R3 showed a distinct upregulation of *FSHR3* within 3 h of FSH treatment and that levels return to basal levels by 15 h. Thus, evidence is available to suggest that the stem cells lodged in the OSE respond to FSH via a growth factor-type receptor isoform FSHR3, which acts via the MAPK/calcium signaling pathway. This is a novel effect of FSH on OSCs recently deciphered by our group and the results are compiled in Table 5.

However, owing to their very small size (smaller than red blood cells), VSELs have remained obscure, controversial, and poorly studied till date (Abbott 2013, Ratajczak *et al.* 2014). Emerging literature suggests that VSELs do exist and similar to mouse bone marrow VSELs that survive total body irradiation (Ratajczak *et al.* 2011), we have observed that both adult mouse testicular (Anand *et al.* 2014) and ovarian VSELs survive chemotherapy (Fig. 4) because of their relatively quiescent state. The VSELs present in the chemo-ablated mouse OSE express nuclear OCT4 (Fig. 4) and also respond to FSH (K Sriraman, D Bhartiya, S Anand, and S Bhutda, unpublished observations). Similarly, Anand *et al.* (2014) have shown that mouse testicular VSELs survive

Table 5 Emerging understanding of FSH modulation of adult mouse ovarian stem cells resulting in neo-oogenesis and primordial follicle assembly (Bhartiya *et al.* 2012).

Our results	Inference
OSE proliferation was noted during the estrous state and also after PMSG treatment associated with increased PCNA staining	Similar to OSE proliferation observed during the normal estrous cycle; PMSG (FSH analog) exerts direct proliferative action on OSE in addition to the well-studied action on granulosa cells of growing follicles
Increased FSHR expression in OSE after PMSG treatment Nuclear OCT4, a marker specific for pluripotent stem cells (VSELs) present in the OSE is also observed in developing oocytes after PMSG treatment	PMSG action is mediated via FSH receptors that are expressed on OSE ^a Oocytes arise from nuclear OCT4-positive VSELs VSELs self-renew, give rise to OGSCs that divide rapidly and give rise to germ cell nests, undergo meiosis, and give rise to oocytes (with nuclear OCT4)
Mouse VASA homologue (MVH) immunolocalization in germ cell clusters and oocytes after PMSG treatment SCP3 staining in oocytes at both protein and transcript levels after PMSG treatment	PMSG treatment results in increased germ cell proliferation, clonal expansion with incomplete cytokinesis, and differentiation Ovarian stem cells differentiate into germ cells, which also undergo meiosis during adult life
PMSG treatment results in increased transcripts of pluripotent stem cells (<i>Oct4A</i> and <i>Nanog</i>), early primordial germ cell-specific markers (<i>Oct4</i> , <i>Stella</i> , <i>Fragilis</i>), germ cell markers (<i>Mvh</i>), early oocyte-specific markers (<i>Nobox</i> and <i>Hoxa10</i>), premeiotic marker (<i>Scp3</i>), meiosis-specific homologous recombination-specific marker (<i>Dmc1</i>), and PMSG action mediator (<i>Fshr</i>)	Pluripotent VSELs undergo proliferation Primordial germ cells persist in adult ovary as VSELs Upon differentiation, germ cell markers are expressed VSELs differentiate into oocytes and also undergo meiosis Neo-oogenesis in adult ovary is mediated via FSH through FSHR expressed on the stem cells in OSE

^aPatel *et al.* (2013) showed by studies on sheep OSE smears that FSHR are selectively expressed on the stem cells (epithelial cells remain negative).

chemotherapy and are able to restore spermatogenesis in chemo-ablated testis when a healthy niche is provided.

We have further studied the underlying mechanism of how these stem cells lodged in the OSE are regulated by FSH and are responsible for neo-oogenesis and PF assembly in adult ovary similar to the mechanism how SSCs are involved during spermatogenesis in testis (Bhartiya *et al.* 2012, Patel *et al.* 2013). Adult mouse OSE proliferation and PF assembly occur in a very subtle manner during the normal estrous cycle and the process gets augmented after PMSG treatment (Table 5). Certain degree of OSE proliferation was observed in the pro-estrous stage of the normal estrous cycle, which became prominent during the estrous state and at a higher magnification – cohorts of PF with cytoplasmic continuity were observed just below the OSE – resembling a ‘nest’. Presence of cytoplasmic continuity among the developing PF suggests that they were newly formed rather than pre-existing follicles from birth because, as per current understanding, germ cell nest breakdown occurs just after birth (Xu & Gridley 2013). Stimulation with recombinant FSH resulted in similar OSE proliferation on day 2 (Fig. 3). Concluding this section, it is indeed the stem cells in the OSE that express FSHR and respond to FSH via FSHR3 resulting in neo-oogenesis and PF assembly during adult life. Any defect in the stem cell biology in the OSE will possibly result in the failure of PF to assemble and this may give rise to a phenotype of absence of follicles characteristic of POF.

FSH–FSHR3–stem cell interaction in OSE is implicated during POF, aging, and cancer

POF is a heterogeneous disorder that occurs at the frequency of <1% in women below 40 years of age.

Besides having a genetic basis and autoimmune etiology, POF may also be caused by surgical removal of ovaries or it occurs as a side effect of oncotherapy for various non-gynecological malignancies. Despite huge efforts, as discussed and elaborated above, genetic basis for POF is still not elucidated. We propose that mutations, specifically in intracellular exon 11 of *FSHR3*, could possibly result in impaired signaling (not binding to FSH) that may result in suppressed stem cell function and, thus, primordial follicle assembly will not occur resulting in POF associated with elevated FSH levels (Fig. 1).

On similar lines, it has been suggested that the microenvironment (niche) supporting the stem cells gets compromised with age (not able to provide the required growth factors/cytokines) and stem cells are not able to differentiate into oocytes thus resulting in menopause (Tilly & Telfer 2009, Massasa *et al.* 2010) with associated elevated FSH levels (Fig. 1). Nikura *et al.* (2009) demonstrated that the stem cells from aged ovary retain the differentiation potential and form oocytes when exposed to young ovarian environment. Similarly, it has been recently concluded that compromised somatic niche due to oncotherapy does not support testicular stem cell differentiation (Oatley & Brinster 2012). Chakkalal *et al.* (2012) also suggested that the niche gets compromised with age in muscles resulting in altered stem cell function. Bukovsky (2011) has reported that it is possibly a functional decline of immune system between 35 and 40 years of age that could also be responsible for termination of neo-oogenesis and follicular assembly and hence menopause. As mentioned earlier, stem cells isolated from adult OSE retain the ability to undergo spontaneous differentiation into oocyte-like structures *in vitro* (Virant-Klun & Skutella 2010, Parte *et al.* 2011, 2014). Thus, despite elevated FSH levels, compromised

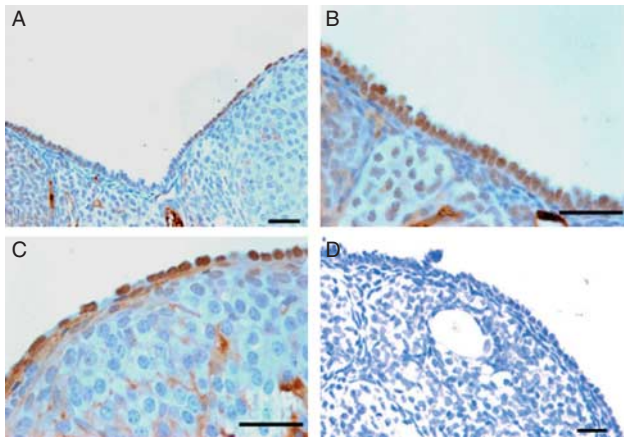


Figure 4 OCT4-positive stem cells in mouse ovary. (A) Low-magnification view of a chemo-ablated (10 mg/kg busulfan daily for 4 days and 100 mg/kg cyclophosphamide for first 2 days) mouse ovary with prominent OSE. A heterogeneous staining pattern with unstained epithelial cells interspersed with distinct nuclear *Oct4*-expressing stem cells is clearly evident. Mouse VSELs survive chemotherapy (Anand *et al.* 2014) as well as radiotherapy (Ratajczak *et al.* 2011) possibly because of their quiescent nature and increase in numbers but do not differentiate in the chemo-ablated gonad because the somatic niche is compromised. These stem cells are not easily evident in untreated mouse ovarian sections but are clearly observed in chemo-ablated ovary and respond to FSH (K Sriraman, D Bhartiya, S Anand, and S Bhutda, unpublished observations). (B and C) Higher magnification showing the presence of nuclear to cytoplasmic OCT4 expression in stem cells located in the OSE. (D) Negative control. Scale bar represents 20 μ m.

somatic microenvironment of the ovary results in menopause (Fig. 1).

Continuous stimulation of stem cells by elevated levels of circulatory FSH may also act as a trigger to stimulate their proliferation and, under certain yet not well-understood conditions, results in cancer. POF cases may not be prone to developing ovarian cancer – mainly because the underlying mechanisms are very different. POF may occur because of a mutation in *FSHR* whereas the FSH–FSHR–stem cell interaction and PF assembly were normal in women for some time before they develop cancer. Cancer patients may not necessarily show any mutation in *FSHR* – rather cancer has been proposed to be a stem cell disease and some change in the somatic microenvironment (niche) results in uncontrolled proliferation of otherwise quiescent VSELs resulting in cancer. Thus, it is not surprising that Schildkraut *et al.* (2001) found no correlation between age at menopause and ovarian cancer. POF cases (unlike cancer patients) are also likely to have associated elevated FSH levels. VSELs express FSHR and their increased numbers may result in excessive binding of FSH with FSHR and, hence, FSH levels are not always elevated in ovarian cancer patients. This could be a likely explanation for lowered FSH levels in ovarian cancer patients (Fig. 1), in addition to the role of neurotrophins as suggested previously (Bose 2005).

The understanding of FSH–FSHR3–stem cells in OSE provides a plausible explanation as to: i) why more than 90% of ovarian cancers arise in the OSE; ii) why FSHR is expressed on the ovarian cancer cells; and iii) why there is an increased incidence of cancers with age, and thus supports the gonadotropin theory of ovarian cancer with a stem cell perspective.

The hypothesis that the stem cells under the effect of elevated FSH levels may result in ovarian cancer gets further credence from the current understanding that cancer is a stem cell disease. VSELs could be the embryonic remnants in adult organs including ovaries responsible for cancers in adult life (Ratajczak *et al.* 2010). Neonatal exposure to estrogen/endocrine disruptors is known to be associated with an increased risk of cancer in adult life and a recent study by our group has implicated altered VSEL biology to be the underlying cause for an increased risk of cancer (Bhartiya *et al.* 2013c). Nuclear OCT4 is a pluripotent stem cell marker expressed by VSELs lodged in OSE and is also reported to be over-expressed in ovarian cancer. Zhang *et al.* (2013) reported over-expression of OCT4 in ovarian carcinoma compared with benign cystadenomas and borderline tumors. OCT4 expression was significantly correlated with the histological grade as staining for OCT4 was increased in serous cystadenocarcinoma, when compared with clear cell carcinoma. FSH stimulation resulted in a significant increase in both nuclear and cytoplasmic OCT4 protein in a dose-dependent manner in both Hey and OVCAR3 cell lines. Ma *et al.* (2013) have implicated Lin28/OCT4/BMP4-mediated mechanism in ovarian tumor cell growth by further extending the earlier report by Peng *et al.* (2010) indicating co-expression of LIN28 and OCT4 in a sub-population of cells in cancer cell lines and tumor tissue and expression intensity correlated with the tumor grade. They proposed that Lin28 and OCT4 may have important roles in the initiation and/or progression of EOCs. Samardzija *et al.* (2012) have discussed OCT4 and ovarian cancer and reported nuclear OCT4-positive cells to be present as a core of tumor spheroids. Thus, it is becoming increasingly apparent that normal ovarian nuclear OCT4-positive VSELs are also implicated in ovarian cancers. A paper published recently by Flesken-Nikitin *et al.* (2013) is also extremely relevant. This group by elegant lineage tracing studies concluded that OSE in the hilum region is the stem cell niche for ovarian cancer stem cells in the mouse ovary and also has the ability to regenerate the OSE.

Conclusions

We need to appreciate the novel action of FSH on the stem cells lodged in the OSE via FSHR3. The research focus should be on exon 11 of *FSHR* to search for mutations in cases of POF and cancer. For this, we need to first sequence FSHR3 in mouse and human and study

FSHR3 biology by raising specific antibodies. This review opens up several new areas for research and also questions several existing paradigms in ovarian biology, including the following: i) initial follicle growth is FSH independent and ii) FSH exerts action on granulosa cells of follicles in ovary. By contrast, we have compiled the available literature suggesting that: i) stem cells are present in OSE and express FSHR; ii) neo-oogenesis and PF assembly occur in adult ovary in response to FSH; and iii) FSH exerts its action via a novel transcript *FSHR3*. Any perturbation in the FSH–FSHR3–stem cell network in OSE may possibly result in menopause and most probably be responsible for various pathologies such as POF and cancer.

On comparing POF in women with azoospermia in men, it is indeed intriguing that similar to POF, genetic basis for azoospermia is also not yet deciphered and no significant association is reported with the *FSHR* gene (or any other) and male infertility. VSELs exist in human (Bhartiya *et al.* 2010) and mouse (Anand *et al.* 2014) testis as in the ovaries and nuclear OCT4 is a specific and sensitive marker for testicular cancers (Jones *et al.* 2004). Studies are currently ongoing in our laboratory to study the effect of FSH on testicular stem cells before we implicate FSH–FSHR3–stem cell interaction in testicular biology and azoospermia as well.

Key messages

1. Stem cells, including VSELs (with nuclear OCT4) and their descendants OSCs (with cytoplasmic OCT4), are localized to the OSE and are regulated by FSH to undergo neo-oogenesis and PF assembly.
2. This novel action of FSH on the stem cells in the OSE is mediated via the alternately spliced FSHR transcript *FSHR3*. *FSHR3* is an important and abundantly expressed transcript involved in follicular dynamics and is also highly expressed in ovarian cancer cells.
3. The *FSHR3* transcript has not yet been searched for mutations in women with POF. *FSHR3* has a unique exon 11, which needs to be sequenced and studied in details, as exons 1–8, which are common with canonical *FSHR1*, have already been screened.
4. Compromised somatic microenvironment (niche) with age does not support normal stem cell proliferation, differentiation, neo-oogenesis, and PF assembly – thus resulting in age-related senescence/menopause.
5. Certain (yet not well understood) changes in the stem cell niche trigger uncontrolled proliferation of stem cells and thus cancer.
6. VSEL biology in the adult ovary needs to be acknowledged, *FSHR3* needs to be sequenced in humans and mice, exon 11-specific antibodies need to be generated, and detailed studies need to be undertaken to better understand ovarian biology, POF, menopause, and also cancer. Deciphering the specific and distinct changes in the niche that result in menopause and

cancer is challenging but will have huge relevance in the field of reproductive medicine.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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