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Ovarian regeneration: The potential for stem cell contribution in the postnatal ovary to sustained endocrine function

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ABSTRACT

The endocrine function of the ovary is dependent upon the ovarian follicle, which on a cellular basis consists of an oocyte surrounded by adjacent somatic cells responsible for generating sex steroid hormones and maintenance of hormonal stasis with the hypothalamic-pituitary axis. As females age, both fertility and the endocrine function of the ovary decline due to waning follicle numbers as well as aging-related cellular dysfunction. Although there is currently no cure for ovarian failure and endocrine disruption, recent advances in ovarian biology centered on ovarian stem cell and progenitor cell populations have brought the prospects of cell- or tissue-based therapeutic strategies closer to fruition. Herein, we review the relative contributions of ovarian stem cells to ovarian function during the reproductive lifespan, and postulate steps toward the development of ovarian stem cell-based approaches to advance fertility treatments, and also importantly to provide a physiological long-term means of endocrine support.

1. Introduction

In female mammals, fertility and endocrine function rely on a tightly regulated synchronicity within the hypothalamic-pituitary-gonadal (HPG) axis, in which the ovary serves as both the primary source of sex steroid hormones and germ cells (oocytes) required to maintain hormonal stasis and fertility throughout the reproductive lifespan. Predominantly localized to the outer cortex, the ovarian follicles serve as the functional units of the ovaries and consist of an oocyte surrounded by granulosa cells or their precursors, enclosed within an extracellular matrix (ECM)-rich basement membrane, composed of a species- and developmental stage-specific combination of predominantly laminins and collagens (Berkholtz et al., 2006; Heeren et al., 2015; Hummitsch et al., 2013). In rodents it has been demonstrated that the majority of the pregranulosa cells enclosed within primordial follicles in the ovarian cortex are non-proliferative, however mitotically active pregranulosa cells are found within follicles of the medulla, which are likely part of the primordial pool active prior to sexual maturity (Hirshfield and DeSanti, 1995). Following a process of 'follicle activation' of quiescent primordial follicles, the granulosa cells transition from squamous to cuboidal and mitotically activate, and a theca cell layer is recruited to surround the basement membrane (Skinner, 2005). In growing follicles, the ovarian granulosa and theca cells work in concert to respond to circulating levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary to generate the sex steroids (*i.e.* estradiol and progesterone), acting through the FSH receptor (FSHR) and LH receptor (LHR), respectively.

In recent years both germline stem cells, termed 'female germline stem cells' (fGSC) or 'oogonial stem cells' (OSCs), and somatic ovarian stem cells or progenitors have been reported, generating a renewed enthusiasm for the exploration of strategies to promote ovarian regeneration and/or sustained ovarian function (reviewed in Woods and Tilly, 2015; Grieve et al., 2015; Silvestris et al., 2015). However, despite the identification of ovarian stem cell and progenitor populations along with evidence to support that adult ovaries are amenable to follicle renewal during the reproductive lifespan, ovarian failure remains inevitable due to pathological conditions such as polycystic ovarian syndrome (PCOS) or depletion of follicles as a consequence of surgical ablation, exposure to environmental toxicants or chemotherapeutic agents, or as a result of age. As a major outcome of ovarian failure (in addition to infertility), steroid biosynthesis ceases and the ability of the ovary to feedback to the hypothalamus via inhibin and estradiol is lost.

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Consequently, circulating levels of FSH (followed by LH) rise sharply in women with menopause, and the pathological conditions associated with ovarian failure ensue. Accordingly, strategies to improve fertility, as well as delay or prevent the endocrine-related symptoms associated with menopause will require a greater understanding of ovarian function and the properties that govern the renewal and regeneration of multiple ovarian cell types. Herein we review germline and somatic stem cell populations in the ovary, a role for pluripotent stem cell-derived somatic cells, and the potential for these cells to maintain ovarian function during the reproductive lifespan, and prospective utility for therapeutics as efforts to extend fertility and prevent or delay menopause come closer to fruition.

2. Ovarian germ cells: primordial germ cells (PGCs) and oogonial stem cells (OSCs) as distinct precursors to oocytes

It has traditionally been accepted that most female mammals, unlike males, or other vertebrate or invertebrate females, are endowed at birth with a non-renewable pool of oocytes, of which a species-specific number will be selected for ovulation throughout the reproductive lifespan until the pool is exhausted (reviewed in Woods and Tilly, 2013c). However, more recent data supports ovarian function and oocyte biology as having a greater degree of plasticity than previously thought. While the oocytes present at birth are the direct progeny of PGCs fetal in origin, an emerging body of work from multiple laboratories worldwide has demonstrated that the ovaries from adult female mammals contain a distinct population of mitotically active germ cells that can generate oocytes during adulthood (Johnson et al., 2004; Zou et al., 2009; Pacchiarotti et al., 2010; Zhang et al., 2011; Zou et al., 2011; White et al., 2012; Imudia et al., 2013; Park et al., 2013; Woods et al., 2013b; Zhou et al., 2014; Xie et al., 2014; Fakih et al., 2015; Grieve et al., 2015; Khosravi-Farsani et al., 2015; Park and Tilly, 2015; Silvestris et al., 2015; Xiong et al., 2015; Ding et al., 2016; Lu et al., 2016; Zhang et al., 2016). Among the defining properties of OSCs are a stable karyotype and germline molecular profile following extended propagation, and, importantly, the ability to spontaneously initiate a differentiation program into oocytes following either culture *in vitro* or transplantation into ovarian tissue (Zou et al., 2009; Pacchiarotti et al., 2010; White et al., 2012; Ding et al., 2016). In mice, the oocytes formed from transplanted OSCs complete maturation to the metaphase-II stage of development, and can be fertilized yielding viable embryos and offspring (Zou et al., 2009; White et al., 2012; Xiong et al., 2015; Zhang and Wu, 2016). While a number of laboratories have independently successfully isolated OSCs using multiple methodologies, there remains some controversy as to the existence or biological significance of OSCs. These counter-claims to OSCs are largely centered on circumstantial negative findings, (Zhang et al., 2012; Lei and Spradling, 2013), or technical difficulties arising from antibody purification strategies (Zhang et al., 2012; 2015). For example, using a transgenic reporter mouse (*Ddx4-Cre:Rosa26r*$^{bw/+}$) in which *Ddx4* positive cells were presumed to fluoresce, putative *Ddx4*-positive cells were identified. However, following subculture, these cells failed to proliferate, inconsistent with what has previously been reported for OSCs (Zhang et al., 2012). Subsequently, the data generated using the *Ddx4-Cre* mouse reporter line was experimentally re-examined, and it was found that fluorescence was not restricted to the germline as previously claimed, with demonstrated promoter 'leakiness' throughout the ovary. Moreover, when ovarian dispersates from this mouse line were combined with antibodies targeting DDX4 and subject to fluorescence activated cell sorting (FACS), a distinct subpopulation of DDX4-tdTm- positive cells having properties consistent with OSCs were isolated and propagated, refuting the earlier claims that *DDX4*-positive cells from the ovary were not OSCs (Park and Tilly, 2015). The data in these studies and others surrounding the controversy on the existence of OSCs has been extensively reviewed in careful detail by us and others (Woods and Tilly, 2013a, 2015; Grieve et al., 2015; Woods and Tilly, 2015). Although to what extent OSCs contribute under normal physiological circumstances to ovarian function has yet to be demonstrated, the potential utility of OSCs for ovarian therapies is broad (Woods and Tilly, 2013a, 2015; Hummitzsch et al., 2015; Woods and Tilly, 2015). As just one illustration, we have developed a clinically validated fertility treatment, termed *au*tologous *g*ermline *m*itochondrial *e*nergy *t*ransfer (AUGMENT^{SM}), which utilizes the isolated mitochondria from a woman's own OSCs injected at the time of intracytoplasmic sperm injection (reviewed in Woods and Tilly, 2015). To date, the findings reported from three clinical sites show a marked improvement in embryo quality and IVF success rates (Casper et al., 2015; Fakih et al., 2015).

Although both PGCs and OSCs give rise to oocytes, differences between the two cell types are clear. During embryonic development PGCs colonize the gonadal ridge and mitotically divide as they populate what will eventually become either an ovary or a testis. In humans, there is a major void in our current understanding of the molecular mechanisms that govern the processes of PGC specification, migration, proliferation, and entry into meiosis due to limitations with sample availability (De Felici, 2013), though histological evidence has shown that PGCs are identifiable during the third week of embryo development in the endoderm of the yolk sac (Witschi, 1948), proliferate extensively between the 3rd and 4th week (Witschi, 1948; Politzer, 1933) and colonize the developing ovary by the end of the 5th or beginning of the 6th week (Makabe et al., 1991). While the earliest stages of embryonic development differ dramatically between humans and mice, much of the contemporary knowledge of mammalian PGC specification, proliferation, migration and colonization has been generated using mice as a model, in which the process is well characterized and can be studied with relative ease. It has been postulated that as *in vitro* methodology and human modeling using pluripotent stem cell cultures progress that many of the knowledge gaps surrounding human ovarian development will be filled (De Felici et al., 2004). Additionally, as advances in 'omics'-based approaches move toward lesser input amounts, valuable information can be garnered from samples limited by sources or size, which will dramatically improve our understanding of the molecular events that drive developmental milestones in human ovarian physiology (Truman et al., 2016).

The biological properties of murine PGCs have been extensively reviewed elsewhere (Saitou et al., 2002; De Felici et al., 2004; Wear et al., 2016). In brief, primordial germ cells are identifiable early as 7.25 days post coitum (dpc) as a small cluster of cells positive for alkaline phosphatase; at the end of gastrulation, this small cluster proliferates to approximately 50–80 cells (Chiquoine, 1954; Ginsburg et al., 1990). Mouse PGC migration occurs in several stages, during which PGCs develop in the hindgut, emerge and invade the body wall to move dorsally, and subsequently begin migration toward the genital ridge, and colonize the indifferent gonad at approximately embryonic day e10.5 (Molyneaux et al., 2001; Molyneaux and Wylie, 2004). Following colonization of the gonadal ridge, PGCs rapidly proliferate, reaching approximately 20,000 in number, and become oogonia (Tam and Snow, 1981; Speed, 1982). During colonization, PGCs form nests of closely associated germ cells organized into long ovigerous cords, bordered by a basal lamina which provides a physical separation between the germ cells and the surrounding pre-granulosa and mesenchymal stroma cells (Konishi et al., 1986; Heeren et al., 2015). In mice, formation of the nests begins at e12.5 and continues until meiotic