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RESEARCH ARTICLE

Conservation and contrast in cell states of echinoderm ovaries

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Abstract

Echinoderms produce functional gametes throughout their lifespan, in some cases exceeding 200 years. The histology and ultrastructure of echinoderm ovaries has been described but how these ovaries function and maintain the production of high-quality gametes remains a mystery. Here, we present the first single cell RNA sequencing data sets of mature ovaries from two sea urchin species (*Strongylocentrotus purpuratus [Sp]* and *Lytechinus variegatus [Lv]*), and one sea star species (*Patiria miniata [Pm]*). We find 14 cell states in the Sp ovary, 16 cell states in the Lv ovary and 13 cell states in the ovary of the sea star. This resource is essential to understand the structure and functional biology of the ovary in echinoderms, and better informs decisions in the utilization of in situ RNA hybridization probes selective for various cell types. We link key genes with cell clusters in validation of this approach. This resource also aids in the identification of the stem cells for prolonged and continuous gamete production, is a foundation for testing changes in the annual reproductive cycle, and is essential for understanding the evolution of reproduction of this important phylum.

KEYWORDS
echinoderms, FoxL2, oogonia, ovary, scRNAseq

1 | INTRODUCTION

Sea stars and sea urchins spawn millions of gametes that produce beautifully transparent embryos and larvae that develop synchronously. These echinoderms, studied for over 150 years by many investigators interested in cell and developmental biology ([Fol, 1879; Hertwig, 1875], see also [Derbès, 1847]) have contributed foundationally to many scientific advancements including cell cycle regulation (Evans et al., 1983), the respiratory burst and mitochondrial function (Warburg, 1931), egg activation (Jaffe, 1976; Just, 1939; Vacquier & Moy, 1977), gene regulatory networks (e.g., [Davidson et al., 2002]), inductive mechanisms (e.g., [Horstadius, 1950]), biomineralization (e.g., [Okazaki, 1965]), innate immunity (e.g., [Smith et al., 2006]), the cytoskeleton in cell division (e.g., [Schroeder, 1990]), and the evolution of major body plans (Arendt & Extavour, 2016; Formery et al., 2023).

Lacking in these accomplishments are studies on gonad function, especially with regard to their unusual lack of reproductive senescence. How do the ovaries of these animals keep producing millions of oocytes annually throughout their lifespan which, depending on the species, can last from 3 to 4 years to over 200 years (Bodnar & Coffman, 2016)? What are the different cell populations in an ovary that maintain this organ's function? Further, what similarities in reproductive mechanisms might be seen between sea stars and sea urchins, which are basal branching deuterostomes, with mammals and other vertebrates? In humans, women are born with an ovarian reserve that contains a finite and ever decreasing number of oocytes. Moreover, the human ovary ages faster than any other organ system of the body, and the number and the quality of oocytes decreases with age. Sea stars and sea urchins have a distinct reproductive strategy that may inform important functional diversity (Wessel et al., 2010).

The histology of the echinoderm ovary has been described by light and electron microscopy in several species (e.g., [Chia & Walker, 1991; Schoenmakers et al., 1981]). Now, 40–50 years later, new technologies allow us to interrogate ovarian function with a molecular perspective. Reich and colleagues (Reich et al., 2015) reported bulk RNA-sequencing of ovaries from 23 echinoderm species to identify foundational transcript accumulation and to map the phylogenomic landscape of the echinoderm clade.

Here, we describe the first ovarian single cell RNA seq data sets obtained in two sea urchins (*Strongylocentrotus purpuratus* [Sp] and *Lytechinus variegatus* [Lv]) and a sea star (*Patiria miniata* [Pm]). The two sea urchin species shared a common ancestor about 50 million year ago, whereas the last common ancestor of *S. purpuratus* and *P. miniata* diverged approximately 500 million years ago. Sea urchin and sea star ovaries were dissociated individually, processed for single-cell RNA-sequencing and aligned to their corresponding genome (echinobase.org). All three data sets were analyzed using Seurat (Figure 1; [Satija et al., 2015]). Gene markers for each cluster and each species is provided as Supporting Information Tables. The clusters have been identified based on their gene markers, and this information is provided as Table S1–S3. One can now pick any of these gene markers and use RNA in situ hybridization to identify its location in the ovary (Figure 2). As an example, Sp Lefty mRNA is enriched in the cluster 4 of Sp Ovary data set (Figure S1). By in situ RNA hybridization, we find Sp Lefty enriched in early stage oocytes (Figure 2). Lefty's function has been described during sea urchin embryonic development; this protein is involved in the oral-aboral axis determination and acts as a Nodal inhibitor (Duboc et al., 2008). Its expression in the ovary is more surprising. Perhaps TGF-beta involvement in oocyte early growth is a shared element in oogenesis of echinoderms and mammals, considering the GDF-9 expression by early mouse oocytes (McGrath et al., 1995). Another important marker for this Sp cluster 4 is Sp Vasa, a well-known germline marker that has been previously used to identify oogonia and early oocyte stages (Juliano et al., 2006; Yakovlev et al., 2010). Altogether, these data suggest that the Sp cluster 4 represents the oogonial stem cells and the early oocyte stages in *S. purpuratus*.

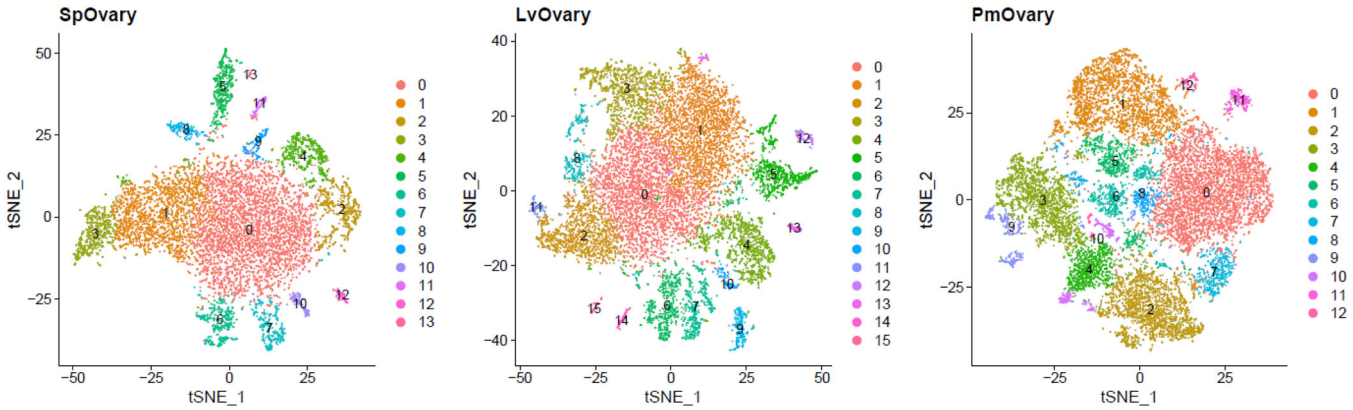

FIGURE 1 tSNE plots representing the scRNA seq data set of *Strongylocentrotus purpuratus*, *Lytechinus variegatus* and *Patiria miniata* ovaries. scRNA seq, single-cell RNA-sequencing.

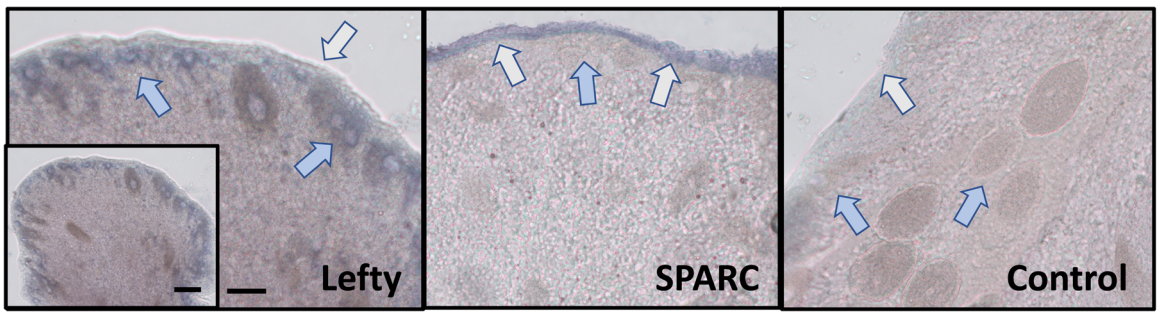

FIGURE 2 Lefty and SPARC transcripts are expressed in specific ovarian cell populations. In situ RNA hybridization of an ovary from *Strongylocentrotus purpuratus*, indicating the expression of Lefty in the early oocyte stages and SPARC in the ovarian epithelium. The ovary of sea urchins and sea stars consists of lobes of reproductive tissue. The outermost layer contains a double walled epithelium (the ovarian capsule) inside of which is the germinal epithelium of stem cells and early oocytes, evident by their proportionally large nucleus (germinal vesicle; blue arrow). As oocytes grow and mature, they are forced center ward through mixed populations of somatic accessory cells, to a common anastomosing lumen (not shown), leading to the gonopore on the adult epidermis where gametes are spawned. Blue arrows indicate young oocytes, and white arrows indicate the ovarian capsule. Bar in Lefty insert figure = 80 micrometers, and in main Lefty figure = 40 micrometers.

The Sp cluster 4 can be subclustered to identify the germline subpopulations (Figure S2). Three subpopulations arise from this analysis. While most of the cells express Vasa and Sox4, the subcluster 0 is enriched in lefty and PRDM9 transcripts, suggesting that subcluster 0 represents the early meiotic oocytes. PRDM9 is a meiosis-specific histone lysine methyltransferase (Diagouraga et al., 2018). In contrast, subcluster 2 is enriched in transcripts coding for the polycomb group protein Pc and for Sox17, suggesting that this subcluster represents the oogonial stem cells. Polycomb group proteins are associated with pluripotency maintenance. For example, a subunit of the polycomb repressive complex 1, Phc1, activates Nanog transcription in pluripotent cells (Chen et al., 2021).

Vasa is also found enriched in cluster 4 of the Pm ovary, suggesting that Pm cluster 4 also represents the oogonial stem cells and early oocyte stages (Figure S1). Subclustering leads to three subpopulations including Pm subcluster 0 that shows an enrichment of transcripts coding for RTF1. The RNA polymerase-associated protein RTF1 is a component of the PAF1 complex (PAF1C) that regulates transcription. PAF1C has been shown to maintain mouse embryonic stem cell renewal (Ding et al., 2021).

Lv ovary cluster 13 shows an enrichment of germline gene markers such as nanos and piwi, suggesting that this cluster corresponds to the germ cells. Two subpopulations were identified: the early meiotic oocytes (Lv subcluster 0 enriched in genes such as boule and HORMA domain-containing protein 1), and the oogonial stem cells (subcluster 1: enriched in transcripts such as geminin and PRDM10). Boule belongs to the Daz family (Deleted in Azoospermia that also includes Dazl and Daz) that plays important function during meiosis (Yang et al., 2020). In mouse, Hormad1 acts with the synaptonemal complex to coordinate meiotic progression (Daniel et al., 2011). PRDM10 plays a critical role in mouse embryonic stem cell maintenance by regulating the transcription of a key translational factor Eif3b (Han et al., 2020).

FoxL2 (Forkhead box transcription factor L2) is expressed by ovarian somatic cells in mammals and is known to be essential for ovarian development. In humans, a mutation in FoxL2 is associated with BPES (Blepharophimosis Ptosis Epicanthus Inversus Syndrome) that includes eyelid malformation and premature ovarian failure in women (De Baere, 2001). FoxL2 expression is enriched in the granulosa cells, the cells that surround and support the developing oocyte in mammalian ovaries (Zhou et al., 2022). These cells correspond to the follicle cells in echinoderms. In our data sets, FoxL2 is enriched in Sp cluster 6, in Lv cluster 4 and in Pm cluster 2 (Figure S3). These three cell states are also enriched in transcripts coding for yolk proteins such as MYP or Vitellogenin related proteins. Vitellogenin has previously been shown in the sea star to be expressed in the follicle cells of the ovary (Zazueta-Novoa et al., 2016), suggesting that Sp cluster 6, Lv cluster 4 and Pm cluster 2 represent the follicle cells.

Sp SPARC mRNA is enriched in clusters 5 and 10 of the Sp ovary (Figure S4). In humans, SPARC is a component of the extracellular matrix and is detected in the ovarian surface epithelium; its expression is regulated by progesterone and its abundance is altered in ovarian cancer (Mok et al., 1996). By in situ RNA hybridization, Sp SPARC is also detected in the sea urchin ovarian epithelium suggesting that its expression is conserved between sea urchin and human ovaries (Figure 2). In Lv, SPARC is detected in clusters 4 and 5, suggesting a broader expression than in Sp ovary; Lv SPARC seems to be detected not only in the epithelium (Lv cluster 5) but also in the follicle cells (Lv cluster 4). This transcript is expressed even more ubiquitously in the sea star Pm ovary (Figure S4). Collagens are among the most abundant proteins in extracellular matrix and here we found that the collagen alpha-1(V) chain (6Afcol) is enriched and co-expressed with SPARC in Sp cluster 5. The 6Afcol collagen transcript is mostly detected in Lv cluster 5 and Pm cluster 3, suggesting that these Lv and Pm cell states could represent the ovarian epithelium in these species. In the Sp sea urchin, cluster 5 also expresses transcripts important for hormonal regulation such as the steroid hydroxylase (CYP17A1), a key enzyme in the steroidogenic pathway, identifying the cell state of putative hormonal regulators to maintain Sp ovaries (Figure S5).

With this resource, investigators will be able to better test gene expression in the echinoderm ovaries, to identify the similarities and differences of the ovaries throughout evolution using multi-species integrated data sets (Tarashansky et al., 2021) and to get one step closer to solving the mystery of how the oogonial stem cells are differentially maintained in animals. In the future, drug inhibitors of ovarian cell communication as well as CRISPR/Cas9 knock-out and knock-in of genes of interest directly in explant ovary cultures will be critical to test how signaling pathways and gene expression affect the different cell states of the ovary and correspondingly in senescing, or nearly immortalized gamete production. Recent progress in animal husbandry in echinoderms (Hodin et al., 2019; Vyas et al., 2022; Wessel et al., 2021) now also facilitates transgenerational studies to address these important issues in reproduction.

AUTHOR CONTRIBUTIONS

Nathalie Oulhen: Conceptualization; investigation; funding acquisition; writing—original draft; methodology; validation; visualization; writing—review and editing; formal analysis; data curation. **Shumpei Morita**: Conceptualization; investigation; methodology; writing—review and editing. **Cosmo Pieplow**: Investigation; validation; data curation. **Thomas M. Onorato**: Conceptualization; investigation; writing—review and editing; data curation. **Stephany Foster**: Conceptualization; investigation; methodology; validation; writing—review and editing; data curation. **Gary Wessel**: Conceptualization; funding acquisition; writing—original draft; validation; writing—review and editing; formal analysis; project administration; supervision; resources.

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DATA AVAILABILITY STATEMENT

The sequencing files and gene expression matrices for the single cell RNA seq analysis presented here have been deposited at NCBI GEO: GSE246430.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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