The Drosophila ovary produces three follicle waves similar to those in mice

Wayne Yunpeng Fu[1,2,3] and Allan C Spradling[1,2,3,4]

[1]Carnegie Institution for Science

[2]Howard Hughes Medical Institute

3520 San Martin Dr.

Baltimore, MD 21218

[3]Department of Biology

Johns Hopkins University

[4]correspondence: spradling@carnegiescience.edu

ABSTRACT

Ovarian follicles in some species develop in subgroups known as "waves." Mouse fetal ovaries generate wave 2 follicles that form an ovarian reserve and wave 1.5 follicles that support early fertility. Wave 1 mouse follicles undergo atresia in juvenile animals that amplifies thecal cells and androgen production during puberty. We identified three similar follicle waves in Drosophila by tracking >6,000 individual lineage-labeled clones from individual germline or somatic cells in developing pupal ovaries. 40 of ~100 larval primordial germ cells (PGCs) associate with forming germline stem cell (GSC) niches. After a delay of >30 hours during early pupal stages, GSCs begin division and ultimately produce cysts that ensure a lifetime supply of wave 2 eggs. Wave 1.5 follicles develop directly from many of the remaining PGCs to generate ~20 cysts and all 80 ovarian follicles present at adult eclosion. Adult-like but unstable follicle stem cells (FSCs) generate most follicle cells for wave 1.5, with a contribution from the basal stalk. Unexpectedly, a true wave 1 subpopulation arises from relatively few posterior PGCs. Drosophila wave 1 germ cells increase rapidly to 250-350 cells by dividing and then forming germline cysts. They subsequently interact with somatic swarm cells before undergoing an atresia-like process and exiting the ovary. Our results argue that production of specific ovarian follicle waves, including an initial wave that undergoes atresia during adult tissue development, has been conserved during evolution.

Keywords

Ovarian follicle wave, germ cell, steroid hormone, evolutionary conservation

SIGNIFICANCE

Here we show that Drosophila oogenesis takes place in three follicle waves matching the follicle waves recently described in mice. Previously, ovaries were thought to generate one follicle type dedicated to reproduction and follicles undergoing atresia were considered defective. By better documenting pupal ovary development at single-cell resolution, we demonstrate that each wave derives from distinct PGCs and interacts with different somatic cells. The previously unknown Drosophila wave 1 follicles, amplify rapidly from ~10 to 300 germ cells, break from the ovary and release their contents into the hemolymph coincident with the pupal ecdysone peak. Follicle wave conservation argues that ovaries produce multiple germ cell types that evolved for distinct roles, including the promotion of adult development.

INTRODUCTION

Some species generate subclasses of ovarian follicles, in spatially or temporally separated groups known as waves (review: DeFalco and Capel, 2009; Eppig and Handel, 2012). For example, the *C. elegans* hermaphrodite gonad initially produces a wave of sperm, and then switches to making oocytes (review: Pazdernik and Schedl, 2013). Mammals such as mice generate wave 1 ovarian follicles that grow immediately at birth in the central, nutrient-rich medullar region, while 95% of follicles, wave 2, reside in the cortex and become quiescent as the ovarian reserve (Mork et al. 2012; review: Rotgers et al. 2018). A recently described third wave of primordial follicles, termed "wave 1.5," develops at the medulla/cortex boundary, arrests for ~ 2 weeks, and produces most the first ovulated oocytes rather than wave 1 (Meinsohn et al. 2021; Yin and Spradling, 2025). Wave 1 follicles generate few if any mature oocytes. They undergo a process classically termed "atresia" (Byskov, 1974), in which most oocytes and granulosa cells turn over, while thecal cells proliferate and interstitial gland cells are recruited. These hormone-producing cells reorganize into a gland-like region that synthesizes mainly androgenic steroids (Yin and Spradling, 2025) during a sensitive period of embryonic development (Trova et al. 2021).

Drosophila oocytes develop from germline stem cells (GSC) located in a structure known as the germarium at the tip of developing follicle strings called ovarioles (Figure 1A). 2-3 GSCs reside in a niche near the germarium's terminal filament (TF) where they give rise to daughters termed cystoblasts (CB) that initiate 16-cell cyst formation (Figure 1B). Two follicle stem cells (FSCs) reside midway through the germarium (Figure 1C) where cysts switch from an early covering of squamous escort cells (ECs) to follicle cells (Margolis and Spradling, 1995). FSCs also reside in a niche (Nystul and Spradling, 2007; Sahai-Hernandez and Nystul, 2011; Rust and Nystul, 2020).

FSC daughters proliferate over a two day period to cover each cyst, allowing the resulting ovarian follicle to exit the germarium.

The development of the adult Drosophila ovary has been well characterized (King et al. 1968, King, 1970; Sahut-Barnola et al. 1995; Forbes et al. 1996; Godt and Laski, 1995; Couderc et al. 2002; Zhu and Xie, 2003; Song et al. 2007; Gilboa and Lehmann, 2006; Panchal et al. 2017; Sladina et al. 2020). The embryonic ovary acquires primordial germ cells (PGCs) which proliferate throughout larval development in association with somatic intermingled cells (Wieschaus and Szabad, 1979; Zhu and Xie, 2003; Gilboa and Lehmann, 2006; Ganz et al. 2011; Rosales-Nieves et al. 2025). Construction of ~16 ovarioles starts during the third larval instar (L3) under control of the steroid hormone ecdysone (Figure 1D) with the assembly of terminal filaments (TFs) (Sahut-Barnola et al, 1995; Godt and Laski, 1996; Sahut-Barnola et al, 1996). Before TF completion, a large population of somatic swarm cells migrate from the anterior to the posterior region of the ovary (Couderc et al. 2002). GSC niches are finished with the addition of cap cells (Forbes et al. 1996; Panchal et al. 2017) beginning at 0h (hours) after puparium formation (APF) mediated by Notch and ecdysone signaling (Song et al. 2007; Yatsenko and Shcherbata, 2018; see Figure 1E). Only a minority of the larval PGCs are required to fill these niches, but the time when they become active GSCs has not been determined. The fate of the additional PGCs is unknown, but they are thought to develop into the first follicles (Asaoka and Lin, 2007), suggesting they constitute a distinct follicle wave resembling mouse wave 1.5. However, no candidate for a Drosophila wave 1 has been described.

Many additional uncertainties remain regarding the pupal development of ovarian follicles. Anterior sheath cells migrate over each terminal filament and encapsulate PGCs to begin ovariole formation, but how initial follicles produced from PGCs acquire a layer of follicle cells is unknown.

The adult FSC niche utilizes escort cells, likely descended from intermingled cells, located adjacent to the FSC or nearby (Sahai-Hernandez and Nystul, 2011). Whenthe approximately 40 ECs in the germarium terminally differentiate and when functional niches and FSC activity begin during pupal development are unknown. Swarm cells are proposed to activate CBs to produce cysts by torso signaling at -20h APF (Banish et al. 2021). A few PGCs express Bam and begin cyst production just before the larval-pupal transition (Zhu and Xie, 2003; Figure 3D). However, the source of the ecdysone pulses that control pupal development are uncertain. The prothoracic gland that produces larval ecdysone pulses (Figure 1D) turns over during early pupal development, leaving the origin of the major peak at 24h APF uncertain (Scanlan et al. 2023). Thus, more detailed study of early pupal development has the potential to clarify many important aspects of ovary formation in additional to clarifying Drosophila follicle waves.

Multiple aspects of oogenesis are proven to be highly conserved in evolution across both vertebrate and invertebrate groups (Tootle, 2013; Bergero et al. 2021; Spradling et al. 2022; Berg et al. 2024; Zhu et al. 2024; Schick et al. 2025). This motivated us to search for follicle waves in Drosophila, focusing on the "extra" PGCs not needed to form GSCs. Lineage labeling represents a powerful way to characterize ovarian development and follicle waves. Our study helps to clarify many of the previous uncertainties in ovary formation. In particular, we find that Drosophila has three follicle waves corresponding to all three mouse waves. The Drosophila analog of wave 1 turns over during 22-26h APF and we propose that it contributes to production of the 24h APF pupal ecdysone peak.

RESULTS

Analyzing ovarian follicle waves by lineage tracing

To obtain a clearer picture of follicle waves, we used a background-free lineage labeling system based on heat shock-induced FLP-mediated recombination between chromosome 2L homologs (Harrison and Perrimon, 1994; Margolis and Spradling, 1995; Figure 1D). After a heat shock, any mitotically cycling cell has a chance to recombine, but only a small, random fraction of candidate cells actually undergo recombination, an important feature that allows the progeny of individual cells to be mapped. Moreover, recombination causes only one of the two daughter cells at the next mitosis to become heritably marked, another property enhancing information yield especially with respect to forming cysts (Figure 1B,D). The short period when a larva stops moving, and begins to tan its puparium cuticle (0h APF) allows induced clones to be precisely timed (see Methods). We induced clones at 4-hour intervals between -2h and 14h APF and analyzed them at two periods. The greatest cellular resolution came from scoring lineage-labeled ovaries after eclosion (see Figure 1A,C), but some lineage-marked pupal ovaries were also scored 24h after clone induction. Our experiments used developmental staging accurate to $\pm$2h. The uncertainly was sometimes further reduced by only collecting a cohort that had entered puparium formation in a time window of $\pm$1h. All times APF represent window midpoints.

All of the ovarioles were analyzed for somatic cell clones in both germaria and follicles, whereas we found it sufficient to analyze germ cells in only about half of each group due to low inter-clone variation. Tables 1 and 2 summarize the clones and Supplemental Table S1 records the individual labeling characteristics of all 2,270 germaria and their ovarioles containing 1,637 labeled wave 2 follicles and 1,476 direct-developing wave 1.5 follicles.

Characterization of wave 2 follicles

We first analyzed the lineage labeling of GSCs that support "wave 2" follicle production throughout adult life. Marked GSC clones are easily recognized in newly eclosed ovaries by their location in the niche in the germarium (Figure 1C; Figure 2A-A') and association with adjacent marked progeny germline cysts. GSC clones with these properties increased by 50% at 6h APF and labeled thereafter at a frequency similar to adult GSC clones: 30% of ovarioles (Figure 2C, blue line). All GSCs underwent an average of just under 4 divisions in the ~110h before analysis time shortly after eclosion (Figure 2C, orange line).

Germaria containing 152 total GSCs (2.7 GSCs/germarium) among 346 ovarioles in our first -2h group (mean of 15.7 ovarioles per ovary) were fully analyzed. While slightly more germaria had multiple labeled GSCs than expected by chance, the increase was slight, indicating that symmetric division played a minor role in filling GSC niches in our experiments. Only about 7% of ovarioles with marked GSCs were fully labeled. Interestingly, overall about the same number of ovarioles contained at least 1 labeled GSC (221 total) as the number that contained a single labeled cyst 4-5 divisions downstream from unlabeled GSCs (199 total). We called these cystoblast (CB) clones (Figure 2B), and believe they result when a recombined GSC in a niche segregates the lineage maker to the CB at its first mitotic division, explaining why CB clones occur at similar frequencies as GSC clones and develop to the same extent as the oldest GSC progeny.

Characterization of direct developing follicles providing early fertility

The most common type of germ cell labeling pattern corresponded to single cysts with 8 or fewer labeled germ cells. In newly eclosed flies these cysts had usually developed into follicles, although some were still in the posterior germarium (Figures 1A, 2D, Table S1). We named them "wave 1.5" follicles after the special wave of mouse follicles that give rise to the earliest offspring

(Yin and Spradling, 2025). Both their labeling pattern and location show that wave 1.5 clones arise from more posterior larval PGCs that developed directly into cysts, explaining the reduced clone size (see Figure 1B). Rarely, follicles downstream from wave 2 clones contained 16-labeled cells (Figure 2E). These likely result from rare symmetric division of PGCs before they undergo cyst formation. Wave 1.5 clones arose 2.5-3 times more commonly than GSC or CB clones, suggesting they derived from a larger pool of PGCs. Marked wave 1.5 follicles randomly occupy follicle positions in newly eclosed fly ovarioles, since their distribution among the 5 positions (S1, S2-3, S4-5 S6-7 and S8-9) follows a binominal distribution. Comparing the labeling pattern of wave 1.5 follicles from -2h to 14h (Figure 2F-G) revealed that wave 1.5 precursor germ cells progress through cyst development in a manner reflective of time elapsed during early pupal stage. Since a cyst or follicle has to belong to either wave 2 or wave 1.5 in an ovariole of an early eclosed fly, by subtracting wave 2 cysts we determined wave 1.5 makes up to 20 cysts and 80 follicles on average in an ovary immediately after eclosion.

A large, novel population of wave 1 germ cells turns over between 22h-26h APF

We carried out a developmental survey to pupal ovary development between -2h and 30h APF to supplement our inferences from lineage tracing (Figure 3A-J). We quantitated germ cells giving rise to wave 2 and wave 1.5 by counting individual germ cells plus cysts located within ovarioles as they become defined by the cellular migration and sheath formation (Figure 3K). The ovariole has surrounded its germ cells by 10h, however, the number of germ cells per ovariole continues to increase, rising from 8.5 to 10 by 18h due to the last PGC symmetric divisions. After this point, no further increase is seen up to 30h (Figure 3K).

Figure 3B-J shows a sequence of pupal ovarian germ cell development from -2h to 30h APF. A bam-GFP construct reports germ cells that have entered cyst formation, and Hts/1B1 antibody

identifies fusomes, diagnostic of cyst stage. Complete z-series were also recorded and consulted. Expression of Bam in germ cells in late larvae and early pupae before 6h APF was confined to germ cells in the center of the PGC population (Figure 3B,C). In 10h APF ovaries we noticed that vacuoles and gaps between cells had appeared involving germ cells (Figure 3E,M,N). These cells include GSCs directly adjacent to TFs (Figure 3N-N'). Vacuoles/gaps disappeared from the apparently uninjured cells within 4 hours (Figure 3F-G).

Beginning as early as 6h APF (Figure 3D), a subpopulation of germ cells were clearly migrating toward the posterior region of the ovary which is highly enriched in somatic swarm cells that moved there late in L3 (Corderic et al. 2002). Posterior somatic cells form a conical structure that protrudes at this time (Figure 3E-F). Germ cell posterior movement continued, producing two distinct groups (Figure 3F-G, L, arrows). The germ cells in the posterior further increase in number as they form cysts and by 18-22h APF they aggregate in a mass of 100 to 200 cells (Figure 3G-H, L; Figure 4D, orange line). Between 22-26h, all the posterior germ cells and many adjacent somatic cells break from the ovary posterior and enter the hemolymph (Figure 3I). We term these germ cells "wave 1" because they develop the earliest and undergo an atresia-like turnover process, akin to wave 1 follicles in the mouse (Yin and Spradling, 2025). All three waves are visible along the a-p axis in Figure 3H. By 30h APF, the ovary has completely lost wave 1 germ cells and its protruding posterior, appearing round in shape and to have been repaired (Figure 3J).

Rapid multiplication and clearance of wave 1 germ cells

We analyzed lineage-labeled germ cells directly in young pupal ovaries 24 hours after clone induction to gain further insight into wave 1 multiplication. Analyzing clones induced at -2h and 2h APF in pupal ovaries 24 hours later showed that individual wave 1 clones typically contained multiple nearby cysts that were fully labeled (Figure 4A-C), indicating wave 1 precursor PGCs

expanded rapidly first by symmetric division and then by forming germline cysts with typical fusomes. The view facing the ovary posterior shown in Figure 4B-C reveals branched 8-16 cell fusomes in >10 remaining wave 1 cysts. We estimated the cumulative total number of wave 1 germ cells released by first determining that at 18h APF when PGC symmetric division had ceased, there were 22 wave 1 cysts of various sizes indicated by fusome structure (Figure S4A). At this time before cells began to be extruded, only 15% of cysts contain 16 cells (Figure 4D, blue line). However, at 22h and 26h APF the percentage of 16-cell cysts continued to rise and reached 75%, indicating continuous cyst division even though the total number of wave 1 cells started to fall due to ongoing cell extrusion (Figure 4D). We estimate that 60%-80% of the ~22 extruded cysts contain 16 cells, indicating that the in total between 250-350 wave 1 germ cells are expelled. Clusters of wave 1 germ cells and associated somatic cells break off in groups at the posterior (Figure 4E-G). Immediately before and as they exit the ovary, wave 1 germ cells become larger and more spherical in shape, changing their appearance in brightfeld; this coincides with reduced vasa expression (Figure 4H, H'). We examined 22-26h APF testes as well, and found a similar sequence of regional germ cell appearance changes (Figure I, I') followed by expulsion from the gonad (J-J"). In the testis, however, cells were lost from the sides of the gonad rather than the posterior, where germ cells are absent.

Origin of pupal follicle cells

In uninjured adult ovarioles, regardless of nutrition level, two long-lived FSCs ($t_{1/2}$ ~10 days) located at the r2a/2b junction coordinately supply follicle cells to newly forming follicles (review: Rust and Nystul, 2020). Daughters of both FSCs alternate (out of phase) to cover cyst surfaces when migrating along the ovariole or inward (Figure 5A; Nystul and Spradling, 2007). This produces a follicular monolayer sufficient for follicle budding in about 48 hours. In contrast, how wave 1.5

pupal follicles acquire follicle cells is poorly known. Electron microscopy (King et al. 1968) and our observations (Figure 1E') show that pupal ovarioles even by 48h lack an adult-like progression of cysts or an r2a/2b junction. Consequently, it was of interest to examine how FSCs arise and function during pupal development.

Lineage analysis scored at eclosion revealed very few FSC or follicle cell clones when induced prior to 6h APF (Figure 5B). However, FSC clones began to be observed frequently staring at 10h APF, and resembled adult clones in proliferation pattern (FSC-L class). These clones started at precisely the same location at the r2a/2b border described for adult clones (Figure 5A vs 5B). Moreover, as in adults, when we used 3D image stacks to quantitate the entire surface of the follicles, about 50% by area of the follicle cells on the surface of a given follicle were part of the FSC-L clones, leaving about 50% unmarked. This suggests that two FSCs with equal activity, as in adults, provide most wave 1.5 follicle cells.

However, FSC clones extending from the r2a/2b junction to the last follicle were rare. Unlike adult FSC clones, around half of all marked pupal FSCs did not remain in the niche for many divisions, they became lost either due to weak affinity or competition from the other competent FSC. This produces an FSC clone that extends a shorter fraction of the ovariole while lacking an active labeled FSC (FSC-M class, Figure 5C-D). Moreover, FSC-M clones do not start at a fixed position, because new follicles formed after replacement continued to develop using the unlabeled FSC and its progeny follicle cells.

Surprisingly, a class of short FSC clones (FSC-S) was also found, where an FSC at the r2a/2b junction produced follicle cells for only one or two downstream cysts (Figure 5E), suggesting it had been arrested and only became active shortly before eclosion. FSC-S clones also make up the majority of labeled FSC clones (Figure 6J). FSCs in the FSC-S clones are likely the cells that

replaced the FSCs lost during pupal stage making the FSC-M clones, since FSC-S clones usually only extend up to the end of germarium while FSC-M clones start at the end of germarium, the two patterns complementing each other.

In addition to FSC-derived clones, other patterns of follicle labeling were observed. The oldest wave 1.5 follicle at the end of each ovariole had a distinctly different pattern of labeling, not seen in other follicles. Terminal wave 1.5 follicles very frequently contain labeled follicle cells in a posterior patch and less often in other positions (Figure 5F-G). Basal stalk cells were previously reported to contribute to pupal follicle cells (Reilein et al. 2021), and we observed efficient clonal labeling of basal stalks at 10h APF (Figure 5K). Movement of basal stalk cells onto the posterior wave 1.5 follicle layer provides an attractive explanation for the bias such clones show toward the posterior region where the stalk attaches. Similar behavior is also documented in the adult ovarioles, where inter-follicular stalk cells are known to sometimes offload extra cells onto the adjoining follicle (Nystul and Spradling, 2010). Long basal stalks may donate multiple cells both labeled and unlabeled, contributing to the variable size and uneven shape of these patches.

Wave 1.5 follicles also manifested small developmental differences compared to adult follicles. They sometimes contained oversize polar cells, suggesting they polyploidize prematurely. The number and size of border cells clusters, which include the anterior polar cells, sometimes were enlarged, and delayed in migration (Figure 5H, arrow). Abnormalities of nurse cell size and number (8 or 32 cells) were also increased (Figure 5I-J).

Analysis of escort cell differentiation

Another attractive opportunity posed by the clonal analysis was to learn more about the differentiation of the germarium's permanent somatic cells, i.e. (Table S1, EC-FC). Escort cells (ECs), which play important roles in the GSC, FSC and differentiation niches (Kirilly et al. 2011;

Gao et al. 2019; Rust et al; 2020) were of particular interest. Figure 6A shows EC labeling by the enhancer trap line PZ1444. We scored six consistently recognizable EC types believed to be part of the GSC and FSC niches based on their spatial proximity, which are denoted EC1-EC6 (Figure 6A). An EC1 wraps the surface of each GSC, while behind it lies EC2 (Figure 6B-C,I). A median EC3 is also present between the GSCs (Figure 6D-E). Three ECs similarly surround both FSC niches (Gao et al. 2019; Rust et al. 2020). EC4 is on the surface of germarium and directly contacts the FSC (Figure 6G-H), more anterior to it is EC5 (Figure F,H). Another median EC type, EC6, is found between the two FSCs located internal in the germarium (Figure 6I). At least in some cases, EC6 cell membranes extend to an adjacent FSC (Sahai-Hernandez and Nystul, 2013).

Our results showed that both cap cells and escort cells were labeled at multiple times times between -2h and 14h, rather than displaying a narrow window indicative of terminal differentiation. The labeling frequency of EC1 and EC2, the most anterior ECs was higher than other ECs at -2h APF (Figure 6K). The FSC-associated EC4 also began to be labeled at -2h, and increased further along with EC5 (Figure 6L). Labeling of other ECs was also detected at lower frequencies (Figure 6M, ECo). We found multiple examples of double and triple EC clones (Figure 6B,H; Table S1). Given the low frequency of individual EC clones, these likely correspond to the labeling of common precursor cells. Cap cells are essential for normal GSC niche function, and they showed a major increase at 10h APF (Figure 6M). This suggested that it may take longer than has often been assumed for GSC niches to fully mature during early pupal development, and their completion may depend on the 10h ecdysone pulse. This might also explain the 10h peak for EC1, which are also a known part of the GSC niche.

DISCUSSION

Drosophila oogenesis starts with two GSC-independent follicle waves

Our experiments demonstrated that the long term GSC-based system of follicle production in Drosophila (wave 2) is preceded by two earlier follicle waves, much like in the mouse ovary. A larger number of PGCs than previously identified, about 160 by 10h, contribute to these waves. Approximately 40 PGCs on average produce GSCs, while 100 PGCs give rise to wave 1.5, and wave 1 is formed from initial posterior PGCs that divide 1-2 times to engender 22 wave 1 founder germ cells. The early Bam expression in medial PGCs (Figure 2B-D) likely reflects immediate cyst formation in posterior wave 1.5 PGCs which have the most demanding developmental schedule, causing some to possibly start in late L3. In contrast, wave 2 PGCs are still quiescent and posterior wave 1 PGCs are still dividing symmetrically before initiating cyst formation. Our findings reveal the different behaviors of the PGC groups that give rise to each of the three follicle waves.

The activation of GSCs is delayed after the onset of pupal development

We concluded that about 40 PGCs associate with the 16 GSC niches (2.7 per niche) based on microscopy of early pupal ovarioles, but additional observations showed that they remain quiescent for more than 30 hours. First, the small average GSC clone size of slightly less than 4 divisions by the time of eclosion (Figure 2C) supports early PGC dormancy. 96 hours of pupal development plus up to 12 hours after eclosion before ovaries were scored would be sufficient for 5-6 rounds of adult GSC division, consistent with a delay of >30h. Second, GSC division would increase the number of single germ cells and cysts per ovariole, but it remained constant until at least 30h APF (Figure 3K). Third, 2-4 single cells with round spectrosomes remained posterior to GSCs in ovarioles at 26h APF, indicating that not only wave 2 GSCs but probably also the last wave 1.5 CBs are temporarily dormant.

Thus, the long pause before GSC activation may simply be due to the time required for ~7 wave 1.5 cysts and follicles per ovariole to develop and move down the ovariole as they acquire a follicle cell layer. This delay in GSC activation may explain why evidence of niche development continued longer than previously expected. Clones marking two important niche components, cap cells and EC1 cells, increased significantly at 10h (Figure 6K,M), suggesting that niches continue to expand and may require the 10h ecdysone pulse to signal their maturation.

The ovary prioritizes rapid production of wave 1.5 follicles

Our studies argue that wave 1.5 follicles are intrinsically important, in contrast to the previous view that they simply represent the direct development of a few excess PGCs present to ensure that GSC niches will all be filled. The majority of PGCs generate wave 1.5 cysts, enough to form some nearly mature cysts and all the follicles present in each ovariole at eclosion. Wave 1.5 PGCs have the capacity to proliferate symmetrically to ensure this level of follicle production, as indicated by the marked wave 1.5 follicles with all 16 germ cells labeled (see Table S1). The time required to fully develop wave 1.5 delays wave 2 cyst production. What might be the special value of these follicles to Drosophila reproduction?

The wave 1.5 production program ensures that females can begin producing progeny with little delay, extending no more than one day after eclosion. This likely requires additional adaptations to allow wave 1.5 follicles to generate enough follicle cells to bud off from the germarium by 40h APF. The normal production of 80+ cells per follicle from only two precursor cells, one from each FSC (Margolis and Spradling, 1995) requires more time, about 48h in adults. Thus, the pupal ovary requires a significant pool of EC, FSC, and follicle cell precursors to support both germarium differentiation and rapid wave 1.5 follicle development. These precursors likely correspond to the intermingled cells and/or FSC precursors identified by scRNA-seq (Sladina et al

2020). At least 4 such precursors per follicle are probably used to speed the development of the most posterior 1-2 wave 1.5 cysts, explaining the rarity of adult-like clonal patterns on these follicles, and the frequent contributions from basal stalk cells.

However, the follicle cells enveloping the remaining wave 1.5 cysts and follicles are mostly produced by two FSCs that are located at the r2a/2b junction as in adults. This is somewhat surprising since early pupal ovarioles (Figure 1E'; King et al. 1968) initially do not match the reproducible organization seen in adult germaria (Figure 1C). The early pupal germarium does not organize cysts two across to create a typical region 2a or an identifiable r2a/2b junction. This may be due to the extended differentiation of FSC-associated escort cells (Figure 6L) whose maturation, like the GSC niche, appears to be stimulated at 10h (Figure 5A'). Early FSCs perhaps for this reason are less tightly bound and frequently replaced in the niche, corresponding to the FSC-M class of clones (Figure 5B-E; Figure 6J). Interestingly, the clonal patterns overall suggest that follicle cells for wave 1.5 and wave 2 follicle development come from different progenitors. In particular, we recovered some ovarioles that contained both GSC and FSC clones, which might reveal if FSC loss correlates with the switch from wave 1.5 to wave 2 follicles (Figure S4). Some of these suggest that FSC-S clones represent new FSCs whose appearance matches the time wave 2 cysts arrive at the r2a/2b junction. A similar switch in the somatic cells associated with different follicle waves is already known to take place between mouse wave 1 and wave 2 (Mork et al. 2012; Niu and Spradling, 2020). Therefore, it is likely that Drosophila wave 1.5 and wave 2 folliclles also have somatic cell coverage derived from. distinct progenitor populations.

Wave 1.5 oocytes may also be adapted to the limited environmental resources confronting the majority of Drosophila females. Wave 1.5 follicles are the first to mature and their yolk probably derives from larval nutrients stored in the fat body, which separates into individual cells that fill the

abdomen of newly eclosed flies and nourish their developing wave 1.5 follicles. In contrast, egg production from wave 2 follicles only begins when wave 1.5 are depleted, allowing them to take maximum advantage of rare sources rich in nutrients. Our precise characterization of these two follicle waves now allows experiments to look for and document molecular and physiological differences that may relate to these two environmental situations.

The defects we observed in a minority of the the most mature wave 1.5 are probably have little impact on reproduction. Many of these follicles were observed in a single ovary, and Drosophila females have checkpoints that allow unfit follicles to be turned over and the nutrients reused. The defect frequencies we observed need to be confirmed in wave 1.5 follicles in wild type flies. Although heat shocks like those used do not cause similar ovarian effects in adulthood, early pupal development may be particularly sensitive due to their atypical acquisition of follicle cells, including critical polar and stalk cells.

Drosophila has wave 1 cysts that turn over during pupal development

In the mouse ovary a small subset of follicles develop first in the nutrient-rich medulla and never undergoes a developmental arrest like other follicles. Instead, after growing rapidly during the first three weeks of juvenile life, wave 1 oocytes undergo atresia, in which oocytes and some somatic cells turn over while a subset of somatic thecal cells expand and generate a gland-like structure within the medullar region that synthesizes steroid hormones especially during the first week of puberty. These hormones may influence structure of tissues including the brain that are still developing during this time (Yin and Spradling, 2025).

Our finding of an early Drosophila germ cell wave producing 250-350 germ cells that turn over rather than maturing into eggs raises the question of whether it contributes in some manner to pupal tissue development. It is still unclear if wave 1 follicles are surrounded by somatic follicle cells like

those surrounding wave 1.5 and wave 2 germ cells. Wave 1 cysts do associate with swarm cells, the abundant, poorly understood cell type that migrates to the ovary posterior in the L3 larva. The Drosophila early pupal stage is equivalent in many ways with mammalian juvenile development, as this stage includes tissue remodeling, brain rewiring, and ends with the production of a fertile adult organism. The fact that this follicle wave escaped detection until now even in a well studied model organism, suggests that similar early waves in many other species may exist, and should be identified.

We propose that wave 1 Drosophila follicles, like their mouse counterparts, are involved by their interaction with surrounding swarm cells and extrusion into the hemolymph, in steroid hormone production at a key developmental transition point. We also observed evidence of similar-appearing testis germ cell extrusion during this interval. Extruded gonadal cells might be important in bringing about sex-specific differences during gonad development (Camara et al. 2019; Zhou et al. 2021). In the mouse, wave 1 follicles reorganize into a gland-like structure highly expressing androgen biosynthetic genes and proteins (Yin and Spradling, 2025) while the brain continues to remodel in puberty. Just as in mouse, alterations in the Drosophila brain that prepare for adulthood are also taking place during the first two days of pupation. From 6h to 20h APF, neurons in the Drosophila mushroom body are undergoing ecdysone-mediated pruning (Boulanger et al. 2011) prior to the establishment of new adult projections. This includes the 10h period when pupal germ cells undergo a short period of dramatic vacuole formation (Figure 3E, L, M). Cell migration and regrowth of neurons continues in the brain until at least 36h APF (review Yaniv and Schuldinger, 2016). Many other adult tissues including derivatives of the genital imaginal disc such as the spermathecae and oviducts are undergoing sex-specific growth and remodeling as well (Shen and Sun, 2020; Berg et al. 2024).

There has been a long-standing mystery regarding how the large adult ecdysone peak is produced at pupal-adult apolysis that peaks about 24h APF (Scanlan et al. 2023). However, gene expression studies make it unlikely that pupal ovarian cells synthesize ecdysone directly (Sladina et al. 2020). The most likely model involves the uptake and storage of ecdysone, a process that takes place during embryogenesis. However, a tissue involved in ecdysone release during pupal development has remained unknown, although eclosion muscles have been proposed (Zhang et al. 2024). Our further studies on this question will be presented elsewhere.

Three follicle waves may have been conserved between mouse and Drosophila

The general similarity of the three Drosophila follicle waves described here to those found in the mouse ovary (Yin and Spradling, 2025) were surprising. At the very least, this shows that follicle waves are a widespread phenomenon in diverse animals. Multiple differences also exist, such as the use of stem cells or follicle quiescence to sustain fertility, or the production of about 110 wave 1.5 follicles in Drosophila but more than 400 in mice. One of the most interesting differences between Drosophila wave 1 and its possible counterpart in mammals is the more direct participation of a substantial number of germ cells in the process leading to their exit from the ovary. It is more common for somatic cells to take on the major role, like thecal cells in mouse wave 1. This suggests that Drosophila germ cells participate directly in wave 1 function, whereas in the mouse, wave 1 oocytes turn over at an early stage.

Reproductive strategies are subject to strong selection and change markedly between animal groups. However, the mechanisms for generating specific subgroups of follicles may have been conserved despite changes in how they broadly promote reproductive system development, as well as produce gametes for both early and sustained gamete production. By better defining Drosophila

follicle waves it should now be easier to test their functional importance and search for evidence of evolutionary conservation.

MATERIALS AND METHODS

We describe in the Results section the methods used to carry out this study based on established techniques (Harrison and Perrimon, 1993; Margolis and Spradling, 1995, Marianis and Spradling, 2013) and SI Appendix, References]. Using low clone induction frequencies, analyzing a substantial number of clones and photographically recording high quality images of ovarioles that reveal cellular architecture at single cell resolution were keys to success. Detailed protocols and materials information are given in SI Appendix, Supplemental Methods.

DATA AVAILABILITY

Complete results from the lineage studies are included in the paper and Supplemental material.

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FIGURE LEGENDS

Figure 1. Strategy for analyzing ovarian follicle waves.

A-B: samples of lineage analysis after ovary development to eclosion. A. Example of an ovariole. One wave 1.5 follicle clone labels 8 (4 visible in the image) of the 16-cells in the germline cyst within a single developing Stage 8 follicle of one ovariole isolated from a newly eclosed fly ovary. B. Lineage labeling system: heat shock (ΔT) activates a constitutive marker gene (green) in one daughter cell of the next round of mitosis. C. The germarium from a newly eclosed fly that was heat shocked at 12h APF. One GSC was labeled (GSC, green) along with its 4 progeny cysts. One GSC remains unlabeled, along with its progeny cysts. Other cell types: TF--terminal filament, CC--cap cells, EC--escort cells (specific ECs are numbered), FSC--follicle stem cell, fc--follicle cell, spectrosome and fusome (magenta). r2A, r2B are germarium regions, S1, S2 are follicle stages. D. Diagram shows the cyst formation trajectory from symmetrically dividing GSC/PGC, to cyst-forming cystoblast (CB) which undergoes 4 rounds of mitotic division to eventually construct a 16-cell cyst (CC), consisting of 1 oocyte (O) and 15 nurse cells (NC). The number of labeled cyst cells expected if activation takes place at the indicated stages is shown below: "clone size". E. Timeline of Drosophila development and ecdysone expression during the third larval instar and pupal stage (modified from Scanlan et al. 2024). Puparium formation occurs coincident with a major peak of ecdysone at 0h APF (after puparium formation). A small peak at 10-12h APF corresponds to pupariation and a broader pharate-adult peak reaches its maximum about 24-28h APF. E'. Images of germarium structure from 30h and 40h APF pupal ovarioles showing the absence of an adult configuration of cyst development.

Figure 2. Documentation of wave 2 and wave 1.5 by lineage tracing.

A. Germarium from a newly eclosed fly heat shocked at -2h APF. One of its two GSCs is labeled (green) and has generated a lineage of 3 progeny cysts (arrowheads) while daughters of the other GSC are unlabeled. A' Ovariole with all GSCs labeled, where all wave 2 cysts from GSCs are labeled green and therefore all unlabeled cysts and follicles are wave 1.5. B. An ovariole showing a cystoblast (CB) clone identified by all 16 of its germ cells being labeled. Its position in region 2b (R2b) corresponds to the first unlabeled cyst in A, A'. C. Summary diagram of wave 2 GSC clones induced at the 5 time points studied. The frequency of clone labeling (blue) rapidly increased at 6h APF and remains at around 30% of ovarioles. The number of divisions the GSCs have undergone (orange) stayed uniform around 4 cysts/GSC across the 16hr duration. D. Ovarioles with unlabeled germaria showing wave 1.5 follicles with 8/16 labeled cyst cells (5 visible), or 4/16 (2 visible) labeled. E. An ovariole showing one 16/16 fully labeled wave 1.5 follicle at stage 4-5, while the less mature wave 1.5 follicle at stage 1 in the same ovariole is only 8/16 labeled. F-G. Summary diagrams showing the labeling status of germ cells in wave 1.5 follicles following induction at -2h (F), or 14h (G).

Figure 3. Time course of pupal germ cell development in three follicle waves.

A. Diagram of a 10h APF pupal ovary oriented as in panel images, anterior to the left and posterior to the right. The wave 2 arrow points to PGCs that become GSCs, wave 1.5 arrow points to PGCs that directly undergo cyst division inside ovarioles, and wave 2 arrow points to PGCs that localize towards the posterior. B-J. A time sequence of pupal ovaries from -2h APF to 30h APF stained for germ cells (Vasa, red), forming cysts (bam-GFP, green), fusomes and somatic cell membranes (Hts/1B1, magenta) and nuclei (DAPI, blue). Arrowheads in D indicate newly formed ovarioles where ovariolar germ cells are separated by the sheath, a feature not observed in earlier time points

(B, C). Yellow arrows in F, G show the posterior movement of wave 1 germ cell precursors, which develop into cysts, aggregate (H) and leave the ovary along with a large number of posterior somatic cells (I). White arrows in E, H, I point to vacuoles devoid of antibody labeling that appear around germ cells. The distinction of 3 waves is outlined in H. K. Quantification of total single germ cells and cysts per ovariole from -2h to 30h APF. Error bars represent standard deviation of average across ovaries. Ovary age at analysis is indicated in hours after puparium formation (APF). L. A stacked image of 10 Z-series planes to show the large aggregation of all wave 1 germ cells into a disk-shaped mass in the posterior (arrow) of a 22h APF ovary. M-N. 10h APF germ cells magnified to show vacuoles and gaps between cells.

Figure 4. Rapid proliferation of ovarian wave 1 germ cells and cysts.

A. Pupal ovary lineage labeled at -2h APF and analyzed at 22h APF. Wave 1 germ cells at the posterior (p) include lineage labeled cysts with fusomes (A', Hts/1B1 channel). A few smaller cysts were labeled within the ovarioles. B. A pupal ovary labeled as in A, but viewed from the posterior to reveal 14 wave 1 cysts, all with fusome (B'); 4 cysts are labeled in all cells. Yellow arrow indicates site where wave 1 germ and somatic cells have broken off the ovary. C. Pupal ovary labeled at 2h APF and analyzed at 26h APF. A large segment of ovary somatic cells have separated (upper right). C' shows that all 4 cysts have 16-cell fusomes, 3 are lineage marked in all cells. D. Quantification of wave 1 cysts and germ cells (orange) from the time they start to become spatially separated from the other 2 more anterior waves (14h APF) until the time they have all extruded from the ovary (30h APF). Points on dashed lines are cumulative total wave 1 germ cells ever produced discounting loss from extrusion and considering the continuous cyst amplification, as shown by the constant increase of 16 cell-cyst percentage out of all cysts in wave 1 (blue). E. Pupal ovary labeled

at 2h APF and analyzed at 26h APF showing significant germ cell extrusion from the posterior, among which two large clusters are lineage positive. F-G. 22h APF bam-GFP fly line pupal ovaries showing germ cell extrusion similar to that seen in lineage tracing fly line ovaries. H-H'. 22h APF ovary with clear vacuoles embedded in the ovary next to wave 1 germ cells. H' is the brightfield image of the same ovary showing morphology of the vacuoles, indicated by arrows. I. A 22h APF testis with a large cluster of dying germ cells. I' shows a magnified view of the dying germ cell cluster under brightfield, revealing the morphological changes of those cells that makes them distinct from surrounding testical tissue. J. A 22h APF testis showing significant amount of cell loss on the upper left corner. J'-J". Magnified images of J. showing dying germ cells that still have some remnant vasa expression.

Figure 5. Adult-like FSCs and additional progenitors produce wave 1.5 somatic follicle cells.
A. Image of an adult ovarian FSC lineage-labeled (green) as in earlier experiments, showing its location in an ovariole at the r2a/2b junction. Two green FSC daughters can be seen at the top that are migrating along above the lens shaped cyst (DAPI, blue). The one green cell on the bottom is another FSC daughter cell. A' shows the frequency of FSC clone induced as a function of induction times in hours APF (blue). Shown also is the percentage of ovarioles with a follicle cell clone (orange). B. A similar lineage-labeled FSC in a pupal ovariole marked at 10h APF and analyzed 4 days later at eclosion. A clone encompassing about 50% of the follicle cells is seen extending over two region 2B cysts and onto the first 4 wave 1.5 follicles. C. A similar FSC-like clone in which the labeled FSC was replaced with an unlabeled FSC after the clone has covered parts of 4 sequential

follicles. D. A clone whose FSC was lost earlier leaving marked follicle cells on the last 3 follicles. E. A short FSC clone generated at 14h APF that by eclosion has only extended two r2B cysts. F. A final wave 1.5 follicle at stage 10 showing a labeled follicle cell clone at its posterior. G. The two last wave 1.5 follicles in an ovariole labeled at 6h APF, showing follicle cell clones, which are only present on these follicles. H. A final wave 1.5 follicle now at stage 9 that was lineage labeled at 14h and contains a follicle cell clone which extends to the previous follicle. The border cell cluster at the posterior is mosaic (magnified in the box below) with 3 marked cells. Other examples show the large size of the polar cells in these clusters and the presence of 3-4 labeled cells. I-J. Ovarioles of newly eclosed flies showing abnormal cyst size of 8 cells and 32 cells in the last two wave 1.5 follicles, and the disproportionate polyploidization of several nurse cells. K. A 10h APF ovary induced for lineage and analyzed at 34h. Wave 1 has been expelled and the ovarioles have developed large basal stalks that show dispersed labeling of individual somatic cells.

Figure 6. Lineage labeling of somatic cells to identify escort cell (EC) differentiation.
A. A germarium stained to mark the escort cells (EC) using the enhancer trap line PZ1444. Two GSCs (unlabeled) are seen at the front associated with cap cells (arrowhead). In addition, 8 specific ECs are labeled and indicated with arrowheads and assigned corresponding names. B-I show germaria following clonal marking to identify EC-specific clones.. B. A germarium with two labeled EC1 cells (arrows). C. An EC2 clone. D. An EC3 clone. A cap cell clone is also shown. E. An EC3 clone. F. An EC5 clone is shown. G. An EC4 clone. H. Two adjacent labeled ECs, E5 and E4 are located directly adjacent to an unlabeled FSC. I. A triple clone that labels EC1, EC2 and EC6. J. Relative frequency of ovarioles that contains an FSC-L, FSC-M, or FSC-S clone, respectively. K. Frequency of ovarioles with clones of ECs surrounding GSCs (EC1-3). L. Frequency of ovarioles

with clones of ECs next to FSCs (EC4-6). M. Clone frequency of cap cells and ECs other than EC1-6 (EC other), contacting neither GSCs nor FSCs. Both peak at 10h APF corresponding to the pupal ecdysone pulse. Y-axis across J-M shows frequency per 100 ovarioles.

Tables

Table 1. Germ cell clones

	GSC		R1	R2A	R2B		stage 1		stage2-3		stage4-5		Stage 6-7		Stage 8-9		Stage 10A	
time	N	P	P	P	4-8	16	4-8	16*	2-8	16	2-8	16	1-8	16	1-8	16	1-8	16
-2h-1	82	70	122	109	6	64	25	13	37	6	34	5	59	4	60	1	2	0
-2h-2																		
2h-1	74	59	108	132	3	78	25	11	51	11	65	7	65	3	81	0	67	0
2h-2																		
6h-1	43	37	70	60	2	36	11	2	15	2	28	1	21	1	48	0	49	0
6h-2																		
10h-1	83	65	116	101	18	71	37	7	51	5	56	4	88	0	116	1	24	0
14h-1	59	45	73	76	17	48	27	6	39	5	56	3	85	1	107	0	1	0
14h-2																		
14h-3																		
Total	**341**	**276**	**489**	**478**	**46**	**297**	**125**	**39**	**193**	**29**	**239**	**20**	**318**	**9**	**412**	**2**	**143**	**0**

w2	1637

w1.5	1476

*includes GSC,CB and (rare) post-mitotic w1.5 PGC clones;

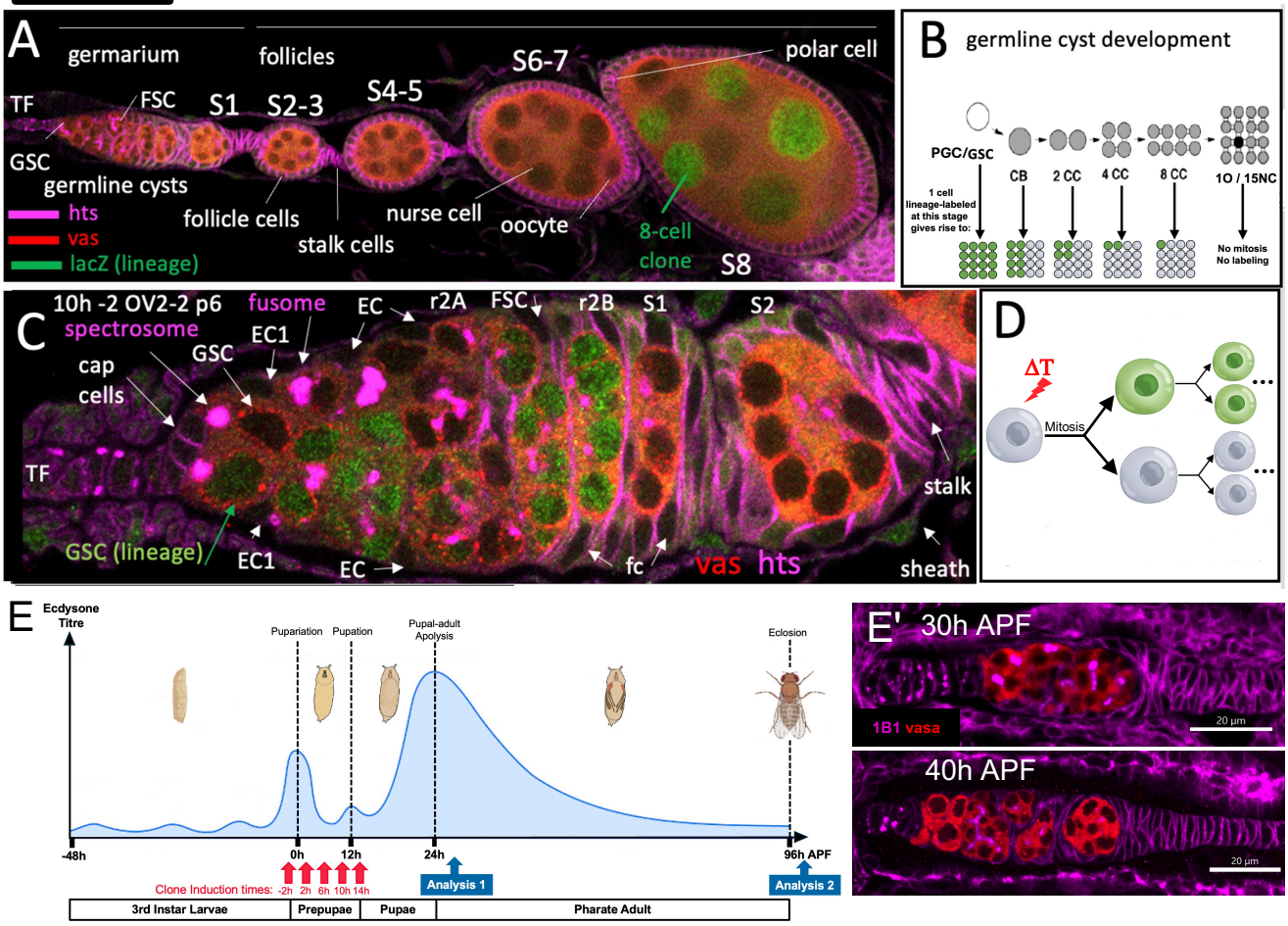
Legend. Numbers of lineage marked cysts and follicles labeled by lineage marking at the time in the first column as described in the text +/- 2 hours. Remaining columns (R to L) are numbers of marked cyst and follicles by developmental stages within ovarioles (R = region). N= unlabeled GSCs along with P = labeled GSCs. For germ cells, only one replicate group is fully quantified due to low level of variation. bottom; w2 = wave 2 w1.5 = wave 1.5.

Table 2. Somatic cell clones

time	Ovaries	Ovarioles	TF	1CC	2CC	1EC	2EC	4EC	FSC-S	FSC-M	FSC-L
0h-1	**22**	**346**		**1**		**2**	**2**	**1**	**6**	**5**	**5**
0h-2	**18**	**275**		**3**		**2**	**3**	**1**	**1**	**2**	**2**
4h-1	**20**	**303**	**2**	**6**	**1**	**5**			**3**		**2**
4h-2	**14**	**214**		**3**		**4**	**5**		**5**	**1**	**1**
8h-1	**8**	**129**	**1**	**1**		**4**			**3**		**1**
8h-2	**21**	**320**	**2**	**5**	**1**	**12**	**4**	**2**	**2**	**3**	**3**
12h-1	**14**	**224**	**10**	**26**	**9**	**24**	**11**	**17**	**16**	**6**	**10**
16h-1	**10**	**164**		**12**	**2**	**10**	**2**		**10**	**11**	**1**
16h-2	**7**	**111**		**5**		**7**	**5**	**1**	**3**	**5**	**6**
16h-3	**12**	**184**		**14**	**1**	**23**	**14**	**1**	**9**	**7**	**7**
Total	**146**	**2270**	**15**	**76**	**14**	**93**	**46**	**23**	**58**	**40**	**38**

Legend. First two columns: number of ovaries and ovarioles scored in the indicated group. The remaining columns (L to R) quantify each type of somatic cell clone. TF: terminal filament, 1CC: clone with 1cap cell, 2CC: clone with 2 cap cells, 1EC: clone with 1 EC, 2EC clone with 2 ECs; 4EC clone with 4 ECs; FSC-S: number of clones with short FSC clone (see text); FSC-M: number of clones with middle FSC clones (see text); FSC-L: number of clones with long FSC clones (see text).

Fig.1


A
germarium
follicles
polar cell
TF
FSC
S1
S2-3
S4-5
S6-7
GSC
germline cysts
hts
vas
lacZ (lineage)
follicle cells
stalk cells
nurse cell
oocyte
8-cell
clone
S8
B
germline cyst development
PGC/GSC
CB
2 CC
4 CC
8 CC
1O / 15NC
1 cell lineage-labeled at this stage gives rise to:
No mitosis
No labeling
C
10h -2 OV2-2 p6
spectrosome
fusome
EC
r2A
FSC
r2B
S1
S2
GSC
EC1
cap cells
TF
stalk
GSC (lineage)
EC1
EC
fc
vas
hts
sheath
D
ΔT
Mitosis

E
Ecdysone Titre
Pupariation
Pupation
Pupal-adult Apolysis
Eclosion
-48h
0h
12h
24h
96h APF
Clone Induction times: -2h 2h 6h 10h 14h
Analysis 1
Analysis 2
3rd Instar Larvae
Prepupae
Pupae
Pharate Adult

E'
30h APF
1B1 vasa
20 µm
40h APF
20 µm

Fig.2

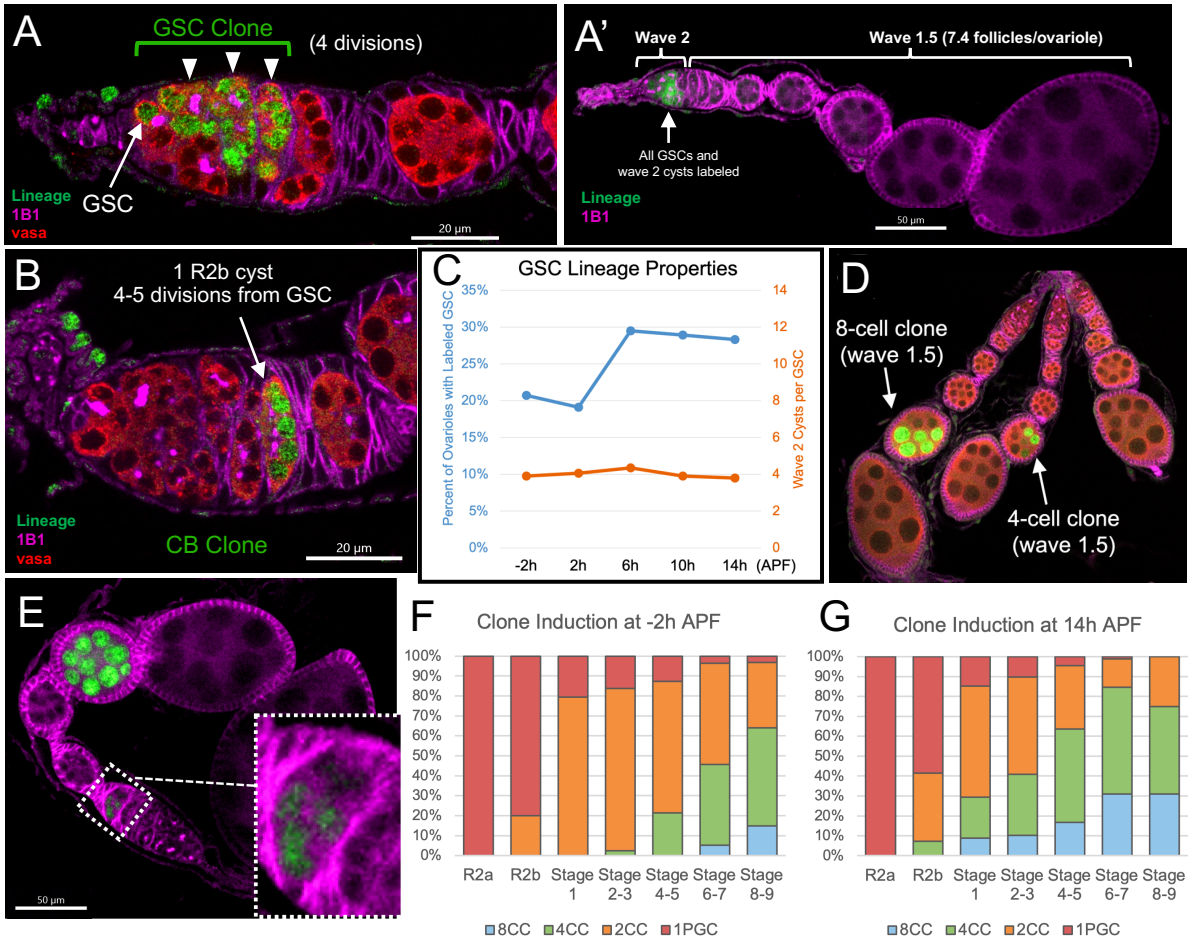

A
GSC Clone
(4 divisions)
GSC
Lineage
1B1
vasa
20 µm
A'
Wave 2
Wave 1.5 (7.4 follicles/ovariole)
All GSCs and
wave 2 cysts labeled
Lineage
1B1
50 µm
B
1 R2b cyst
4-5 divisions from GSC
Lineage
1B1
vasa
CB Clone
20 µm
C
GSC Lineage Properties
Percent of Ovarioles with Labeled GSC
Wave 2 Cysts per GSC
35%
30%
25%
20%
15%
10%
5%
0%
14
12
10
8
6
4
2
0
-2h
2h
6h
10h
14h
(APF)
D
8-cell clone
(wave 1.5)
4-cell clone
(wave 1.5)
E
50 µm
F
Clone Induction at -2h APF
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
R2a
R2b
Stage 1
Stage 2-3
Stage 4-5
Stage 6-7
Stage 8-9
8CC
4CC
2CC
1PGC
G
Clone Induction at 14h APF
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
R2a
R2b
Stage 1
Stage 2-3
Stage 4-5
Stage 6-7
Stage 8-9
8CC
4CC
2CC
1PGC

Fig.3

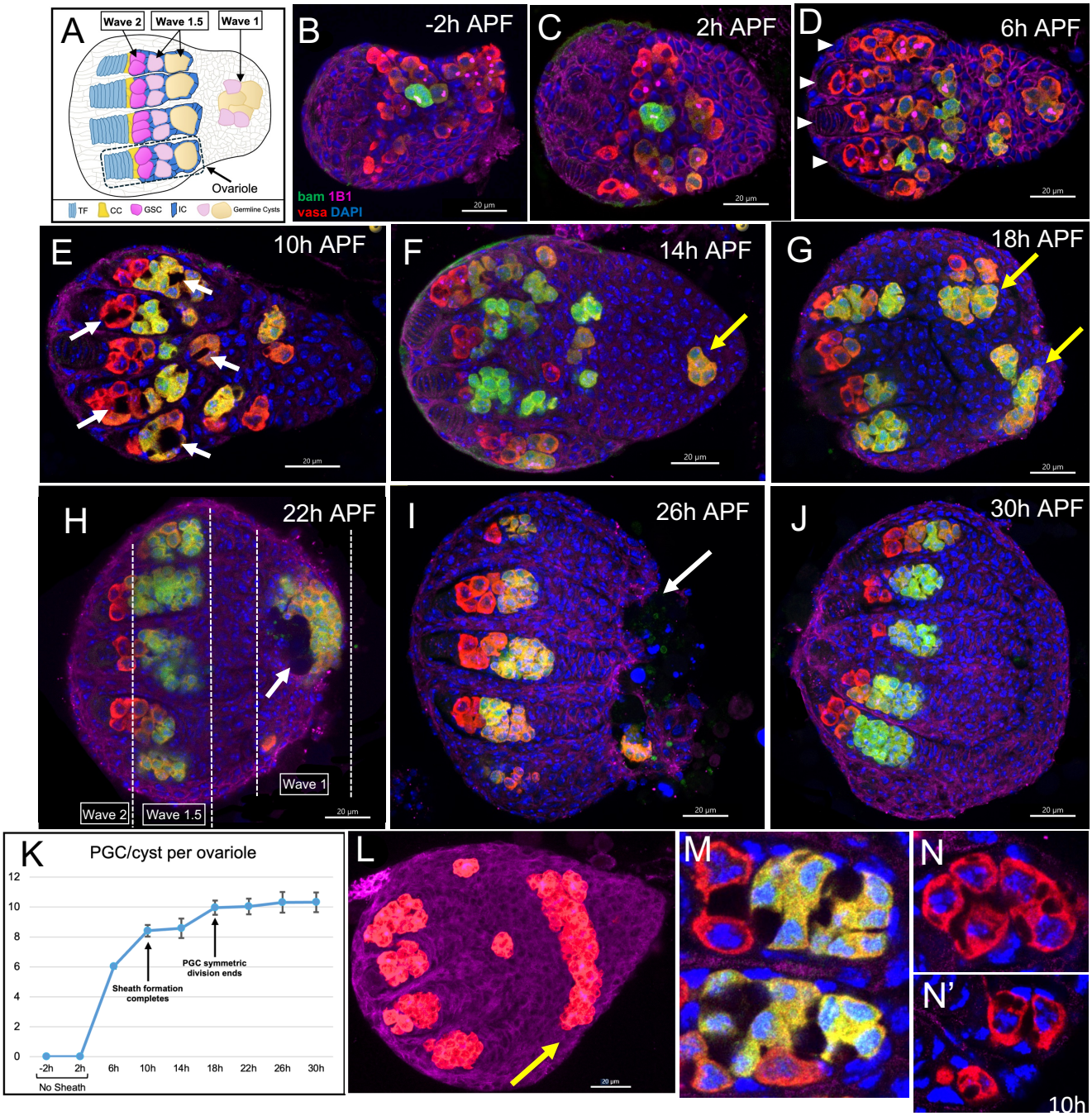

A
Wave 2
Wave 1.5
Wave 1
Ovariole
TF
CC
GSC
IC
Germline Cysts
B
-2h APF
bam 1B1
vasa DAPI
20 μm
C
2h APF
D
6h APF
E
10h APF
F
14h APF
G
18h APF
H
22h APF
Wave 2
Wave 1.5
Wave 1
I
26h APF
J
30h APF
K
PGC/cyst per ovariole
12
10
8
6
4
2
0
-2h
2h
6h
10h
14h
18h
22h
26h
30h
No Sheath
Sheath formation completes
PGC symmetric division ends
L
M
N
N'
10h

Fig.4

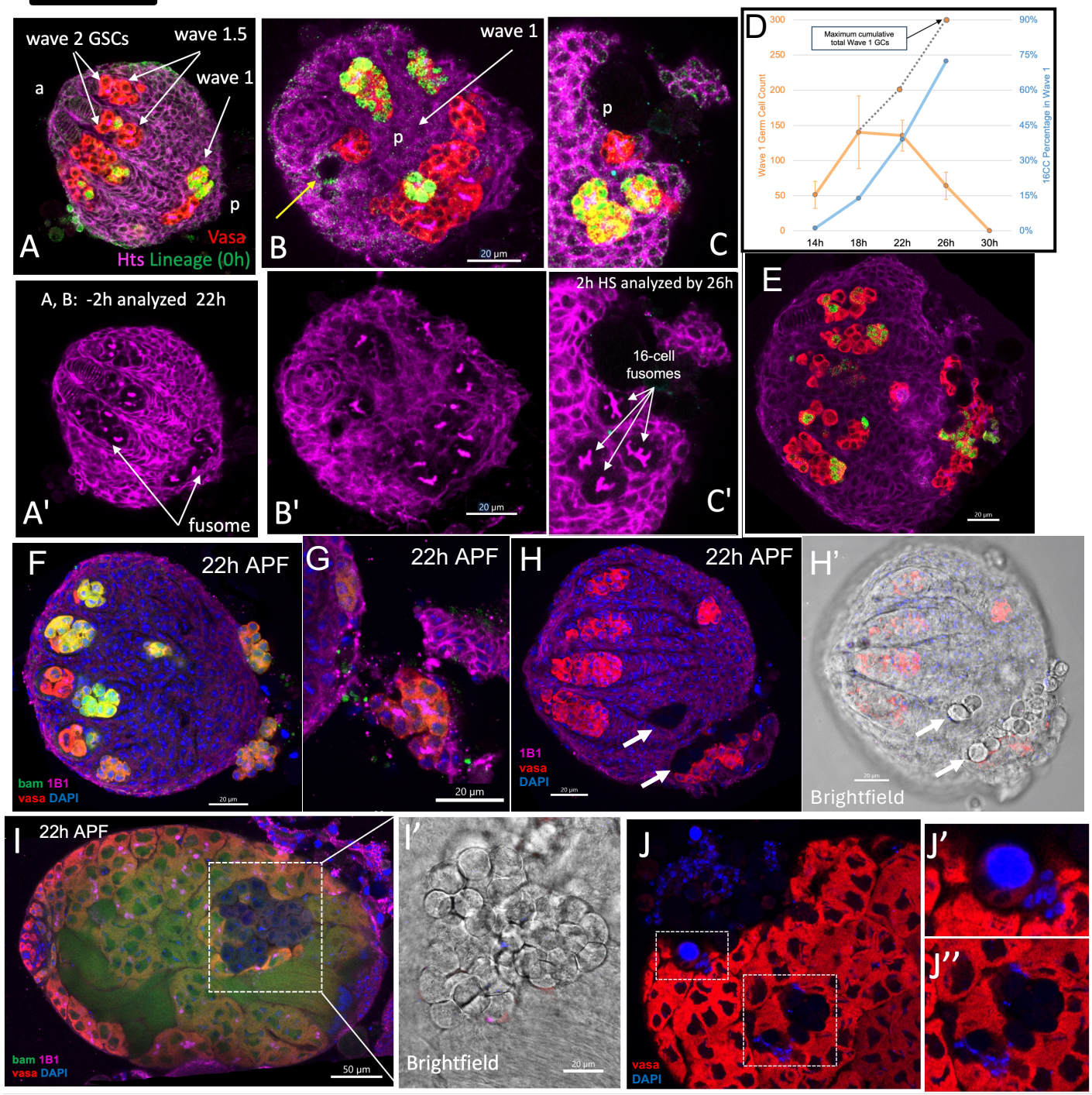

wave 2 GSCs
wave 1.5
wave 1
a
p
Vasa
Hts Lineage (0h)
A
wave 1
p
B
20 µm
p
C
D
Maximum cumulative total Wave 1 GCs
Wave 1 Germ Cell Count
16CC Percentage in Wave 1
300
250
200
150
100
50
0
90%
75%
60%
45%
30%
15%
0%
14h
18h
22h
26h
30h
A, B: -2h analyzed 22h
A'
fusome
B'
20 µm
2h HS analyzed by 26h
16-cell fusomes
C'
E
20 µm
F
22h APF
bam 1B1
vasa DAPI
20 µm
G
22h APF
20 µm
H
22h APF
1B1
vasa
DAPI
20 µm
H'
Brightfield
I
22h APF
bam 1B1
vasa DAPI
50 µm
I'
Brightfield
20 µm
J
vasa
DAPI
J'
J''

Fig.5

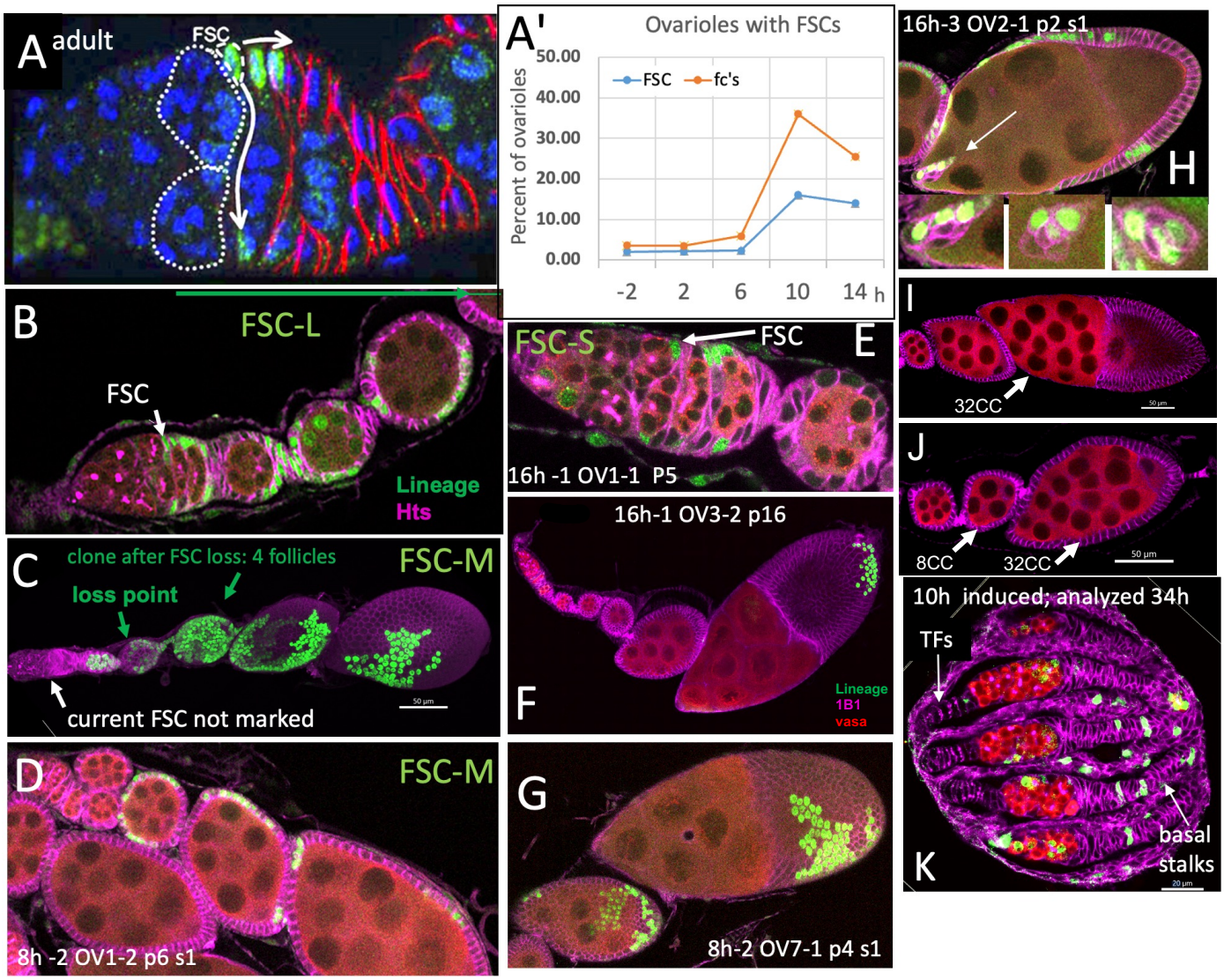

A
adult
FSC
A'
Ovarioles with FSCs
Percent of ovarioles
50.00
40.00
30.00
20.00
10.00
0.00
FSC
fc's
-2
2
6
10
14 h
16h-3 OV2-1 p2 s1
H
B
FSC-L
FSC
Lineage
Hts
FSC-S
FSC
E
16h -1 OV1-1 P5
I
32CC
J
8CC
32CC
C
clone after FSC loss: 4 follicles
loss point
FSC-M
current FSC not marked
16h-1 OV3-2 p16
F
Lineage
1B1
vasa
10h induced; analyzed 34h
TFs
basal
stalks
K
D
FSC-M
8h -2 OV1-2 p6 s1
G
8h-2 OV7-1 p4 s1

Fig.6

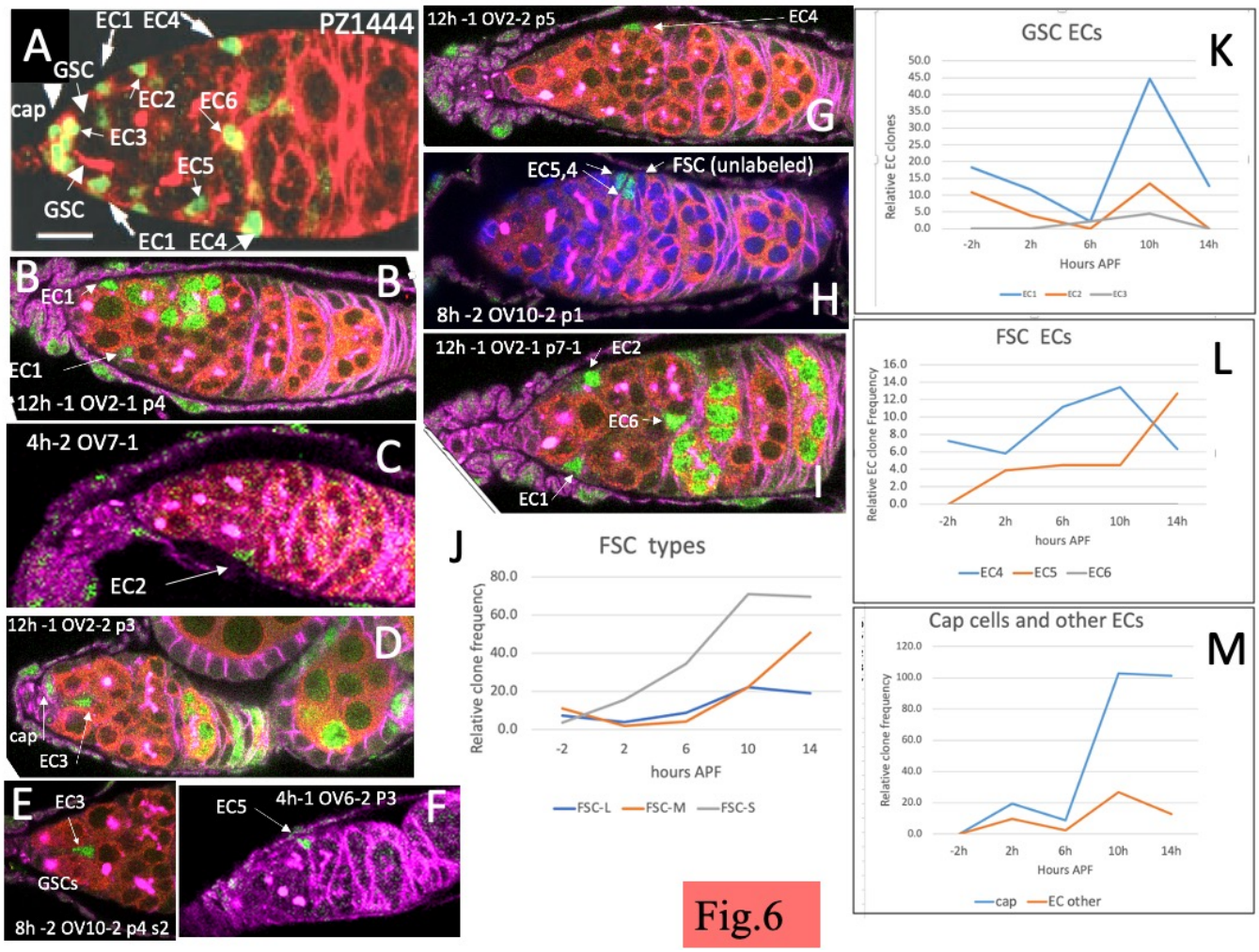

A
PZ1444
EC1 EC4
GSC
cap
EC2
EC6
EC3
EC5
GSC
EC1 EC4
B
EC1
EC1
12h -1 OV2-1 p4
4h-2 OV7-1
C
EC2
12h -1 OV2-2 p3
D
cap
EC3
E
EC3
GSCs
8h -2 OV10-2 p4 s2
EC5
4h-1 OV6-2 P3
F
12h -1 OV2-2 p5
EC4
G
EC5,4
FSC (unlabeled)
8h -2 OV10-2 p1
H
12h -1 OV2-1 p7-1
EC2
EC6
EC1
I
J
FSC types
Relative clone frequency
hours APF
FSC-L
FSC-M
FSC-S
GSC ECs
K
Relative EC clones
Hours APF
EC1
EC2
EC3
FSC ECs
L
Relative EC clone Frequency
hours APF
EC4
EC5
EC6
Cap cells and other ECs
M
Relative clone frequency
Hours APF
cap
EC other

Fig.6