Maintenance of the ovarian line fGS/OSS

Lucy Cherbas

INTRODUCTION

fGS/OSS is a line consisting of a mixture of somatic sheath cells and germ cells; OSS (which is in the DGRC collection) and OSC (which is not) are derivative lines from which the germ cell component has been lost. fGS/OSS is a somewhat tricky line to grow, much more finicky than OSS; because we and others have found it difficult to grow, we provide this protocol. The methods described here were worked out in the DGRC, using the original protocol from Yuzo Niki and suggestions from Daniel Lakatos, and using them, we have had little trouble growing this line. fGS/OSS carries a nos-EGFP marker, which produces green fluorescence in the germ cells but not in the somatic cells. Since most tissue culture labs, including ours, use phase contrast inverted microscopes to monitor growing cultures, we use phase-contrast photos in this protocol; the germ cells are not distinguishable by color in phase contrast, but they are easily identifiable as the round cells lying on top of the layer of flat, surface-adherent somatic cells.

APPEARANCE OF A HEALTHY CULTURE

fGS/OSS cells are quite sensitive to overcrowding. In a healthy culture, much of the surface area is covered by a flat layer of somatic cells, but at least half of the surface area is still free of cells. Figure 1 illustrates a culture at a stage suitable for transfer. A few cells have already begun to form tight clumps which are not adhering to the surface; these clumps, according to Dr. Niki, are composed of somatic cells surrounding a group of germ cells. I recommend transferring the cells before such clumps become prominent; the culture shown in the photo is at about the maximum density that the cells should be allowed to reach. For the most part, the clumps will die, and will not contribute to the further growth of the culture.

Protocol: Maintenance of the ovarian line fGS/OSS

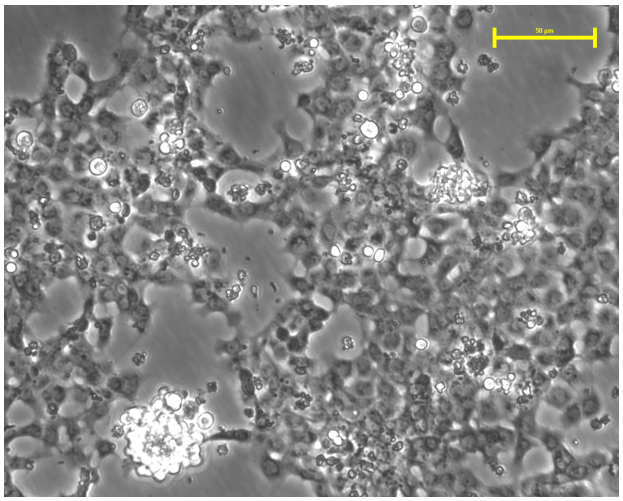

Figure 1. Cells prior to transfer. Germ cells are the round, refractile cells, often in doublets, on top of the layer of flat germ cells that cover much of the surface

TRANSFER OF CELLS

fGS/OSS cells should be trypsinized at each transfer (see separate protocol for our trypsinization procedure). It is not difficult to remove the cells from the substrate without trypsinization, but apparently there is excessive damage to the cells if trypsin is not used, and the resulting cultures have at best small regions of healthy cells. When cells are trypsinized, removed from the plate, centrifuged, and then resuspended in fresh medium (which should always contain about 25% medium from the old culture), the cells are mostly in very tight clumps, as illustrated in Figure 2. These clumps tend to have a smooth surface initially, but very quickly cells begin to crawl out of the clumps and spread on the surface of the plate; this is already apparent in the photo in Figure 2. Within a day, most of the somatic cells are well spread, and the germ cells are easily visible (Figure 3).

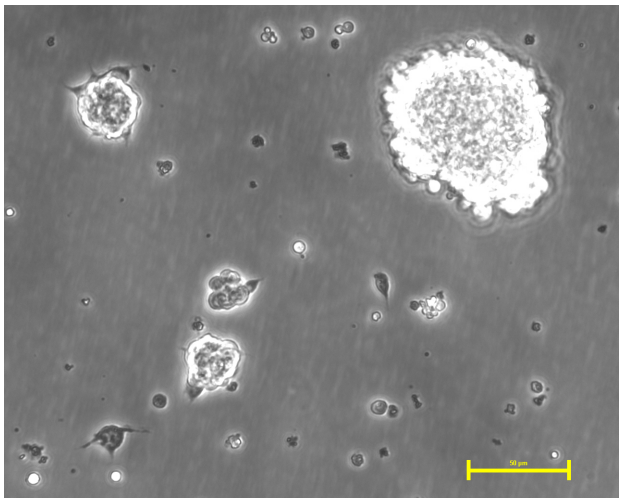

Figure 2. A culture of fGS/OSS 2 hr after transfer.

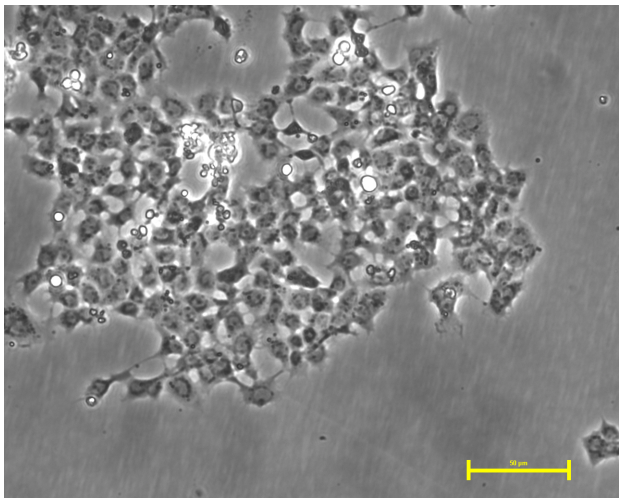

Figure 3. The same culture (not the same field) as Figure 2, photographed approximately 24 hr later.

Because of fGS/OSS cells' tendency to form tight clumps when they are removed from the substrate, we have not found a way to accurately estimate the density of the cells. For this reason, we do not have an accurate estimate of their doubling time; as judged by the frequency of transfers and the dilution at each transfer, we conclude that they double roughly every two days. We recommend transferring cells, with a 2-4 fold dilution, somewhere between the density shown in Figure 3 and that shown in Figure 1.

FREEZING AND THAWING AMPOULES OF CELLS

To make a frozen stock of fGS/OSS cells, use exactly the same procedure as for any other line, described in our protocol for freezing cells. The cells should be removed from the surface of the culture plates with trypsinization, before they are centrifuged and taken up in freezing medium.

Thawing cells from a frozen stock is also similar to the procedure for any other line (described in our protocol for thawing cells). Not surprisingly, the cells are clumped in the frozen ampoule, and they remain in clumps when thawed (see Figure 4). In our experience, the clumps are less tight than those seen immediately after a transfer with trypsinization (Figure 2), but otherwise, they behave very similarly.

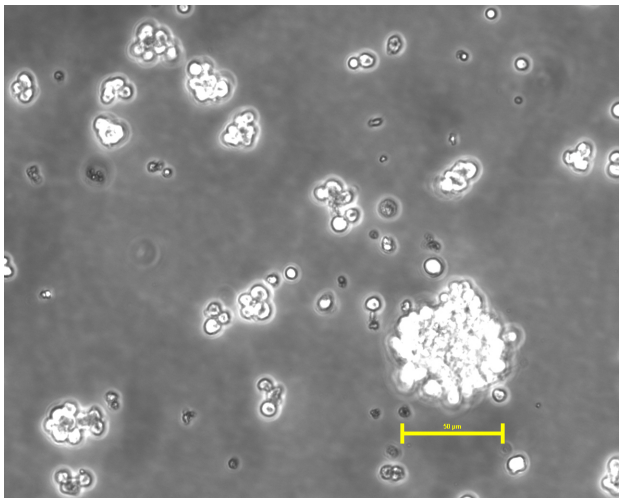

Figure 4. fGS/OSS cells 30 min. after thawing.

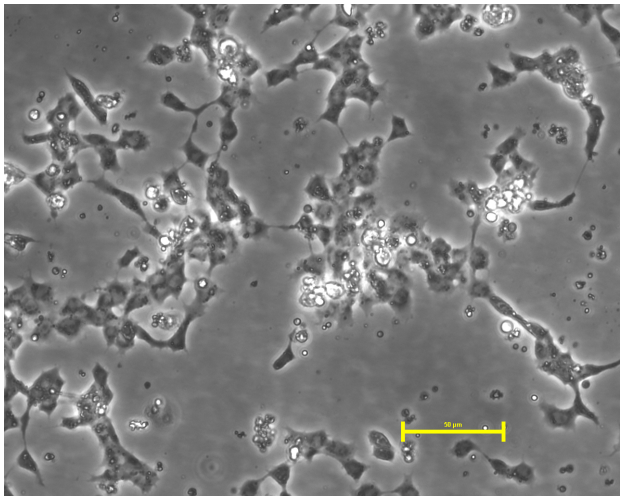

Figure 5. The same culture as Figure 4 (but a different field) 1 day after thawing.