SUPPLEMENTAL MATERIAL: ***Germ cell differentiation and lipids***

Fig. 8. Phase and fluorescence photomicrographs of pure isolated germ cells, pachytene spermatocyte (PS), round spermatids (RS) and a fraction containing residual bodies (RB) that are released from elongating forms of spermatids. The cell elements were identified by their size and by the typical aspect of their nucleus, stained with 5 μM of H_{33342} (Reyes et al., 1997). Because they have no nuclei, RB were not marked with H_{33342}.

Fig. 9. Tetraenoic and pentaenoic fatty acids of the n-6 series present in triacylglycerols (TAG), alkyl-diacylglycerols (ADG) and cholesterol esters (CE) of seminiferous tubules, germ cells and residual bodies. The fatty acids are represented by their number of carbon atoms. All belong to the n-6 series. The bars show that the composition of TAG and ADG in seminiferous tubules was mostly determined by the TAG and ADG contained in round spermatids and residual bodies. By contrast, the CE of seminiferous tubules were far richer in PUFA and VLCPUFA than those recovered from isolated cells or RB, supporting the view that CE with 22:5n-6 and VLCPUFA mainly belong to Sertoli cells. In the progression pachytene spermatocytes → round spermatids CE were progressively poorer, and TAG were progressively richer, in 22:5n-6 and longer PUFA.

Fig. 10. Distribution of molecular species of SM according to their fatty acids by TLC. PS, pachytene spermatocytes; RS, round spermatids; RS, round spermatids; r, residual bodies. *Top panel*: Detail of two-dimension TLC of phospholipids from each cell fraction showing the large spot of choline glycerophospholipids (CGP) as a reference, and the separation of SM into two clearly delimited bands (SM1 and SM2, respectively, with different staining intensities). *Bottom panels*: At the center, a TLC plate showing the two kinds of fatty acid methyl esters (FAME and 2-OH FAME) produced after methanolysis of each of the above SM bands. As illustrated by the corresponding GC tracings, the SM1 band included SM species with common saturated fatty acids (16:0, 18:0) and SM species with 2-OH VLCPUFA (after methanolysis, the corresponding FAME were widely separated by TLC). The SM2 band had SM species with common saturated fatty acids and N-VLCPUFA (the corresponding methyl esters were not non-separable by TLC). Also illustrated is the abundance of N-VLCPUFA in

pachytene spermatocytes in contrast with the prevalence of 2-OH VLCPUFA in round spermatids and residual bodies.

Figure 8

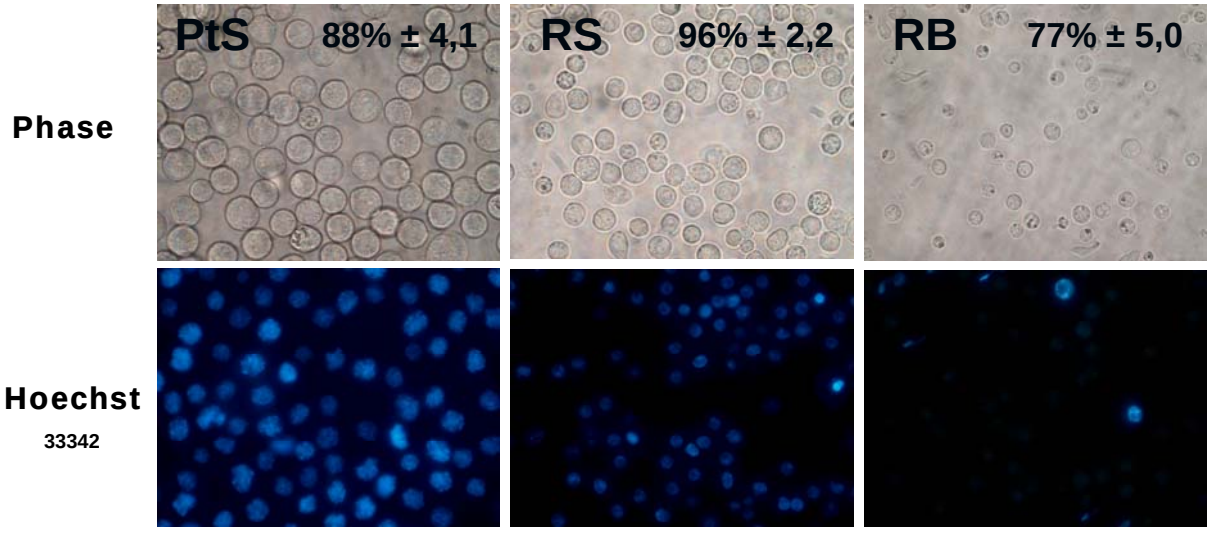


Figure 9

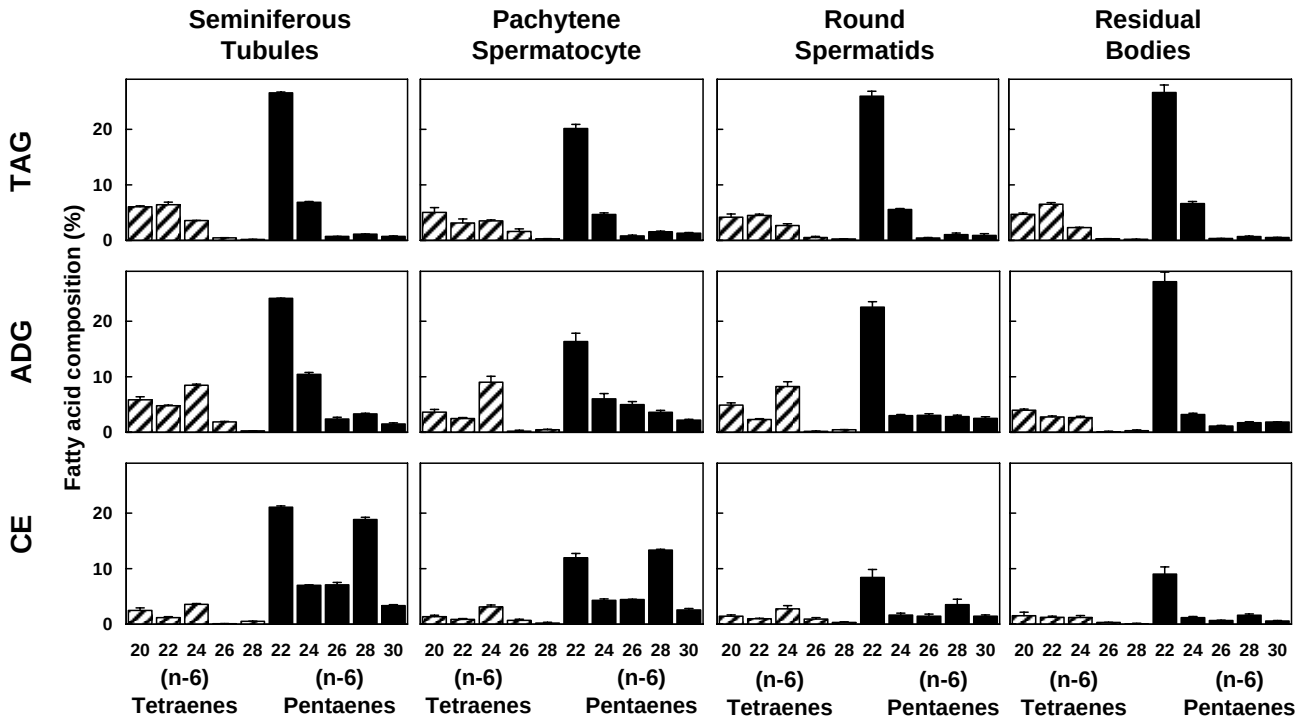
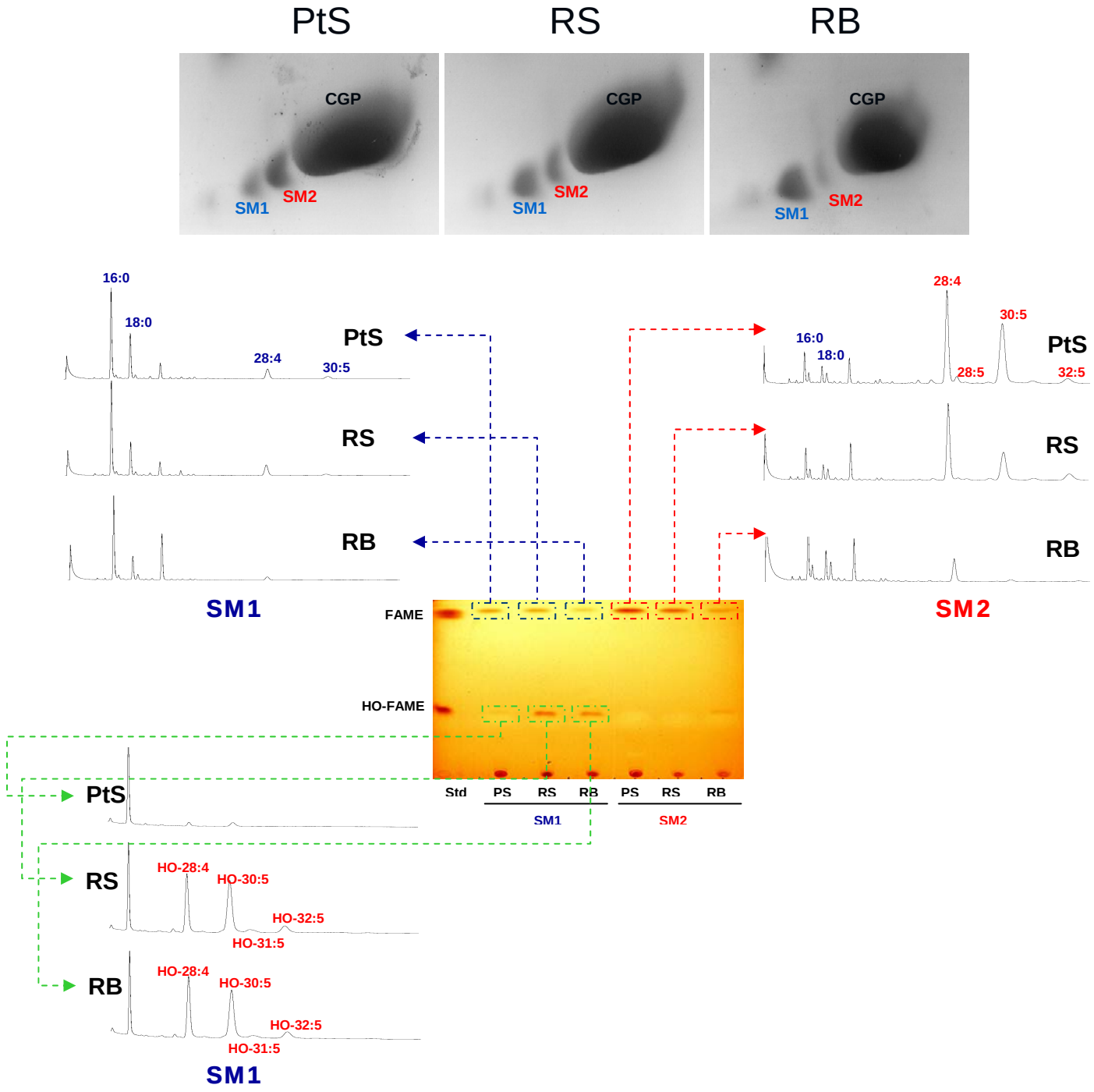

Seminiferous Tubules
Pachytene Spermatocyte
Round Spermatids
Residual Bodies
TAG
ADG
CE
Fatty acid composition (%)
0
10
20
20 22 24 26 28 22 24 26 28 30
(n-6) Tetraenes
(n-6) Pentaenes

Figure 10


PtS
RS
RB
CGP
SM1
SM2
16:0
18:0
28:4
30:5
28:5
32:5
FAME
HO-FAME
Std
PS
RS
RB
HO-28:4
HO-30:5
HO-32:5
HO-31:5