SUPPLEMENTAL MATERIAL: FABP, DGAT and PLIN2 during spermatogenesis

SUPPLEMENTAL DATA LEGENDS

TABLE S1: Statistical analysis of the relative expression of Fabp, Dgat and Plin2 genes in mouse testis during postnatal development and in cells isolated therefrom, including two germ cell types and residual bodies. The significance of the differences was analyzed by ANOVA followed by the Bonferroni’s post-test. The differences were classified as: $p \leq 0.001$ (***) ; $p \leq 0.01$ (**) ; and $p< 0.05$ (*). The mean ± SD of normalized expression is indicated in shaded boxes.

FIGURE S1. Phase and fluorescence photomicrographs of germ cells and residual bodies from adult mouse testis. Pachytene spermatocytes, round spermatids, and a fraction containing the residual bodies that are released from elongating, condensing forms of spermatids are shown. The cell elements were identified by their size and by the typical aspect of their nucleus, stained with 5μM of Hoechst 33342. Residual bodies were not marked with Hoechst as they lack chromatin. Nile Red was used to stain cellular membrane lipids and lipid droplets. Note the faint discoloration of the two germ cell fractions in contrast with the bright staining of residual bodies, which contain residual materials in a densely packed form. These include left-behind membranes and neutral lipid droplets. (bar = 50 μm).

FIGURE S2. Photomicrographs showing the immunolocalization of FABP3 (green) at P6, P18 and adult testis (AD). Note the immunolocalization of FABP3 in interstitial cells at P6 decreasing to virtually undetectable levels at P18, in contrast with the abundant expression of the protein in adult testis (bar = 50 μm).

Supplementary Materials and Methods,

Immunofluorescence and Confocal Microscopy:

Testes fixed at 4% in paraformaldehyde at 4°C were rinsed overnight in PBS containing 30% sucrose at 4°C for cryoprotection, placed in a small amount of OCT compound Tissue Tek (Sakura Finetek U.S.A) and stored at -80°C. Ten μm thick frozen sections were prepared with a cryostat and picked up on poly-L-lysine-coated glass coverslips (Sigma). The tissue sections thus obtained were post-fixed with
acetone:methanol (1:1), permeabilized with 0.1% Triton X-100 and then incubated with anti-FABP3 (66E2) antibody (Novus Biologicals). After incubation with fluorochrome-conjugated (Alexa488) secondary antibodies (Jackson ImmunoResearch), samples were mounted with Vectashield Mounting Medium with DAPI (Vector Laboratories), and images were captured using a Leica TCS-SP5-AOBS confocal microscope with 40X oil immersion objectives.