Supplemental material

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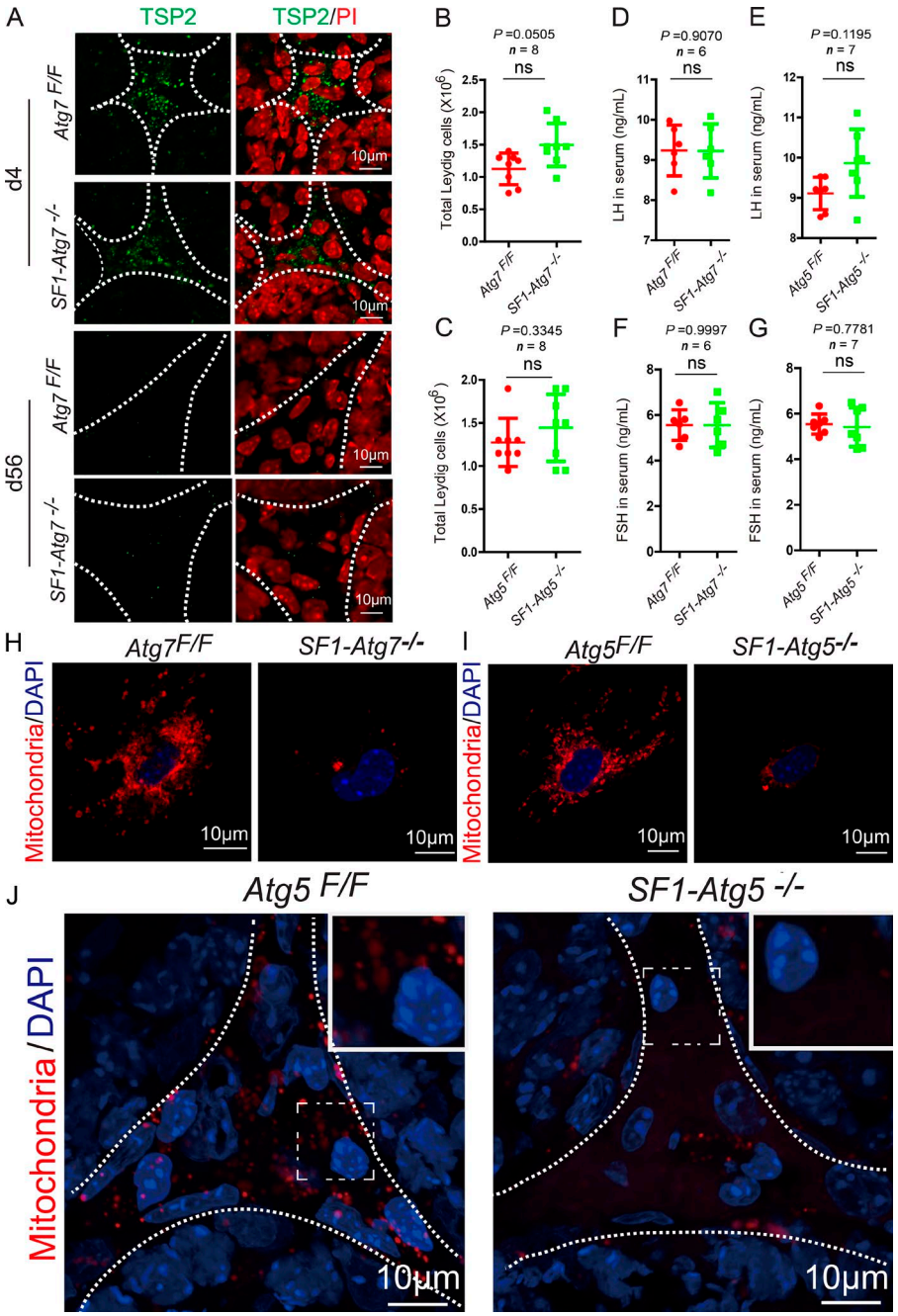

Figure S1. **The disruption of autophagy does not affect the fetal–adult Leydig cell transition, proliferation, and Gn secretion, but it influences the number of mitochondria.** Related to Fig. 3. **(A)** Immunofluorescence staining of TSP2 (green) in *Atg7*$^{Flox/Flox}$ and *SF1-Atg7*$^{-/-}$ mouse testes at d4 and d56. Nuclei were stained with PI (red). **(B and C)** The total number of Leydig cells was not reduced in *SF1-Atg5*$^{-/-}$ and *SF1-Atg7*$^{-/-}$mice. **(B)** *Atg7*$^{Flox/Flox}$ (red, 1.13 ± 0.09 × 10^6 per testis) and *SF1-Atg7*$^{-/-}$ (green, 1.50 ± 0.12 × 10^6 per testis). **(C)** *Atg5*$^{Flox/Flox}$ (red, 1.28 ± 0.10 × 10^6 per testis) and *SF1-Atg5*$^{-/×}$ (green, 1.44 ± 0.14 × 10^6 per testis). **(D and E)** The serum concentration of LH was not significantly changed in autophagy-deficient mice. **(D)** *Atg7*$^{Flox/Flox}$ mice (red, 9.24 ± 0.26 ng/ml) and *SF1-Atg7*$^{-/-}$ mice (green, 9.23 ± 0.28 ng/ml). **(E)** *Atg5*$^{Flox/Flox}$ mice (red, 9.11 ± 0.15 ng/ml) and *SF1-Atg5*$^{-/-}$ mice (green, 9.86 ± 0.32 ng/ml). **(F and G)** The serum concentration of FSH was not significantly changed in autophagy-deficient mice. **(F)** *Atg7*$^{Flox/Flox}$ mice (red, 5.69 ± 0.27 ng/ml) and *SF1-Atg7*$^{-/-}$ mice (green, 5.56 ± 0.40 ng/ml). **(G)** *Atg5*$^{Flox/Flox}$ mice (red, 5.54 ± 0.17 ng/ml) and *SF1-Atg5*$^{-/-}$ mice (green, 5.41 ± 0.32 ng/ml). **(H and I)** The mitochondria of isolated autophagy-deficient Leydig cells and control groups were labeled with MitoTracker red and observed using fluorescence microscopy. **(J)** DsRed-labeled mitochondria (red) were directly detected in *Atg5*$^{Flox/Flox}$; *CAG/su9-DsRed2* mice and *SF1-Atg5*$^{-/-}$; *CAG/su9-DsRed2* mice. Insets are marked by boxes and are 15 μM wide. Nuclei were labeled with DAPI (blue).

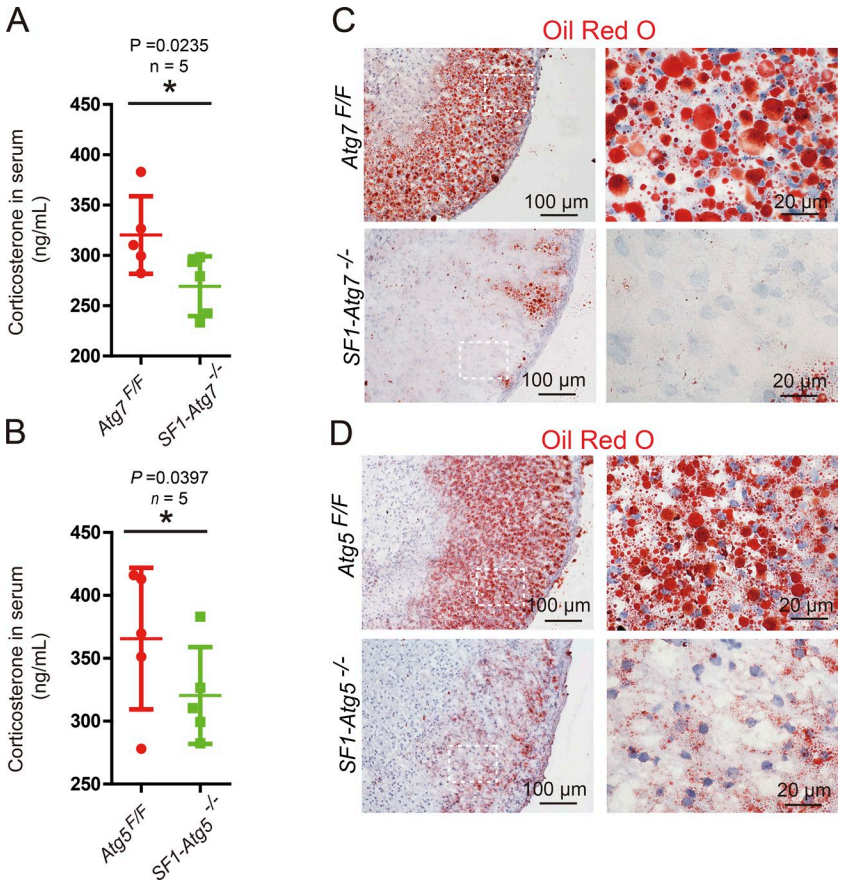

Figure S2. **The lipids in the adrenal cortex and corticosterone in the serum decrease in the steroidogenic cell–specific autophagy disruption mice. (A and B)** The serum concentration of corticosterone was decreased in autophagy-deficient mice. **(A)** *Atg7*$^{Flox/Flox}$ mice (red, 320.4 ± 17.23 ng/ml) and *SF1-Atg7*$^{-/-}$ mice (green, 269.4 ± 13.28 ng/ml). **(B)** *Atg5*$^{Flox/Flox}$ mice (red, 345.6 ± 31.50 ng/ml) and *SF1-Atg5*$^{-/-}$ mice (green, 261.5 ± 27.56 ng/ml). *, P < 0.05. **(C and D)** LDs dramatically decreased in the adrenal cortex of the steroidogenic cell–specific autophagy disruption mice. **(C)** ORO (red) staining in *Atg7*$^{Flox/Flox}$ (top) and *SF1-Atg7*$^{-/-}$ (bottom) adrenal cortex frozen sections. **(D)** ORO (red) staining in *Atg5*$^{Flox/Flox}$ (top) and *SF1-Atg5*$^{-/-}$ (bottom) adrenal cortex frozen sections. Insets are marked by boxes.

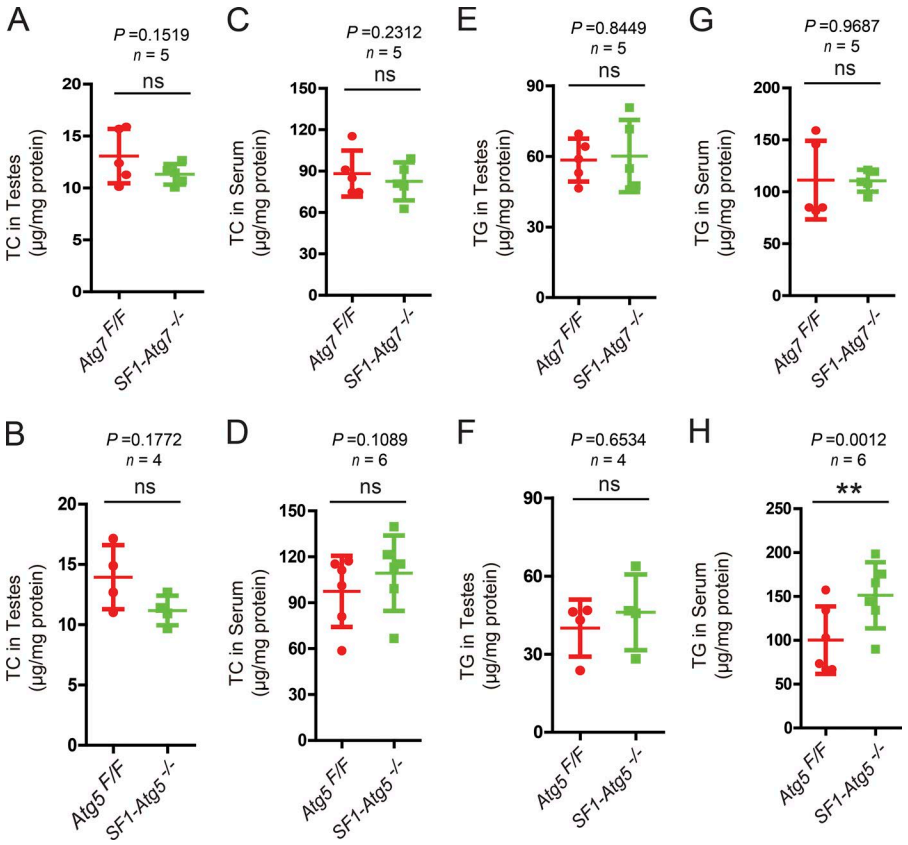

Figure S3. **The steroidogenic cell–specific disruption of autophagy does not affect the concentrations of TGs and TC in testes and serum.** Related to Fig. 3. **(A and B)** The concentrations of TC in autophagy-deficient mouse testes were not reduced compared with control groups. **(A)** *Atg7*$^{Flox/Flox}$ mice (red, 12.42 ± 1.12 μg/mg protein) and *SF1-Atg7*$^{-/-}$ mice (green, 11.29 ± 0.51 μg/mg protein). **(B)** *Atg5*$^{Flox/Flox}$ mice (red, 13.95 ± 1.33 μg/mg protein) and *SF1-Atg5*$^{-/-}$ mice (green, 11.18 ± 0.61 μg/mg protein). **(C and D)** The concentrations of TC in autophagy-deficient mice serum were not changed compared with the control groups. **(C)** *Atg7*$^{Flox/Flox}$ mice (red, 91.55 ± 7.70 μg/mg protein) and *SF1-Atg7*$^{-/-}$ mice (black column, 82.94 ± 7.09 μg/mg protein). **(D)** *Atg5*$^{Flox/Flox}$ mice (red, 97.45 ± 10.58 μg/mg protein) and *SF1-Atg5*$^{-/-}$ mice (green, 109.27 ± 11.01 μg/mg protein). **(E and F)** The concentrations of TGs in autophagy-deficient mouse testes were similar to those in the control groups. **(E)** *Atg7*$^{Flox/Flox}$ mice (red, 56.99 ± 4.38 μg/mg protein) and *SF1-Atg7*$^{-/-}$ mice (green, 54.98 ± 5.24 μg/mg protein). **(F)** *Atg5*$^{Flox/Flox}$ mice (red, 39.99 ± 5.47 μg/mg protein) and *SF1-Atg5*$^{-/-}$ mice (green, 46.10 ± 7.26 μg/mg protein). **(G and H)** The concentrations of TGs in autophagy-deficient mouse serum were not reduced compared with the control groups. **(G)** *Atg7*$^{Flox/Flox}$ mice (red, 117.51 ± 18.02 μg/mg protein) and *SF1-Atg7*$^{-/-}$ mice (green, 114.56 ± 3.01 μg/mg protein). **(H)** *Atg5*$^{Flox/Flox}$ mice (red, 100.29 ± 12.04 μg/mg protein) and *SF1-Atg5*$^{-/-}$ mice (green, 151.32 ± 13.63 μg/mg protein). **, P < 0.01.

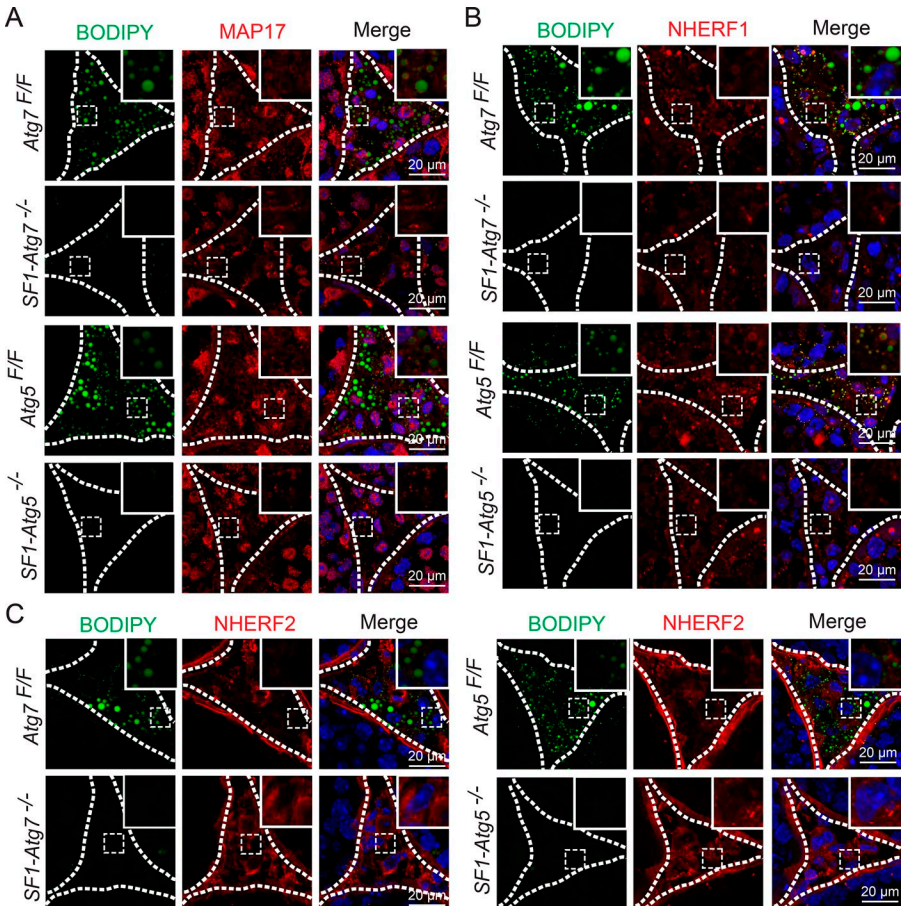

Figure S4. **Only NHERF2 is accumulated in autophagy-deficient Leydig cells.** Related to Fig. 5. **(A)** Immunofluorescence staining of MAP17 (red) and BODIPY staining (green) in autophagy-deficient mouse testes and control groups. **(B)** Immunofluorescence staining of NHERF1 (red) and BODIPY staining (green) in autophagy-deficient mouse testes and control groups. **(C)** Immunofluorescence staining of NHERF2 (red) and BODIPY staining (green) in autophagy-deficient mouse testes and control groups. Nuclei were stained with DAPI (blue). Insets are marked by boxes and are 10 μM wide.

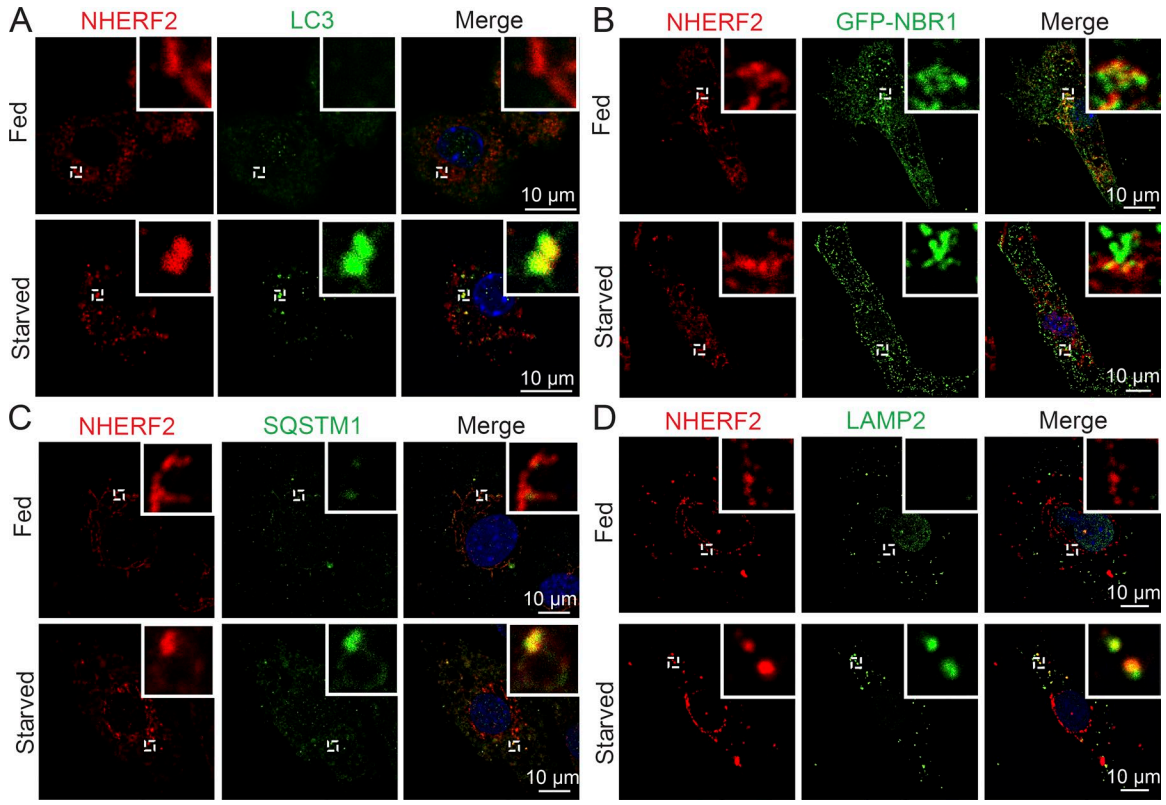

Figure S5. **NHERF2 partially colocalizes with LC3, NBR1, SQSTM1, and LAMP2 in starved Leydig cells.** Related to Fig. 5. **(A)** Immunofluorescence analysis of NHERF2 (red) and LC3 (green) in fed and starved Leydig cells. **(B)** Immunofluorescence analysis of NHERF2 (red) and NBR1 (green) in fed and starved Leydig cells. **(C)** Immunofluorescence analysis of NHERF2 (red) and SQSTM1 (green) in fed and starved Leydig cells. **(D)** Immunofluorescence analysis of NHERF2 (red) and LAMP2 (green) in fed and starved Leydig cells. Nuclei were stained with DAPI (blue). Insets are marked by boxes and are 2.3 μM (A) or 2.8 μM (B–D) wide.

Table S1. **Characteristics of human patients in the normal serum testosterone level study**

Medical record no.	Age	Testosterone level	Clinical diagnosis	Relative LC3 intensity	LC3 puncta numbers	Relative NHERF2 intensity	Relative SR-BI intensity
	yr	*ng/ml*					
14-01671	29	4.66	Azoospermia, varicocele	11609.6	193	13328.80322	19754.55277
14-03119	33	4.22	Azoospermia, varicocele	11613.3	345	7791.443628	13478.35806
14-00366	22	3.6	Oligospermia	20941.4	565	14613.92317	25473.66523
15-0375	39	5.86	Azoospermia, bilateral vas ligation	17618.1	503	5292.289558	18960.77936
15-0822	24	5.23	Azoospermia, varicocele	12687.3	258	8866.248787	12547.83248
14-02870	25	4.83	Obstructive azoospermia	6362.15	404	3390.952711	6247.871168
15-0609	36	5.16	Azoospermia, redundant prepuce	6931.48	274	6444.235196	16471.26612
13-00024	35	4.95	Obstructive azoospermia	12846.8	224	6287.177923	8750.533907
13-00766	30	9.28	Azoospermia	14762.9	377	9610.275967	15822.30204
13-02023	25	8.65	Obstructive azoospermia	7428.2	290	14154.23104	16899.10137
13-04042	39	8.24	Azoospermia	9278.64	393	7790.09014	12280.15254
13-01038	24	8.51	Azoospermia	26997.3	345	13929.16378	8917.463576

Table S2. **Characteristics of human patients in the low-serum testosterone level study**

Medical record no.	Age	Testosterone level	Clinical diagnosis	Relative LC3 intensity	LC3 puncta numbers	Relative NHERF2 intensity	Relative SR-BI intensity
	yr	*ng/ml*					
13-04219	30	2.23	Azoospermia, Syndrome de Klinefelter	9213.743	148	7329.233116	18429.15762
13-04318	33	2.53	Azoospermia, varicocele	8090.767	480	10645.91041	10427.57981
14-03056	23	1.83	Azoospermia, varicocele	5339.931	267	6261.456109	8077.143815
14-04072	22	2.98	Azoospermia, varicocele	6535.413	348	9649.846069	14360.84123
14-04586	23	2.03	Azoospermia, varicocele	21604.6	380	7247.250035	9360.477541
14-04810	35	2.83	Azoospermia, varicocele	7774.267	171	4442.429621	7062.881463
14-5014	30	1.02	Azoospermia, varicocele	9390.367	221	7783.239495	8627.456741
15-1030	27	2.76	Azoospermia	10771.94	207	16436.69117	13241.79725
15-1062	30	2.97	Azoospermia	12039.47	165	16203.42532	18358.20742
15-4484	24	2.7	Azoospermia, varicocele	7886.856	263	4182.662599	9823.974483
15-5117	25	1.05	Azoospermia	9075.124	332	3207.438635	6871.272486
13-01037	27	2.87	Azoospermia	10082.77	157	30832.90528	9401.55781
13-01248	27	2.39	Azoospermia, varicocele	9692.442	202	4702.462904	11041.75586
13-03512	33	1.9	Azoospermia, varicocele	11858.4	160	18615.64554	15382.11758
15-5118	24	2.54	Azoospermia	13997.03	374	5448.697823	10035.51038
15-5120	25	2.26	Azoospermia, varicocele	9084.047	360	4447.607821	8107.178843
16-1748	28	2.78	Azoospermia	8994.569	79	8273.113299	9587.513348
16-1750	30	2.24	Azoospermia, varicocele	9828.537	306	5134.353072	11075.36678
14-00256	33	2.88	Oligospermia, seminal vesiculitis, seminal vesicular stone	8493.194	165	7307.822771	8248.74636
16-1350	25	2.68	Oligospermia, seminal vesiculitis	11997.56	182	7230.115102	10222.96491