### Supplementary Table 2: Overview of different combinations of 3D culture methodology and signaling factors on best outcomes per study

| AUTHORS       | DATE    | START | CULTURE METHODOLOGY | TIME IN CULTURE | CELL REORGANIZATION       | SPERMATOGENESIS | BASAL MEDIUM | SERUM | GONADOTROPINS | TRIG-SUPERFAMILY | GROWTH FACTORS | VITAMIN A         | OTHER INGREDIENTS |
|---------------|---------|-------|---------------------|-----------------|--------------------------|-----------------|---------------|-------|---------------|-----------------|----------------|------------------|------------------|
| Zenes and Engel | 1981    | 8, 10, 12, 25, 30, 40, 60 dpp rTCoS | Rotation culture | -               | Tubule-like structures   | N/A             | MEM           | -     | -             | -               | -              | -                | -                |
| Hadi et al.   | 1995    | 10 dpp rat Sertoli cells | Matrigel | 5 weeks | Cord-like structures | FaS | DMEM | -     | 100 ng/ml FSH | -               | -              | 10 ng/ml EGF | 2 μg/ml tranamin A: 5 μg/ml tranamin 200 ng/ml vitamin E | 10 μM hydrocortisone | 2 μM glutamine | 1 μM sodium selenate | 1 μM sodium pyruvate | 22 μM sodium lactate | 3 μM cystine | 10 μM sodium lactate | 50 ng/ml vitamin C | 50 ng/ml vitamin A |
| Hadi et al.   | 1996    | 10 dpp rat SGCs | Matrigel | 5 weeks | Cord-like structures | N/A | DMEM | -     | 100 ng/ml FSH | -               | -              | 10 ng/ml EGF | 2 μg/ml tranamin A: 5 μg/ml tranamin 200 ng/ml vitamin E | 10 μM hydrocortisone | 2 μM glutamine | 1 μM sodium selenate | 1 μM sodium pyruvate | 22 μM sodium lactate | 3 μM cystine | 10 μM sodium lactate | 50 ng/ml vitamin C | 50 ng/ml vitamin A |
| Yu et al.     | 2005    | 5 dpp rat SGCs and SGCs | Matrigel overlay | 2 weeks | Cellular aggregates | N/A | MEM | -     | -             | -               | -              | -              | -              | 0.1 μM NEAA | 1 μM sodium pyruvate | 3 μM sodium lactate | 1% ITS | -              | -              |
| Gass et al.   | 2006    | 7 dpp rat TCoS | Matrigel | 5 weeks | Tubule-like structures | N/A | DMEM | -     | -             | -               | -              | -              | -              | -              | NEAA | -              | -              |
| Lee et al.    | 2006    | 18 dpp rat TCoS | CGM | 22 days | Cellular aggregates | RS | DMEM/F12 | 10% FCS | 2.5 x 10^6 IU FSH | 10 μM testosterone | -               | 3.3 x 10^7 M RA | 3.3 x 10^7 M retinol | 10 ng/ml ITS | 10 μM vitamin C | 10 μg/ml vitamin E | 2 μM pycocytol | -              | -              |
| Lee et al.    | 2007    | Adult human TCoS | Colagen | 12 days | Cellular aggregates | SpT | DMEM/F12 | 10% FCS | 100 μIU FSH | 10 μM testosterone | 10 μM DHT | -               | 3.3 x 10^7 M RA | 3.3 x 10^7 M retinol | 10 ng/ml ITS | 10 μM vitamin C | 10 μg/ml vitamin E | 2 μM pycocytol | -              | -              |
| Gass et al.   | 2008    | 7 dpp rat TCoS | Matrigel | 5 days | Cellular aggregates | N/A | DMEM | -     | 10 - 100 ng/ml FSH | -               | -              | -              | -              | -              | -              | 1% NEAA | 10 ng/ml - 100 ng/ml NTF3 |
| Stukenberg et al. | 2008 | 7-10 dpp mouse TCoS | SOFT agarose | 15 days | Cellular aggregates | RS | DMEM/F12 | -     | -             | -               | -              | -              | -              | -              | -              | -              |
| Stukenberg et al. | 2009 | 7-10 dpp mouse TCoS | SOFT agarose | 31 days | Cellular aggregates | ES | DMEM/F12 | -     | 5 IU/ hCG | 5 IU/ FSH | -               | -              | -              | 5 IU/ hCG | 5 IU/ FSH | -              | -              | -              |
| Stukenberg et al. | 2009 | 7-9 dpp mouse TCoS | Methylcellulose | 31 days | Cellular aggregates | ES | DMEM/F12 | -     | 5 IU/ hCG | 5 IU/ FSH | -               | -              | -              | 5 IU/ hCG | 5 IU/ FSH | -              | -              | -              |
| Yu et al.     | 2009    | 5 dpp rat SGCs and SGCs | Matrigel | 7 days | Cellular aggregates | N/A | MEM | -     | -             | -               | -              | -              | -              | -              | -              | -              |
| Legendre et al. | 2010 | 18 dpp rat TCoS | Matrigel, collagen I (bioxemal chamber) | 22 days | Tubule-like structures | RS | DMEM/F12 | 5% FCS | 100 μIU FSH | 10 μM testosterone | -               | 3.3 x 10^7 M retinol | 6.25 μg/ml ITS | 10 μM vitamin C | 10 μg/ml vitamin E | 2 μM pycocytol | -              | -              |
| Gass et al.   | 2010    | 7 dpp rat TCoS | Matrigel | 9 days | Tubule-like structures | N/A | DMEM | -     | -             | -               | -              | -              | -              | 0.1% NEAA | -              | -              |
CGM, collagen gel-Matrigel; ddp; days postpartum; DHT, dihydrotestosterone; ES, elongates spermatids; GFR, growth factor reduced; LN, laminin; NEAA, non-essential amino acids; PLGA, poly(D,L-lactic-co-glycolic acid); PaS, pachytene spermatocytes; PMDS, polydimethylsiloxane; PTMC, peritubular myoid cells; RS, round spermatids; SACS, soft-agarose culture system; SC, Sertoli cells; SG, spermatogonia; ST, seminiferous tubule; TC, testicular cell