

Supplementary Table 1: Genes, gonadal function or localization, accession number of rainbow trout sequences, and qPCR primers

Sequences of the Sigenae contigs can be found at the following address: http://public-contigbrowser.sigenae.org:9090/Oncorhynchus_mykiss/index.html

Most of the information about genes function or localization referred here are based on studies in mammal species.

Gene	Full Name	Gonadal function or localization	Ref.	Accession number	Primer Forward	Primer Reverse
wnt2	wingless-type MMTV integration site 2	- expressed in granulosa cells during folliculogenesis - regulates granulosa cells proliferation	1; 2; 3	Sigenae: FYV3OTN01B98XS.p.om.8	GGAGTGCCAACATCAGTTTCAG	ATACACAAAGGCTGCTTCACGA
wnt3	wingless-type MMTV integration site 3	- detected in mouse ovary at birth	15	Sigenae: GB5RBPX01DPZJD.p.om.8	CTGTGGTCCATCATTCTGTCC	ATCTTCCACTGGTGCTGCTAC
wnt4a1	wingless-type MMTV integration site 4a1	- expressed in differentiating gonads of various vertebrates species - key gene in ovarian differentiation in mammals inhibits testosterone synthesis, and testicular vasculature formation - implicated in folliculogenesis in mammals - required for testis development	4; 5; 6; 7; 8 4; 9; 10; 11; 12 13	Genbank: JF815553	CCACGGAGTCAGTCCAGAA	TACTCCTCTCCCTCACATCAACA
wnt5a	wingless-type MMTV integration site 5	- expressed in both male and female embryonic gonads in mouse - in granulosa cells	14 15	Genbank: CU074815	GCATCAGGGAGTGTCACTATCA	CTGCTTCCTATCTGCATGACC
wnt6	wingless-type MMTV integration site 6	- expressed in both male and female genital ridges in mouse	16	Genbank: JN187949	CTCAACTTGCCCTGTTTTTCAT	GCTTGGTCTTCCTGCAGATACT
wnt7b	wingless-type MMTV integration site 7b	- expressed in mouse developping ovary	15	Genbank: JN187950	AAGGATGAGGAAGATCTGTGGA	AGAAAGCGGACATTGTTCAT
wnt9b	wingless-type MMTV integration site 9b	- expressed in mouse developping ovary	15	Genbank: CU066549	ACTAGCAAAGGCTTGCAGCTC	TATTTCAAGTTGTCACCGCAGA
wnt11	wingless-type MMTV integration site 11	- expressed in granulosa cells and in oocytes	15; 17	Genbank: CU063997	GAGACGTTGAGTGGCAGTGA	AAAGCTGCAGTGGCATTCT

<i>rspo1</i>	<i>r-spondin 1</i>	<ul style="list-style-type: none"> - over-expressed in differentiating ovary of various vertebrates - key gene in ovarian differentiation in mammals - positively regulates Wnt4 signaling 	5; 18; 19 20; 21; 22; 23	Genbank: JN187947	GGTCAGCACCAGGGACTATG	CTGTCCCTTTGAGAGATTGAGG
<i>fzd1</i>	<i>frizzled 1</i>	<ul style="list-style-type: none"> - expressed in granulosa cells during late folliculogenesis - in theca cells 	11	Sigenae: CA381602.p.om.8	ATGACTCTGATCGTGGGGATAA	CAGACCATTACACTGTGGTCT
<i>fzd2</i>	<i>frizzled 2</i>	- expressed in human cumulus cells	2	Sigenae: CB498179.p.om.8	CAACAGCCTGAACCCCATAC	AAGGAGGTACCGATGAAGAGGT
<i>fzd3</i>	<i>frizzled 3</i>	- expressed in human cumulus cells	2	Sigenae: GAY7CUQ01A5IU3.p.om.8	GATGTCCCTCTGGACTTCCTC	GCAAAGATAGTGTGGTGAACGA
<i>fzd4</i>	<i>frizzled 4</i>	<ul style="list-style-type: none"> - expressed in granulosa cells - implicated in corpus luteum formation 	1; 11; 24	Genbank: BX875207	CGACGAGACAGAGGAGATGAC	CGTCTGACTGCAGGACGTT
<i>fzd7</i>	<i>frizzled 7</i>	- expressed in mouse developping ovary and oocytes	15	Genbank: CX030042	CAGAGTTGGTGCAGGTTTTACA	TCCTCCACATTAACTTCCACA
<i>fzd8</i>	<i>frizzled 8</i>	unknown		Genbank: CA363645	AATTGCTGGACATGAACAAGTG	AAGTAGAAAAACAATGGTACAAAGC
<i>lrp5</i>	low density lipoprotein receptor-related protein 5	- expressed in mouse developping ovary and oocytes	15	Genbank: CX033605	TGGAGACAAGAGGAGGGAGA	ACTGCAGGGAGTTGGATGT
<i>lrp6</i>	low density lipoprotein receptor-related protein 6	<ul style="list-style-type: none"> - expressed in mouse developping ovary and oocytes - in granulosa cells 	15	Genbank: BX879658	ACCCGGGAAGAGGGTACAT	TTGTCAATGATCATGGCTCTGT
<i>tcf7</i>	<i>t-cell factor 7</i>	- over-expressed in differentiating ovary in rainbow trout	25	Genbank: JN187948	GCATCATTCACCCAGGTTCT	TTCAGTGTGAGGCAGTCCAG
<i>tcf7l2</i>	transcription factor 7-like 2 (T-cell specific, HMG-box)	unknown		Genbank: CA343767	CCCTAACACGAGAGGAGCAG	CTGGGTAGAGCTGCATGTGA
<i>lef1</i>	lymphoid enhancer-binding factor 1	- expressed in granulosa cells	15	Genbank: CX036511	TTACAGGACGGAATTAAATTGG	CCTGTATTGTGGCAGTGACATT
<i>axin2</i>	<i>axin2</i>	- target gene of the canonical Wnt pathway, over-expressed in differentiating ovary	20; 26	Genbank: CU071534	GTGGATCCTGGAGAGTGACC	GCCACCATACGCCTTCTTAG

<i>ctnnb1</i>	<i>beta-catenin</i>	<ul style="list-style-type: none"> - implicated in ovarian differentiation - expressed in granulosa cells during folliculogenesis - promotes preovulatory follicular development - activated during testis development - promotes proliferation and stemness regulation of spermatogonial stem/progenitor cells 	27; 28 29 30	Genbank: CA341830	CACAGAGCTGCTTCACTCCA	GTGAGCTCCACAGACAGACG
<i>fst</i>	<i>follicle-stimulating hormone receptor</i>	<ul style="list-style-type: none"> - implicated in ovarian differentiation, acts downstream of Wnt4 - expressed in rainbow trout differentiating ovary 	20; 28; 31 32	Genbank: CA364762	ACAAAGACGAGTGCGCGTTGCT	CGGCAGGTCTTCTTGCATTGTC

References (Ref):

- 1- Ricken A, Lochhead P, Kontogiannia M, Farookhi R: Wnt signaling in the ovary: identification and compartmentalized expression of wnt-2, wnt-2b, and frizzled-4 mRNAs. *Endocrinology* 143:2741-2749 (2002).
- 2- Wang HX, Tekpetey FR, Kidder GM: Identification of WNT/beta-CATENIN signaling pathway components in human cumulus cells. *Mol Hum Reprod* 15:11-17 (2009).
- 3- Wang HX, Li TY, Kidder GM: WNT2 regulates DNA synthesis in mouse granulosa cells through beta-catenin. *Biol Reprod* 82:865-875 (2010).
- 4- Vainio S, Heikkila M, Kispert A, Chin N, McMahon AP: Female development in mammals is regulated by Wnt-4 signalling. *Nature* 397:405-409 (1999).
- 5- Smith CA, Shoemaker CM, Roeszler KN, Queen J, Crews D et al: Cloning and expression of *R-Spondin1* in different vertebrates suggests a conserved role in ovarian development. *BMC Dev Biol* 8:72 (2008).

- 6- Tripathi V, Raman R: Identification of *Wnt4* as the ovary pathway gene and temporal disparity of its expression vis-a-vis testis genes in the garden lizard, *Calotes versicolor*. *Gene* 449:77-84 (2010).
- 7- Yu H, Pask AJ, Shaw G, Renfree MB: Differential expression of WNT4 in testicular and ovarian development in a marsupial. *BMC Dev Biol* 6:44. (2006).
- 8- Wu GC, Chang CF: wnt4 Is associated with the development of ovarian tissue in the protandrous black Porgy, *Acanthopagrus schlegeli*. *Biol Reprod*. 81:1073-1082 (2009).
- 9- Jordan BK, Shen JH, Olaso R, Ingraham HA, Vilain E: Wnt4 overexpression disrupts normal testicular vasculature and inhibits testosterone synthesis by repressing steroidogenic factor 1/beta-catenin synergy. *Proc Natl Acad Sci U S A* 100:10866-10871 (2003).
- 10- Heikkilä M, Prunskaitė R, Naillat F, Itäranta P, Vuoristo J, Leppäluoto J, Peltoketo H, Vainio S: The partial female to male sex reversal in Wnt-4-deficient females involves induced expression of testosterone biosynthetic genes and testosterone production, and depends on androgen action. *Endocrinology* 146:4016-4023 (2005).
- 11- Hsieh M, Johnson MA, Greenberg NM, Richards JS: Regulated expression of Wnts and Frizzleds at specific stages of follicular development in the rodent ovary. *Endocrinology* 143:898-908 (2002).
- 12- Boyer A, Lapointe E, Zheng X, Cowan RG, Li H, Quirk SM, DeMayo FJ, Richards JS, Boerboom D: WNT4 is required for normal ovarian follicle development and female fertility. *FASEB J* 24:3010-3025 (2010).
- 13- Jeays-Ward K, Dandonneau M, Swain A: Wnt4 is required for proper male as well as female sexual development. *Dev Biol* 276:431-440 (2004).
- 14- Bouma GJ, Hart GT, Washburn LL, Recknagel AK, Eicher EM: Using real time RT-PCR analysis to determine multiple gene expression patterns during XX and XY mouse fetal gonad development. *Gene Expr Patterns* 5:141-149 (2004).
- 15- Harwood BN, Cross SK, Radford EE, Haac BE, De Vries WN: Members of the WNT signaling pathways are widely expressed in mouse ovaries,

oocytes, and cleavage stage embryos. Dev Dyn 237:1099-1111 (2008).

16- Cory AT, Boyer A, Pilon N, Lussier JG, Silversides DW: Presumptive pre-Sertoli cells express genes involved in cell proliferation and cell signalling during a critical window in early testis differentiation. Mol Reprod Dev 74:1491-1504 (2007).

17- Tao Q, Yokota C, Puck H, Kofron M, Birsoy B et al: Maternal *wnt11* activates the canonical wnt signaling pathway required for axis formation in *Xenopus* embryos. Cell 120:857-871 (2005).

18- Kocer A, Pinheiro I, Pannetier M, Renault L, Parma P, Radi O, Kim KA, Camerino G, Pailhoux E: R-spondin1 and FOXL2 act into two distinct cellular types during goat ovarian differentiation. BMC Dev Biol 8:36 (2008).

19- Zhang Y, Li F, Sun D, Liu J, Liu N et al: Molecular analysis shows differential expression of *R-spondin1* in zebrafish (*Danio rerio*) gonads. Mol Biol Rep 38:275-282 (2011).

20- Chassot AA, Ranc F, Gregoire EP, Roepers-Gajadien HL, Taketo MM et al: Activation of beta-catenin signaling by Rspo1 controls differentiation of the mammalian ovary. Hum Mol Genet 17:1264-1277 (2008).

21- Parma P, Radi O, Vidal V, Chaboissier MC, Dellambra E et al: R-spondin1 is essential in sex determination, skin differentiation and malignancy. Nat Genet 38:1304-1309 (2006).

22- Tomizuka K, Horikoshi K, Kitada R, Sugawara Y, Iba Y et al: R-spondin1 plays an essential role in ovarian development through positively regulating Wnt-4 signaling. Hum Mol Genet 17:1278-1291 (2008).

23- Tomaselli S, Megiorni F, Lin L, Mazzilli MC, Gerrelli D, Majore S, Grammatico P, Achermann JC: Human RSPO1/R-spondin1 is expressed during early ovary development and augments β -catenin signaling. PLoS One 6:16366 (2011).

24- Hsieh M, Boerboom D, Shimada M, Lo Y, Parlow AF, Luhmann UF, Berger W, Richards JS: Mice null for *Frizzled4* (*Fzd4*^{-/-}) are infertile and exhibit impaired corpora lutea formation and function. Biol Reprod 73:1135-1146 (2005).

25- Cavileer T, Hunter S, Okutsu T, Yoshizaki G, Nagler JJ: Identification of novel genes associated with molecular sex differentiation in the embryonic gonads of rainbow trout (*Oncorhynchus mykiss*). Sex Dev 3:214-224 (2009).

26- Lustig B, Jerchow B, Sachs M, Weiler S, Pietsch T et al: Negative feedback loop of Wnt signaling through upregulation of *conductin/axin2* in colorectal and liver tumors. Mol Cell Biol 22:1184-1193 (2002).

- 27- Maatouk DM, DiNapoli L, Alvers A, Parker KL, Taketo MM et al: Stabilization of beta-catenin in XY gonads causes male-to-female sex-reversal. Hum Mol Genet 17:2949-2955 (2008).
- 28- Liu CF, Bingham N, Parker K, Yao HH: Sex-specific roles of beta-catenin in mouse gonadal development. Hum Mol Genet 18:405-417 (2009).
- 29- Fan HY, O'Connor A, Shitanaka M, Shimada M, Liu Z et al: Beta-catenin (CTNNB1) promotes preovulatory follicular development but represses LH-mediated ovulation and luteinization. Mol Endocrinol 24:1529-1542 (2010).
- 30- Golestaneh N, Beauchamp E, Fallen S, Kokkinaki M, Uren A, Dym M: Wnt signaling promotes proliferation and stemness regulation of spermatogonial stem/progenitor cells. Reproduction 138:151-162 (2009).
- 31- Yao HH, Matzuk MM, Jorgez CJ, Menke DB, Page DC et al: Follistatin operates downstream of Wnt4 in mammalian ovary organogenesis. Dev Dyn 230:210-215 (2004).
- 32- Vizziano D, Randuineau G, Baron D, Cauty C, Guiguen Y: Characterization of early molecular sex differentiation in rainbow trout, *Oncorhynchus mykiss*. Dev Dyn 236:2198-2206 (2007).

