Published in final edited form as:

Mol Cell Endocrinol. 2005 January 31; 230(0): 87–93. doi:10.1016/j.mce.2004.11.003.

The pathway to femaleness: current knowledge on embryonic development of the ovary

Humphrey Hung-Chang Yao*

Department of Veterinary Biosciences, University of Illinois at Urbana-Champaign, IL 61802, USA

Abstract

Increasing evidence indicates that organogenesis of the ovary is not a passive process arising by default in the absence of the testis pathway. A coordinated interaction is actually in force between somatic cells and female germ cells in embryonic ovaries, thus creating a unique microenvironment that facilitates the formation of follicles. Identification of the functional roles of several novel regulatory elements such as *Figα*, *Foxl2*, *follistatin*, and *Wnt4* reveals the complexity of early ovarian organization. Challenges await us to establish the molecular connections of these molecules as well as to discover new candidates in the pathway of early ovarian development.

Keywords

Sex determination; Gonad; Ovary; Follicle; Germ cells; Oocyte; Estrogen; Follistatin; *Figα*; *Foxl2*; *Wnt4*

1. Introduction

Species with sexual reproduction develop distinct mechanisms to establish maleness and femaleness. In mammals, sex is determined at the time of fertilization based on the chromosomal composition of the sperm (X or Y). The presence or absence of the Y chromosome decides the sex of the gonads (XX for the ovary and XY for the testis), which eventually produce hormones to sculpture the phenotypic sex of the embryo. In the male or XY individual, the *Sry* gene (Sex-determining region of the Y chromosome) induces differentiation of discrete cell lineages in the gonad that eventually leads to organization of the testis. Genetic experiments have unveiled a complex cascade of signaling and morphogenic events downstream of *Sry* (Swain et al., 1996; Tilmann and Capel, 2002; Brennan and Capel, 2004). In contrast to the testis, genetic mechanisms underlying early ovarian development remain a mystery in biology. The goals of this review are to summarize current knowledge on organogenesis of the mammalian ovary using the mouse as an example, which is the most studied model in the field. Specific focuses will be made from the stage of initial gonad formation in embryos to the establishment of the primordial follicles around birth.

2. Building the foundation: formation of the gonad

Before the onset of sex determination, a “unisex” gonad develops in both female and male embryos. This “unisex” gonad is also referred to as “bipotential” because of its ability to develop into a testis or an ovary depending upon the genetic makeup of the embryo. The bipotential gonads in male and female embryos are morphologically indistinguishable and are established by similar molecular mechanisms. In mice, the bipotential gonad arises on the surface of the mesonephros at mid-gestation (embryonic day 10 or E10). Both gonad and mesonephros are derived from the intermediate mesoderm, which runs longitudinally on the dorsal aspect of the coelomic cavity. At around E10, the coelomic epithelium starts to thicken on the ventro-medial surface of the mesonephros to form the gonad. The initial process of gonad formation is regulated by several transcription factors such as *Emx2* (Miyamoto et al., 1997), *Wilms tumor 1 (Wt1)* (Kriedberg et al., 1993), *Lhx9* (Birk et al., 2000), and *steroidogenic factor 1 (Sf1)* (Luo et al., 1994). Null mutations of any of these genes result in similar phenotypes with degeneration of gonads in both sexes, indicating that these transcription factors either work synergistically or form a hierarchical pathway to initiate and/or to maintain the differentiation of the bipotential gonad. These transcription factors appear to act on somatic cells, but not germ cells, to trigger the establishment of gonadal primordia. In embryos that the germ cell population is lost during development, bipotential gonads still form normally (Merchant, 1975; McLaren, 1984), demonstrating that somatic cells are the key player that set up the stage for the dimorphic development of gonads.

3. Parting the way: sexually dimorphic development of the gonad

The drama of sexually dimorphic development commences soon after the bipotential gonad is established. Although the bipotential gonads are morphologically indistinguishable between E10.5–12.0, divergence at the molecular level has already begun. Most notably, the XY gonad starts to express *Sry* at ~E10.5–11.0, the only gene on the Y chromosome required for initiation of testis development (Gubbay et al., 1992; Hawkins et al., 1992). *Sry* triggers differentiation of the Sertoli cell lineage, initiating a cascade of events to coordinate the construction of the testicular architecture and specification of other somatic cell lineages. In contrast, in the XX embryo where the *Sry* gene is absent, the bipotential gonad appears relatively quiescent with no obvious tissue remodeling or cell differentiation with the exception of germ cells (discussed below). Due to this dominant role of *Sry*, sex determination in most mammals is referred to as “testis” determination. The ovary only arises when the *Sry*-induced testis pathway is absent or loses its command. Tremendous progress has been made to identify molecular and cellular components in the testis pathway. However, mechanisms for ovarian development remain a black box.

4. Ovary-determining gene versus the Z theory

The finding of *Sry* for testis determination leads to the assumption that an ovarian counterpart of *Sry* must exist for ovarian determination. Thus far, the ovary-determining gene has not been identified. Nonetheless, it is premature to claim that ovarian development is a passive or “default” process. Considering that the ovary is as structurally and

functionally complex as other organs, there is little doubt that there must be an active, genetic pathway controlling its formation (Eicher and Washburn, 1986).

Dax1 (dosage sensitive sex-reversal-adrenal hypoplasia congenital-critical region of the X chromosome gene1) was originally considered as the candidate for the ovary-determining gene because of its location on the X chromosome and its ovary-specific expression (Swain et al., 1996; Muscatelli et al., 1994; Zanaria et al., 1994). However, knockout and transgenic studies in mice indicate that *Dax1* is not required for normal ovarian development (Yu et al., 1998). Ironically, it is critical for testicular development. Null mutation of *Dax1* has little effect on formation of the ovary; in contrast, it causes severe testicular dysgenesis (Jeffs et al., 2001; Meeks et al., 2003a, 2003b). Furthermore, when the *Dax1* null allele is crossed to a mouse strain carrying a hypomorphic *Sry* allele (*Mus domesticus poschiavinus*, Y^{Pos}), a complete male to female sex reversal arises (Meeks et al., 2003a, 2003b). This evidence strongly disputes the possible role of *Dax1* in ovarian development. A few other genes also exhibit an ovary-specific expression pattern but so far none of them have been implicated in the initial formation of the ovary (Bullejos et al., 2002; Menke and Page, 2002).

Alternatively, a “Z” gene hypothesis is proposed to explain the cases where XX individuals develop testes in the absence of *Sry*. McElreavey and colleagues postulate that in these affected XX individuals, a hypothetical “Z” gene is mutated (McElreavey et al., 1993). This Z gene is normally expressed in the XX gonad to repress the testis pathway and therefore allows ovarian development, whereas in the XY gonad, the Z gene is suppressed by SRY (inhibitor of an inhibitor) resulting in emergence of the testis pathway. When the Z gene in the XX gonad is inactivated or mutated as in the female-to-male sex reversal cases, testis development occurs even without the *Sry*. The Polled/intersex syndrome (PIS) in goats is a perfect example of this case. Two genes (*Pisrti* and *Foxl2*) have been linked to PIS and one of these genes *Foxl2*, a forkhead transcription factor, emerges as a potential candidate for the “Z” gene (Pailhoux et al., 1994, 2001, 2002, 2003; Vaiman et al., 1999; Nikic and Vaiman, 2004; Pannetier et al., 2003).

Foxl2 is expressed in an ovary-specific manner not only in mouse embryos, but also in chickens, turtles, and fish at the time of sex determination (Loffler et al., 2003; Wang et al., 2004), suggesting a conserved role of *Foxl2* in vertebrate ovarian development. In humans, *FOXL2* is linked to BPES (Blepharophimosis Ptosis Epicanthus inversus Syndrome), which in some cases is associated with premature ovarian failure (Harris et al., 2002; Kosaki et al., 2002; De Baere et al., 2003; Fokstuen et al., 2003; Loffler et al., 2003; Udar et al., 2003). However, unlike PIS in goats, female to male sex-reversal is never reported in BPES cases. Similarly, null mutations of *Foxl2* in mice do not affect initial ovary formation (Schmidt et al., 2004; Uda et al., 2004), indicating that at least in humans and mice, *Foxl2* is not the putative Z gene.

Dax1 and *Wnt4* are the other candidates that fit the profile of the Z gene. These two genes are able to antagonize testis development in a dosage sensitive manner in humans (Swain et al., 1996; Jordan et al., 2001). However, null mutation of *Dax1* or *Wnt4* in mice does not cause female to male sex reversal (Yu et al., 1998; Vainio et al., 1999), a critical feature of the loss of the Z gene. *Wnt4* also has another “anti-male” property based on the initial

finding that androgen-producing Leydig cells appeared ectopically in *Wnt4* null XX gonad (Vainio et al., 1999). But further investigation revealed that the steroid-producing cells in *Wnt4* null XX gonads were not Leydig cells; instead, they express markers specific for adrenal steroidogenic cells. In addition, these ectopic adrenal cells are present in both XX and XY gonads in the absence of *Wnt4* (Heikkila et al., 2002). Precursors of adrenal cells and Leydig cells both derive from the primitive adrenal-gonadal primordium. *Wnt4* is likely to regulate proper separation of adrenal cells from the gonadal primordium and does not repress fetal Leydig cell differentiation in the ovary. Although no apparent ovary-determining factor or Z factor has been identified, it neither approves nor disputes these two hypotheses. A far more complex pathway probably exists, which may possess the characteristics of both hypothetical factors (discussed below).

5. Female germ cells: the key to femaleness

Sexually dimorphic development of the gonads creates two distinct somatic environments that support maturation of male and female gametes. The somatic environment of the testis is established immediately after the onset of sex determination whereas the ovarian structure does not appear until birth. The most striking difference between testis and ovary organization is the involvement of germ cells. Testis development progresses normally in the absence of germ cells (Merchant, 1975). In contrast, when germ cells are absent from the XX gonad, ovarian follicles, the functional units in the ovary, never form (McLaren, 1984). Additionally, if germ cells are lost after formation of follicles, follicles rapidly degenerate (Behringer et al., 1990; Hashimoto et al., 1990; Couse et al., 1999). Germ cells appear to be a critical regulator in organizing and maintaining the ovarian structure.

The sex-specific role of germ cells in gonad development may result from the dimorphic development pattern of the germ cells. Female germ cells follow an intrinsic clock to enter the first meiosis around E13.5 and arrest at birth at dictyate of meiosis (Fig. 1) (McLaren, 1984). Conversely, male germ cells are prevented from entering meiosis after they are enclosed in testis cords, where they will resume meiosis at puberty. It has been postulated that the XY gonad has to prevent germ cells from entering meiosis because meiotic germ cells may interfere with initial testis formation. Indeed, some testis-specific events such as migration of mesonephric cells and formation of testis cords can be induced in XX gonads only before germ cells enter meiosis. But once germ cells enter meiosis, the XX gonad loses its plasticity and no longer allows these events to occur (Tilman and Capel, 1999; Yao et al., 2003). These observations suggest that meiotic germ cells antagonize the occurrence of the certain testis events in the XX gonad, which in turn ensure the progression of ovarian development. Furthermore, *Sry* in the testis has to operate earlier to “beat” the intrinsic meiotic clock to ensure the proper progression of testicular pathways.

As mentioned earlier, female germ cells enter meiosis following an intrinsic clock. When germ cells (regardless the sex) develop in ectopic regions such as adrenal glands or the mesonephros (Zamboni and Upadhyay, 1983), or when they are assembled in lung aggregates in culture (McLaren and Southee, 1997), they still enter meiosis around E13.5, the same time as they do in the ovary. This indicates that germ cells will enter meiosis independent of the somatic environment with the exception of that in the testis. However,

recent studies reveal that germ cells in the XX gonad do not enter meiosis synchronously (Menke et al., 2003; Yao et al., 2003; Bullejos and Koopman, 2004). They progress into meiosis in a subtle anterior-to-posterior pattern in parallel with the expression of certain markers in somatic cells. It is therefore proposed that somatic cell environment influences meiotic entry of female germ cells, further challenging the current “intrinsic clock” model. It remains to be examined whether this anterior-to-posterior pattern of germ cell meiosis is controlled by somatic cues or it simply reflects the developmental status of female germ cells (i.e., germ cells in the anterior may migrate into the gonad earlier and are more mature than their posterior counterparts, Molyneaux et al., 2001).

Once female germ cells enter the first meiosis, they are committed to the ovarian fate and develop into oocytes. Perinatally, individual oocyte becomes surrounded by a single layer of squamous granulosa cells to form the primordial follicle. Not surprisingly, this first stage of folliculogenesis is controlled by oocytes. An oocyte-specific basic helix-loop-helix transcription factor, *Figa* (factor in germ line a), is essential for the recruitment of granulosa cells to form the primordial follicle. Without *Figa*, primordial follicles never form and massive depletion of oocytes occurs after birth (Soyal et al., 2000). *Figa* stimulates the expression of zona pellucida proteins (ZP1, 2, and 3) in the oocyte (Liang et al., 1997), which are the components of the extracellular glycoprotein matrix deposited between the oocyte and its surrounding granulosa cells. Because of its unique juxtaposed localization, the zona pellucida is thought to be critical for the coupling of oocyte and granulosa cells. It is therefore proposed that absence of zona pellucida may cause the failure to form primordial follicles in the *Figa* null ovary. However, loss of either one of the three ZP proteins does not affect formation of primordial follicles. It is possible that the remaining zona pellucida may still be able to recruit granulosa cells in the absence of one ZP protein. Alternatively, *Figa* could regulate other oocyte-specific genes to facilitate primordial follicle formation.

Figa is expressed in female, but not male germ cells, starting around E13.5, when sexual dimorphism of germ cell meiosis occurs (Fig. 1). This unique temporal relationship between *Figa* expression and germ cell meiosis implies a functional connection between these two events. However, *Figa* appears not to be involved in meiosis because female germ cells in *Figa* null ovaries progress into first meiosis normally (Soyal et al., 2000). It remains unknown whether the female-specific expression of *Figa* is a germ-cell autonomous event following meiosis or it is regulated independently by somatic cells.

While *Figa* activates the folliculogenesis program in oocytes, another transcription factor, *Foxl2*, become active in somatic cells, specifically pregranulosa cells. Although *Foxl2* appears to be nonessential for early formation of the ovary as discussed above, it is critical for proper differentiation of granulosa cells during folliculogenesis. Null mutations of *Foxl2* in mice recapitulate the human BPES cases with premature ovarian failure. In *Foxl2* null ovaries, granulosa cells in primordial follicles fail to undergo squamous to cuboidal transition leading to arrest of folliculogenesis and early depletion of the follicular pool (Schmidt et al., 2004; Uda et al., 2004). Abnormal oocyte development and loss of steroidogenic cells in the interstitium were also observed. These genetic evidence indicate that loss of *Foxl2* could affect proper differentiation of granulosa cells and other cell types in the ovary, resulting in loss of follicular pool and consequent premature ovarian failure.

6. Emerging pathways in early development of mouse ovary

In contrast to the testis-determining pathway, which is orchestrated by pre-Sertoli cells, female somatic cells (pregranulosa cells) seem to play little or no role in early ovarian development until folliculogenesis starts. However, recent findings indicate that somatic cells are initiators of a novel signaling pathway critical for early ovary development. Somatic cells in the XX, but not XY gonad, express *Wnt4* and *folliculin* at the time of sex determination (Vainio et al., 1999; Menke and Page, 2002). *Wnt4* is further shown to be the upstream regulator that induces the expression of *folliculin* based on epistatic analysis (Yao et al., 2004). In addition, similar defects arise in *Wnt4* and *folliculin* null ovaries, indicating that these two molecules are constituents of a common signaling cascade.

Loss of *Wnt4* or *folliculin* causes two unique defects in ovaries. A testis-specific vessel, referred to as the coelomic vessel, appears on the surface of the *Wnt4* or *folliculin* null ovaries (Jeays-Ward et al., 2003; Yao et al., 2004). This coelomic vessel is an early morphological landmark of testis development and is normally absent in the ovary. WNT4 and folliculin appear to inhibit a specific aspect of the testis pathway in the ovary (Fig. 1). Another phenotype in ovaries from *Wnt4* or *folliculin* null mice is the loss of germ cells. Without *Wnt4* or *folliculin*, female germ cells enter meiosis normally but undergo massive apoptosis at E16.5, leading to loss of the entire population at birth. Intriguingly, germ cell apoptosis normally occurs in the medullary region of the ovary, whereas in the affected ovaries, apoptotic germ cells appear in both medullary and cortical regions (Vainio et al., 1999; Yao et al., 2004). WNT4 and folliculin produced by somatic cells seem to create a protective niche in the ovarian cortex to maintain survival of female germ cells, which may explain why under normal circumstances, follicle formation occurs primarily in the cortex of the ovary.

The WNT4/folliculin signaling cascade is unique in that it has both anti-testis (inhibition of the coelomic vessel) and pro-ovary properties (survival of meiotic germ cells). *Wnt4* and *folliculin* do not completely fit the profile of the putative “Z” factors because they antagonize only one aspect of testis development. *Wnt4* and *folliculin* do have properties consistent with the ovary-determining gene such that they maintain survival of female germ cells, which are the organizers of the ovarian follicles. Nevertheless, these two molecules constitute the first organized signaling cascade in early ovarian development. The somatic cell-origin of these two molecules in the ovary further defines a cellular pathway comparable to that in testis development, which is dictated by somatic cells.

7. Maintenance of ovarian differentiation: essential roles of estrogen

Eutherian mammals have evolved a distinct sex-determination mechanism entirely different from other vertebrate species. Sexes of the eutherian mammals are genetically controlled and completely insensitive to sex steroids such as estrogen and androgen, whereas in fish, amphibians, reptiles, birds, and marsupials, sex steroids control gonadal sexes, especially the fate of the ovary. In these species, estrogen is the ovary-determining factor. Estrogen treatment of male embryos during the critical period of development in fish (Guiguen et al., 1999; Kobayashi et al., 2003), frogs (Mackenzie et al., 2003; Kato et al., 2004), reptiles

(Pieau and Dorizzi, 2004), chicks (Abinawanto et al., 1996; Mittwoch, 1998; Akazome and Mori, 1999), or in marsupial newborns (Shaw et al., 1988; Baker et al., 1993; Fadem, 2000; Coveney et al., 2001; Renfree et al., 2001) can cause testis-to-ovary sex reversal. In addition, inhibition of estrogen synthesis in female embryos leads to testis development in these species. On the other hand, in eutherian mammals, XY embryos do not develop ovaries in response to estrogen treatment (Greene et al., 1940). Neither does blockage of estrogen action in XX embryos cause testis formation (Couse et al., 1999; Dupont et al., 2000; Britt et al., 2004). Considering that eutherian embryos are constantly exposed to the maternal-derived steroids, they must evolve a sex-determination mechanism independent of the influence of steroids.

However, once maternal influences wane after birth, estrogen regains its power in controlling the fate of eutherian ovaries. In the absence of estrogen signaling by either blocking its production (aromatase knockout or ArKO) (Britt et al., 2004) or removing its receptors (ER α β KO) (Couse et al., 1999; Dupont et al., 2000), ovaries form normally but start to develop testicular tissues after puberty. Granulosa cells in maturing follicles progressively trans-differentiate into Sertoli cells that express Sertoli cell markers such as *Sox9* (Dupont et al., 2003). Seminiferous tubule-like structures subsequently emerge and cells resembling Leydig cells appear in the interstitium (Britt et al., 2002, 2004; Britt and Findlay, 2003). Clearly estrogen signaling is critical in maintaining the ovarian structure in the mature ovary by suppressing the emergence of the testicular pathway. These data are consistent with the idea that initial formation of eutherian ovaries does not require estrogen. They also reveal that estrogen, which is the ovary-determining factor conserved among other vertebrates, becomes active again in adult eutherian ovaries. Further comparative studies should provide more insights into the evolutionary process of this unique system in eutherian ovaries.

8. Summary

We have just begun to glimpse into the mechanisms underlying ovarian development. Convincing evidence challenges us to reconsider the existing paradigm that describes ovarian development as a default system. The default concept was first proposed in the early 1950s when Jost performed the groundbreaking experiments to demonstrate mechanisms of sex differentiation of reproductive tracts (Jost, 1947, 1953, 1970). The term “default” was not originally intended to describe the developmental status of the ovary. Instead, it is referred to the female reproductive tract or the Mullerian duct based on the fact that the female reproductive tract forms in both XX and XY individuals in the absence of gonads. Indeed, now it has become evident that early ovarian development is an active process involving intrinsic cell fate decisions and complex crosstalks between germ cells and somatic cells. Most intriguingly, the appearance of testicular structures in XX individuals where *Sry* and its downstream components are absent further raises the improbable question: Could the testicular development be default after all?

Undoubtedly, we need more efforts and tools to reexamine the current paradigm and to complete the picture of ovarian development. We have added several novel constituents to the ovarian pathway (Fig. 1) but at the same time, raised more questions than answers. Does

the elusive “ovary-determining” factor exist? What controls the sexually dimorphic development of germ cells? How does the Wnt4/follistatin pathway elicit its anti-testis and pro-ovary effects? How does estrogen signaling suppress testicular development in adult ovaries? With the advancement of modern genetic techniques, we are in a great position to answer these questions and hopefully, to put together all the missing pieces in the pathway to femaleness.

Acknowledgments

I would like to thank Drs. Janice Bahr and Joan Jorgensen and people in the lab for their critical comments on the manuscript and the support from the March of Dimes Birth Defects Foundation (Basil O'Connor Starting Scientist Award) and grants from Research Board and Veterinary Medical Research Funds at University of Illinois.

References

- Abinawanto, Shimada K, Yoshida K, Saito N. Effects of aromatase inhibitor on sex differentiation and levels of P450 (17 alpha) and P450 arom messenger ribonucleic acid of gonads in chicken embryos. *Gen Comp Endocrinol*. 1996; 102:241–246. [PubMed: 8998968]
- Akazome Y, Mori T. Evidence of sex reversal in the gonads of chicken embryos after oestrogen treatment as detected by expression of lutropin receptor. *J Reprod Fertil*. 1999; 115:9–14. [PubMed: 10341717]
- Baker PJ, Moore HD, Burgess AM, Mittwoch U. Gonadal sex differentiation in embryos and neonates of the marsupial. *Monodelphis domestica*: arrest of testis development in postterm embryos. *J Anat*. 1993; 182 (Pt 2):267–273. [PubMed: 8376201]
- Behringer RR, Cate RL, Froelick GJ, Palmiter RD, Brinster RL. Abnormal sexual development in transgenic mice chronically expressing Mullerian inhibiting substance. *Nature*. 1990; 345:167–170. [PubMed: 2336108]
- Birk O, Casiano D, Wassif C, Cogliati T, Zhao L, Zhao Y, Grinberg A, Huang S, Kreidberg J, Parker K, Porter F, Westphal H. The LIM homeobox gene *Lhx9* is essential for mouse gonad formation. *Nature*. 2000; 403:909–913. [PubMed: 10706291]
- Brennan J, Capel B. One tissue, two fates: molecular genetic events that underlie testis versus ovary development. *Nat Rev Genet*. 2004; 5:509. [PubMed: 15211353]
- Britt KL, Findlay JK. Regulation of the phenotype of ovarian somatic cells by estrogen. *Mol Cell Endocrinol*. 2003; 202:11–17. [PubMed: 12770724]
- Britt KL, Kerr J, O'Donnell L, Jones ME, Drummond AE, Davis SR, Simpson ER, Findlay JK. Estrogen regulates development of the somatic cell phenotype in the eutherian ovary. *FASEB J*. 2002; 16:1389–1397. [PubMed: 12205030]
- Britt KL, Stanton PG, Misso M, Simpson ER, Findlay JK. The effects of estrogen on the expression of genes underlying the differentiation of somatic cells in the murine gonad. *Endocrinology*. 2004; 145:3950–3960. [PubMed: 15142992]
- Bullejos M, Bowles J, Koopman P. Extensive vascularization of developing mouse ovaries revealed by caveolin-1 expression. *Dev Dyn*. 2002; 225:95–99. [PubMed: 12203725]
- Bullejos M, Koopman P. Germ cells enter meiosis in a rostrocaudal wave during development of the mouse ovary. *Mol Reprod Dev*. 2004; 68:422–428. [PubMed: 15236325]
- Couse JF, Hewitt SC, Bunch DO, Sar M, Walker VR, Davis BJ, Korach KS. Postnatal sex reversal of the ovaries in mice lacking estrogen receptors alpha and beta. *Science*. 1999; 286:2328–2331. [PubMed: 10600740]
- Coveney D, Shaw G, Renfree MB. Estrogen-induced gonadal sex reversal in the tammar wallaby. *Biol Reprod*. 2001; 65:613–621. [PubMed: 11466233]
- De Baere E, Beyesen D, Oley C, Lorenz B, Cocquet J, De Sutter P, Devriendt K, Dixon M, Fellous M, Fryns JP, Garza A, Jonsrud C, Koivisto PA, Krause A, Leroy BP, Meire F, Plomp A, Van Maldergem L, De Paepe A, Veitia R, Messiaen L. *Foxl2* and BPES: mutational hotspots,

- phenotypic variability, and revision of the genotype-phenotype correlation. *Am J Hum Genet.* 2003; 72:478–487. [PubMed: 12529855]
- Dupont S, Dennefeld C, Krust A, Chambon P, Mark M. Expression of Sox9 in granulosa cells lacking the estrogen receptors, ERalpha and ERbeta. *Dev Dyn.* 2003; 226:103–106. [PubMed: 12508230]
- Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P, Mark M. Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. *Development.* 2000; 127:4277–4291. [PubMed: 10976058]
- Eicher EM, Washburn LL. Genetic control of primary sex determination in mice. *Ann Rev Genet.* 1986; 20:327–360. [PubMed: 3545061]
- Fadem BH. Perinatal exposure to estradiol masculinizes aspects of sexually dimorphic behavior and morphology in gray short-tailed opossums (*Monodelphis domestica*). *Horm Behav.* 2000; 37:79–85. [PubMed: 10712860]
- Fokstuen S, Antonarakis SE, Blouin JL. *Foxl2*-mutations in blepharophimosis-ptosis-epicanthus inversus syndrome (BPES); challenges for genetic counseling in female patients. *Am J Med Genet.* 2003; 117A:143–146. [PubMed: 12567411]
- Greene R, Burrill M, Ivy A. Experimental intersexuality. the effects of oestrogens on the antenatal development of the rat. *Am J Anat.* 1940:305–345.
- Gubbay J, Vivian N, Economou A, Jackson D, Goodfellow P, Lovell BR. Inverted repeat structure of the *Sry* locus in mice. *Proc Natl Acad Sci USA.* 1992; 89:7953–7957. [PubMed: 1518820]
- Guiguen Y, Baroiller JF, Ricordel MJ, Iseki K, McMeel OM, Martin SA, Fostier A. Involvement of estrogens in the process of sex differentiation in two fish species: the rainbow trout (*Oncorhynchus mykiss*) and a tilapia (*Oreochromis niloticus*). *Mol Reprod Dev.* 1999; 54:154–162. [PubMed: 10471475]
- Harris SE, Chand AL, Winship IM, Gersak K, Aittomaki K, Shelling AN. Identification of novel mutations in *Foxl2* associated with premature ovarian failure. *Mol Hum Reprod.* 2002; 8:729–733. [PubMed: 12149404]
- Hashimoto N, Kubokawa R, Yamazaki K, Noguchi M, Kato Y. Germ cell deficiency causes testis cord differentiation in reconstituted mouse fetal ovaries. *J Exp Zool.* 1990; 253:61–70. [PubMed: 2313242]
- Hawkins JR, Taylor A, Berta P, Levilliers J, Van Der Auwere B, Goodfellow PN. Mutational analysis of *SRY*: nonsense and missense mutations in XY sex reversal. *Hum Genet.* 1992; 88:471–474. [PubMed: 1339396]
- Heikkila M, Peltoketo H, Leppaluoto J, Lives M, Vuolteenaho O, Vainio S. Wnt-4 deficiency alters mouse adrenal cortex function, reducing aldosterone production. *Endocrinology.* 2002; 143:4358–4365. [PubMed: 12399432]
- Jeays-Ward K, Hoyle C, Brennan J, Dandonneau M, Alldus G, Capel B, Swain A. Endothelial and steroidogenic cell migration are regulated by WNT4 in the developing mammalian gonad. *Development.* 2003; 130:3663–3670. [PubMed: 12835383]
- Jeffs B, Meeks JJ, Ito M, Martinson FA, Matzuk MM, Jameson JL, Russell LD. Blockage of the rete testis and efferent ductules by ectopic Sertoli and Leydig cells causes infertility in *Dax1*-deficient male mice. *Endocrinology.* 2001; 142:4486–4495. [PubMed: 11564714]
- Jordan BK, Mohammed M, Ching ST, Delot E, Chen XN, Dewing P, Swain A, Rao PN, Elejalde BR, Vilain E. Up-regulation of WNT-4 signaling and dosage-sensitive sex reversal in humans. *Am J Hum Genet.* 2001; 68:1102–1109. [PubMed: 11283799]
- Jost A. Recherches sur la différenciation sexuelle de l'embryon de lapin. *Arch Anat Microsc Morph Exp.* 1947; 36:271–315.
- Jost A. Problems of fetal endocrinology: the gonadal and hypophyseal hormones. *Recent Prog Horm Res.* 1953; 8:379–418.
- Jost A. Hormonal factors in the sex differentiation of the mammalian foetus. *Philos Trans R Soc Lond.* 1970; 259:119–130. [PubMed: 4399057]
- Kato T, Matsui K, Takase M, Kobayashi M, Nakamura M. Expression of P450 aromatase protein in developing and in sex-reversed gonads of the XX/XY type of the frog *Rana rugosa*. *Gen Comp Endocrinol.* 2004; 137:227–236. [PubMed: 15201061]

- Kobayashi T, Kajiura-Kobayashi H, Nagahama Y. Induction of XY sex reversal by estrogen involves altered gene expression in a teleost, tilapia. *Cytogenet Genome Res.* 2003; 101:289–294. [PubMed: 14684997]
- Kosaki K, Ogata T, Kosaki R, Sato S, Matsuo N. A novel mutation in the *Foxl2* gene in a patient with blepharophimosis syndrome: differential role of the polyalanine tract in the development of the ovary and the eyelid. *Ophthalmic Genet.* 2002; 23:43–47. [PubMed: 11910558]
- Kriedberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, Jaenisch R. Wt-1 is required for early kidney development. *Cell.* 1993; 74:679–691. [PubMed: 8395349]
- Liang L, Soyal SM, Dean J. FIGalpha, a germ cell specific transcription factor involved in the coordinate expression of the zona pellucida genes. *Development.* 1997; 124:4939–4947. [PubMed: 9362457]
- Loffler KA, Zarkower D, Koopman P. Etiology of ovarian failure in blepharophimosis ptosis epicanthus inversus syndrome: *Foxl2* is a conserved, early-acting gene in vertebrate ovarian development. *Endocrinology.* 2003; 144:3237–3243. [PubMed: 12810580]
- Luo X, Ikeda Y, Parker K. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell.* 1994; 77:481–490. [PubMed: 8187173]
- Mackenzie CA, Berrill M, Metcalfe C, Pauli BD. Gonadal differentiation in frogs exposed to estrogenic and antiestrogenic compounds. *Environ Toxicol Chem.* 2003; 22:2466–2475. [PubMed: 14552012]
- McElreavey K, Vilain E, Abbas N, Herskowitz I, Fellous M. A regulatory cascade hypothesis for mammalian sex determination: SRY represses a negative regulator of male development. *Proc Natl Acad Sci USA.* 1993; 90:3368–3372. [PubMed: 8475082]
- McLaren A. Meiosis and differentiation of mouse germ cells. *Symp Soc Exp Biol.* 1984; 38:7–23. [PubMed: 6400220]
- McLaren A, Southee D. Entry of mouse embryonic germ cells into meiosis. *Dev Biol.* 1997; 187:107–113. [PubMed: 9224678]
- Meeks JJ, Crawford SE, Russell TA, Morohashi K, Weiss J, Jameson JL. Dax1 regulates testis cord organization during gonadal differentiation. *Development.* 2003a; 130:1029–1036. [PubMed: 12538527]
- Meeks JJ, Weiss J, Jameson JL. Dax1 is required for testis determination. *Nat Genet.* 2003b; 34:32–33. [PubMed: 12679814]
- Menke D, Page D. Sexually dimorphic gene expression in the developing mouse gonad. *Gene Expr Patterns.* 2002; 2:359–367.
- Menke DB, Koubova J, Page DC. Sexual differentiation of germ cells in XX mouse gonads occurs in an anterior-to-posterior wave. *Dev Biol.* 2003; 262:303–312. [PubMed: 14550793]
- Merchant H. Rat gonadal and ovarioan organogenesis with and without germ cells. An ultrastructural study. *Dev Biol.* 1975; 44:1–21. [PubMed: 1132581]
- Mittwoch U. Phenotypic manifestations during the development of the dominant and default gonads in mammals and birds. *J Exp Zool.* 1998; 281:466–471. [PubMed: 9662833]
- Miyamoto N, Yoshida M, Kuratani S, Matsuo I, Aizawa S. Defects of urogenital development in mice lacking *Emx2*. *Development.* 1997; 124:1653–1664. [PubMed: 9165114]
- Molyneaux KA, Stallock J, Schaible K, Wylie C. Time-lapse analysis of living mouse germ cell migration. *Dev Biol.* 2001; 240:488–498. [PubMed: 11784078]
- Muscatelli F, Strom T, Walker A, Zanaria E, Recan D, Meindl A, Bardoni B, Guioli S, Zehetner G, Rabl W, Schwarz H, Kaplan JC, Camerino G, Meitinger T, Monaco A. Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature.* 1994; 372:672–676. [PubMed: 7990958]
- Nikic S, Vaiman D. Conserved patterns of gene expression in mice and goats in the vicinity of the Polled Intersex Syndrome (PIS) locus. *Chromosome Res.* 2004; 12:465–474. [PubMed: 15252242]
- Pailhoux E, Crihiu EP, Chaffaux S, Darre R, Fellous M, Cotinot C. Molecular analysis of 60,XX pseudohermaphrodite polled goats for the presence of SRY and ZFY genes. *J Reprod Fertil.* 1994; 100:491–496. [PubMed: 8021868]

- Pailhoux E, Vigier B, Chaffaux S, Servel N, Taourit S, Furet JP, Fellous M, Grosclaude F, Cribiu EP, Cotinot C, Vaiman D. A 11.7-kb deletion triggers intersexuality and polledness in goats. *Nat Genet.* 2001; 29:453–458. [PubMed: 11726932]
- Pailhoux E, Vigier B, Vaiman D, Servel N, Chaffaux S, Cribiu EP, Cotinot C. Ontogenesis of female-to-male sex-reversal in XX polled goats. *Dev Dyn.* 2002; 224:39–50. [PubMed: 11984872]
- Pannetier M, Servel N, Cocquet J, Besnard N, Cotinot C, Pailhoux E. Expression studies of the PIS-regulated genes suggest different mechanisms of sex determination within mammals. *Cytogenet Genome Res.* 2003; 101:199–205. [PubMed: 14684983]
- Pieau C, Dorizzi M. Oestrogens and temperature-dependent sex determination in reptiles: all is in the gonads. *J Endocrinol.* 2004; 181:367–377. [PubMed: 15171684]
- Renfree MB, Coveney D, Shaw G. The influence of estrogen on the developing male marsupial. *Reprod Fertil Dev.* 2001; 13:231–240. [PubMed: 11800162]
- Schmidt D, Ovitt CE, Anlag K, Fehsenfeld S, Gredsted L, Treier AC, Treier M. The murine winged-helix transcription factor *Foxl2* is required for granulosa cell differentiation and ovary maintenance. *Development.* 2004; 131:933–942. [PubMed: 14736745]
- Shaw G, Renfree MB, Short RV. Experimental manipulation of sexual differentiation in wallaby pouch young treated with exogenous steroids. *Development.* 1988; 104:689–701. [PubMed: 3268410]
- Soyal SM, Amleh A, Dean J. FIGalpha, a germ cell-specific transcription factor required for ovarian follicle formation. *Development.* 2000; 127:4645–4654. [PubMed: 11023867]
- Swain A, Zanaria E, Hacker A, Lovell-Badge R, Camerino G. Mouse *Dax1* expression is consistent with a role in sex determination as well as in adrenal and hypothalamus function. *Nat Genet.* 1996; 12:404–409. [PubMed: 8630494]
- Tilmann C, Capel B. Cellular and molecular pathways regulating mammalian sex determination. *Recent Prog Horm Res.* 2002; 57:1–18. [PubMed: 12017538]
- Tilmann K, Capel B. Mesonephric cell migration induces testis cord formation and Sertoli cell differentiation in the mammalian gonad. *Development.* 1999; 126:2883–2890. [PubMed: 10357932]
- Uda M, Ottolenghi C, Crisponi L, Garcia JE, Deiana M, Kimber W, Forabosco A, Cao A, Schlessinger D, Pilia G. Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. *Hum Mol Genet.* 2004
- Udar N, Yellore V, Chalukya M, Yelchits S, Silva-Garcia R, Small K. Comparative analysis of the FOXL2 gene and characterization of mutations in BPES patients. *Hum Mutat.* 2003; 22:222–228. [PubMed: 12938087]
- Vaiman D, Schibler L, Oustry-Vaiman A, Pailhoux E, Goldammer T, Stevanovic M, Furet JP, Schwerin M, Cotinot C, Fellous M, Cribiu EP. High-resolution human/goat comparative map of the goat polled/intersex syndrome (PIS): the human homologue is contained in a human YAC from HSA3q23. *Genomics.* 1999; 56:31–39. [PubMed: 10036183]
- Vainio S, Heikkila M, Kispert A, Chin N, McMahon A. Female development in mammals is regulated by Wnt-4 signaling. *Nature.* 1999; 397:405–409. [PubMed: 9989404]
- Wang D, Kobayashi T, Zhou L, Nagahama Y. Molecular cloning and gene expression of Foxl2 in the Nile tilapia, *Oreochromis niloticus*. *Biochem Biophys Res Commun.* 2004; 320:83–89. [PubMed: 15207705]
- Yao HH, DiNapoli L, Capel B. Meiotic germ cells antagonize mesonephric cell migration and testis cord formation in mouse gonads. *Development.* 2003; 130:5895–5902. [PubMed: 14561636]
- Yao HH, Matzuk MM, Jorgez CJ, Menke DB, Page DC, Swain A, Capel B. Follistatin operates downstream of Wnt4 in mammalian ovary organogenesis. *Dev Dyn.* 2004; 230:210–215. [PubMed: 15162500]
- Yu R, Ito T, Saunders S, Camper S, Jameson J. Role of Ahch in gonadal development and gametogenesis. *Nat Genet.* 1998; 20:353–357. [PubMed: 9843206]
- Zamboni L, Upadhyay S. Germ cell differentiation in mouse adrenal glands. *J Exp Zool.* 1983; 228:173–193. [PubMed: 6663256]
- Zanaria E, Muscatelli F, Bardoni B, Trom ST, Guioli S, Guo W, Lalli E, Moser C, Walker A, McCabe E, Meitinger T, Monaco A, Sassone-Corsi P, Camerino G. An unusual member of the nuclear

hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature*. 1994; 372:635–641. [PubMed: 7990953]

Fig. 1.

A time course of events during embryonic development of the mouse ovary from embryonic day 11 (E11) to birth. Female germ cells (in pink) enter meiosis I around E13.5 and arrest at the dictyate at birth. At ~E15, *Figα*, a transcription factor specific for female germ cells, begins to be expressed and is essential for production of the zona pellucida (ZP) proteins and formation of primordial follicles at birth. In the somatic cell lineage (in green), WNT4 is produced which is postulated as an autocrine factor to induce the subsequent production of follistatin (FST) starting at E11.5. WNT4 and FST antagonize the formation of testis vasculature and at the same time, maintain the survival of female germ cells at ~E16. FOXL2, a transcription factor specific for female somatic cells, start to appear at ~E12. FOXL2 is critical for further differentiation of pregranulosa cells. Horizontal bars represent the expression of specific genes and occurrence of molecular events.