Ovarian Organogenesis in Mammals: Mice Cannot Tell Us Everything

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Development • Gonad regionalisation • Interstitial gland • Mammals • Organogenesis • Ovarian evolution • Ovary

Abstract

The mammalian ovary shows extensive variation mainly in relation to the interstitial tissue of the ovary, the so-called interstitial gland, and the degree of gonad regionalisation, which implies the existence of a cortex and a medulla. Three mammalian species, mouse, human, and mole, have been reviewed here as representative animal models for ovarian variability. Whereas the human ovary may be considered to have a conventional pattern of development, the mouse and the mole represent the two extremes of the variation range. The mouse is exceptional among mammals because ovarian regionalisation is much less relevant than in other species. Contrarily, the mole ovary is very similar to that of humans regarding the cortical region, but shows a testis-like pattern of development in the medullary region. Thus, the mole ovary is in fact an ovotestis, a phenomenon also described in other mammals. Accordingly, current studies on the development of the mouse ovary are not sufficient to understand the process in a more general context, because ovarian organogenesis is exceptionally simple in the mouse. From an evolutionary perspective, mammals show a tendency to

eliminate or reduce gonad regionalisation, in contrast with the situation in other vertebrates, where this trait has been preserved. Since developmental variants may not be associated with particular taxonomic groups, their origin seems to be adaptive rather than phylogenetic. The demonstration that gonad development is a rather plastic process in mammals helps to explain how some mammals could have evolved towards more primitive gonad developmental models in response to selective pressure.

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The existence of females and males, a pre-requisite for sexual reproduction, implies that two alternative developmental paths are possible for individuals with identical or nearly identical genetic backgrounds. In many reptiles, there seems to be no genetic difference between males and females as sex is environmentally determined [see Wilkins 2002; Barske and Capel, 2008 for reviews]. In birds, recent studies on the role of *DMRT1*, a very well-conserved gene involved in vertebrate sex development, support the Z-chromosome-dosage hypothesis for avian sex determination [Smith et al., 2009]. In mammals, on the other hand, a single gene, *SRY*, located on the Y chromosome, is the switch for sex determination [Sinclair et al., 1990]. Male development depends on the presence of

androgens produced by the testes, whereas female development occurs when androgens (testes) are absent [Jost, 1947]. According to this picture, sex determination in mammals is equivalent to testis determination, and the function of *SRY* would then be to trigger testis development in genetic males (XY individuals). Hence, the genetic sex of a mammalian individual (established at conception, according to the sex-chromosome complement, XX or XY) determines its gonadal sex (testes or ovaries), which in turn determines its phenotypic sex (male or female). Nevertheless, differences between males and females are quantitative rather than qualitative, as a wide range of intersexual phenotypes are possible, from true hermaphrodites (individuals with both testicular and ovarian tissue in their gonads) to normal males and females, which would then represent the extremes of such a phenotypic range [see Nabhan and Lee, 2007].

The concept of sex determination involves the genetic mechanisms by which the bipotential gonadal primordia of the embryo are committed to differentiate as either testes or ovaries, thus deciding the sex of the individual. Many genes in addition to *SRY* are known to be involved in the gene-regulation cascade subjacent to the processes of mammalian sex determination and differentiation. Most of these genes are involved in testis differentiation, which has been intensely studied, whereas substantially less is currently known about ovarian development.

The consequence of sex determination is gonad differentiation. At the time of sex differentiation, the gonadal primordia differentiate as testes in XY individuals, whereas the absence of the masculinising action of the *SRY* gene product allows ovarian differentiation to proceed in XX individuals. The embryology of the gonads of vertebrates has been investigated since the late nineteenth century, when Waldeyer [1870] established the basis of the 'classical theory' of gonad development (see below). Hundreds of embryological studies have been performed afterwards, describing many different aspects of gonad embryology in most vertebrate taxa, including mammals, birds, reptiles, amphibians, and others. Current knowledge indicates that, apart from some differences between species in the spatio-temporal pattern of differentiation [Carmona et al., 2009a], testis development is a quite well-conserved process, whereas ovarian development is much more heterogeneous, with substantial differences between taxa, including those belonging to the same class. There is also controversy concerning the embryological origin of the different gonadal components and the general model of gonad development. Some of

these issues have been approached recently in studies using modern embryological and molecular methods, which have helped elucidate particular aspects of these processes, for example, the origin of the different cell types of the gonad (reviewed below).

In contrast to previous times, most of current research on mammals is performed using the laboratory mouse (*Mus musculus*) and, much less frequently, the laboratory rat (*Rattus norvegicus*) as animal models. This is especially outstanding in the field of Developmental Biology, where research on species other than the mouse is almost anecdotal and serves generally to add an evolutionary perspective to particular issues. This is also the case with current research on mammalian sex determination and gonad differentiation [see Wilkins, 2002, for a review]. The reason for this situation is evident: current research on developmental biology is focused mainly on unravelling the genetic basis of developmental pathways and, in this field, the mouse is unrivalled as this is the only laboratory animal model in which transgenesis and gene targeting may be applied according to well-standardised methods. However, we might ask to what extent current results found in the laboratory mouse may be extrapolated to other species, including humans. Old reports on the ovarian differentiation in a variety of mammals are recovered here, which together with more recent findings suggest that, probably, the most reasonable answer to this question is 'not sufficiently'. This review will focus on this and other aspects of mammalian gonad development, mainly on the heterogeneity of ovarian development and gonad regionalisation.

An Overview of Mammalian Gonad Development

Either testes or ovaries differentiate from pre-existing bipotential gonadal primordia, which emerge in the ventro-medial surface of the mesonephros. In the mouse, gonadal primordia appear by the thickening of the coelomic epithelium between 10.5 and 11.5 days post-coitum (dpc). Several genes, including *EMX2* [Miyamoto et al., 1997], *WT1* [Kreidberg et al., 1993], *LHX9* [Birk et al., 2000] and *SFI* [Luo et al., 1994], are essential for the formation of the mammalian bipotential gonad. Detailed description of the formation of the primordial gonad has been performed in the rat [Merchant, 1975], where a disorganised gonadal blastema including the epithelial-type, mesenchymal, endothelial and primordial germ cells (PGCs) is initially present. Gonadal cords (also called sex cords) containing epithelial-type somatic cells and PGCs are

Table 1. Differential features of ovarian development in mouse, human and mole

Process/structure of gonad organogenesis	Mouse	Human	Mole
Primary sex cords in the indifferent gonad	no	yes	yes
Primary tunica albuginea	no	no	yes
Conspicuous gonad regionalisation	no	yes	yes
Secondary sex cords containing germ cells	no	yes	yes
Germ cell proliferation period	fast/short	slow/long	slow/long
Time of meiosis onset	pre-natal	pre-natal	post-natal
Persistence of medullary cords	no	no	yes
Time of primordial follicle formation	post-natal (2 dpp)	pre-natal (28 weeks)	post-natal (15 dpp)
Formation of medullary testis-like cords/spherules	no	no	yes
Massive growth of the medullary interstitial gland	no	no	yes
Differentiation of myoid cells	no	no	yes
Differentiation of Leydig cells (testosterone release)	no	no	yes
Testis-like medullary vasculature	no	no	yes
Differentiation of an external tunica albuginea	no	no	yes
Rudimentary epididymis	no	no	yes

dpp = Days post-partum

soon formed in the gonads of both sexes in most species.

In males, testis differentiation begins with the expression of the Y-linked, testis-determining gene *SRY* in somatic cells which, as a consequence of this, become pre-Sertoli cells located in the testis cords, which enclose PGCs. Subsequently, several cellular events take place outside the testis cords, including mesonephric-cell migration, testis-specific vascularisation, and differentiation of Leydig and peritubular myoid cells. In addition to *SRY*, the most relevant genes involved in testis differentiation are *SOX9*, *FGF9*, *DHH* and *SF1*, the functional relationships of which are currently under study and are not yet fully understood [see Brennan and Capel, 2004; Kim and Capel, 2006; Sekido and Lovell-Badge, 2009, for reviews].

Proliferation of the PGCs is the first morphological sign of ovarian differentiation in most studied mammals, but not in the mouse. Afterwards, PGCs enter meiosis and give rise to the formation of primordial ovarian follicles, composed of a single oocyte surrounded by a monolayer of epithelial-type somatic cells, the follicle cells. During puberty and adulthood, follicles grow and mesenchymal cells aggregate around them and differentiate into theca cells, which produce female steroid hormones [see Edson et al., 2009]. The most relevant genes involved in ovarian development in the mouse are *Rspo1*, *Wnt4*, *Ctnnb1* and *Fst* [reviewed by Di Napoli and Capel, 2008].

Divergent Patterns of Mammalian Ovary Development

The high variability of the mammalian ovary was clearly evidenced in the huge review reported by Mossman and Duke [1973]. Referring to mammalian gonad development, the authors stated: ‘... *there are several fundamental points of much practical and theoretical interest about which there is still considerable disagreement and uncertainty. In fact, these are so numerous that an attempt to describe gonad development in a critical fashion involves so many qualifications that it is almost impossible to present the subject with reasonable continuity and clarity.*’ During the past 30 years we have continuously increased our knowledge on testis development, but less effort has been comparatively dedicated to elucidate the organogenesis of the ovary. Consequently, the above sentences by Mossman and Duke remain equally valid today with regard to ovarian development.

The variability of ovarian development in mammals may be illustrated by comparing mice, humans, and moles as representative species, although many additional variants exist. Table 1 summarises the differences between these three distinct models of ovarian development. The mouse shows the simplest pattern of ovarian organogenesis (fig. 1). It is characterised by the absence of gonadal cords in the undifferentiated gonad, between 10.5 and 11.5 dpc [Kanai et al., 1989], the germ and somatic cells maintaining the initial structure of gonadal

Fig. 1. Ovarian development in the mouse. It is fast and very simple, lacking gonadal cords prior to sex differentiation. G = Gonad; M = mesonephros.

Fig. 2. Development in the human ovary. This gonad is regionalised, showing a cortex with cords enclosing the germ cells, and a medulla without cords. Development is slow, compared with that of the mouse. G = Gonad; M = mesonephros.

blastema until the onset of meiosis, which occurs at 13.5 dpc, very soon compared with other mammals. The absence of early sex cords is an exceptional feature, as even other closely related murine rodents, such as rats, do form sex cords before sex differentiation of the gonad [Merchant, 1975]. Cord-like cysts of interconnected germ cells have been described in the mouse ovary at later prenatal stages of development [Pepling and Spradling, 1998]. The formation of primordial follicles, composed of a single oocyte surrounded by a monolayer of somatic (follicle) cells, is delayed until early postnatal stages. In the mouse ovary, the medulla appears late during prenatal development and is not conspicuous in the adult, although an initial functional regionalisation actually exists, as discussed below.

The human ovary shows a more conventional pattern of development [Gillman, 1948; Pinkerton et al., 1961] (fig. 2). The human gonadal blastema is formed at the ovulation age of 5 weeks and most germ cells concentrate in the outer half of the gonad. A phase of germ cell proliferation begins at week 10, which results in the formation of sex cords. Mitotic cells are more frequent beneath the surface epithelium. In 15-week-old human embryos, a medullary region containing blood vessels but not medullary cords is clearly visible. At this stage, a second phase of germ cell proliferation occurs in the outer cortex (but not in the inner cortex or in the medulla), inducing sex cords to grow further. By week 20, germ cells located in

the inner cortex of the ovary enter meiosis, whereas those located in the outer cortex still maintain some proliferative activity. The medulla is larger and contains abundant blood vessels immersed in foetal connective tissue, but no germ cells. In 28-week embryos, most primordial follicles are already formed and occupy most of the cortical region of the ovary. Some growing follicles can be seen in the deeper regions of the cortex, in direct contact with the medullary region, which is even larger and more vascularised.

Most species of moles (family Talpidae) show a very peculiar and complex pattern of ovarian development, which results in the formation of ovotestes with both ovarian and testicular tissue, instead of normal ovaries. This kind of XX ovotestes have been described in four European species of genus *Talpa*, *T. europaea*, *T. occidentalis*, *T. romana*, and *T. stankovici* [Popoff, 1911; Matthews, 1935; Jiménez et al., 1993; Sánchez et al., 1996], the desman *Galemys pyrenaicus* [Peyre, 1962], the star-nosed mole *Condylura cristata* [Mossman and Duke, 1973; Rubenstein et al., 2003], the American shrew mole *Neurotrichus gibbsii* [Rubenstein et al., 2003] and the large Japanese mole *Mogera wogura* [Carmona et al., 2008]. Barriónuevo et al. [2004] reported a detailed morphological description of gonad development in the Iberian mole *T. occidentalis*, where XX gonads develop according to a testis-like pattern (fig. 3). In this species the XX gonads become regionalised quite rapidly, with a cortex and a

Fig. 3. Ovarian development in the mole. This is the most complex among mammals, showing very early regionalisation. One medullary and two cortical regions are formed. The cortical regions originate the ovarian tissue, according to an exceptionally slow process. In the medullary portion, primary sex cords give rise to testis cords, together with others derived from the inner cortical region. After birth, the medullary cords fractionate, giving rise to testicular spherules immersed in massive interstitial tissue composed of Leydig cells and blood vessels.

medulla clearly separated by a thin septum of mesenchymal tissue at 17 dpc. This structure corresponds to the so-called primary tunica albuginea, which is absent in the ovaries of many mammalian species, including the human [Gillman, 1948]. Regionalisation is not morphologically observable in the gonads of male moles at this stage. One day later (18 dpc), primary sex cords appear in the medullary region of both XX and XY gonads. Testicular differentiation begins this way in male embryos and proceeds normally in subsequent days [Barrionuevo et al., 2004; Carmona et al., 2009a]. Ovarian tissue, which develops very slowly in the female moles, derives from the cortical region where all primordial germ cells concentrate. In the gonads of 19 dpc mole embryos, germ cells aggregate and form large cortical cords which also contain epithelial-type somatic cells. These cortical cords, which are separated from each other by mesenchymal tissue, appear as a consequence of a first wave of germ cell proliferation occurring in the region just beneath the surface epithelium of the gonad. In 22 dpc female gonads, a second period of germ cell proliferation takes place, resulting in a regionalised cortex, which now comprises an external and an internal compartment, separated as well by a layer of mesenchymal cells. In the female mole, the onset of meiosis is considerably delayed until postnatal

stages of development (about 4–5 days post-partum, dpp) and the first primordial follicles begin to be formed 10 days later (15 dpp infants), a delay which is quite exceptional among mammals [Barrionuevo et al., 2004; Zurita et al., 2007].

The portion of testicular tissue in mole ovotestes derives from the medullary region of the female gonad. After the first wave of somatic cell proliferation that gives rise to the primary sex (medullary) cords, testicular development is transiently retarded for about 1 week before birth. Nevertheless, during this time the number of medullary cords increases by the projection of masses of somatic cells entering the medulla from the deepest zones of the cortical cords. Germ cells never enter this way into the medulla. Shortly before birth, the medullary cords begin to fractionate and form medullary spherules. After birth, these begin to be surrounded by peritubular myoid cells (a cell which has no homologue in the ovarian tissue of mammals) and the interstitial cells begin to differentiate as Leydig cells, which produce testosterone. The testicular portion of the ovotestis becomes enveloped by a thick tunica albuginea, identical to that of the XY testes, and develops a testis-like vasculature as well [Barrionuevo et al., 2004; Carmona et al., 2009b]. During the juvenile life of moles the testicular portion of the ovotes-

tis grows to a size of more than 10 times larger than the ovarian portion.

According to these developmental patterns, ovarian organogenesis in the mouse is significantly different from that in moles and humans. These differences are evident in the medullary region, which is very small in mice, whereas it is evident in humans and becomes a big portion of testicular tissue in moles. However, differences are also substantial regarding the cortical region of the gonad, for which the transformation into morphologically identifiable ovarian tissue is exceptionally quick in mice and occurs without the early formation of sex cords. In contrast, the development of the ovarian tissue in humans and moles is quite similar, including the formation of large cortical sex cords as a consequence of two successive waves of germ cell proliferation, although the process is exceptionally delayed in moles. However, differences between human and mole ovaries are evident in the medullary region.

From these data, it can be concluded that, whereas the laboratory mouse is a reliable animal model to study testis organogenesis, given that this is quite a well-conserved process among mammals, it is not entirely suitable to study the development of the mammalian ovary because the mouse ovary is quite exceptional in several relevant aspects of the process when compared, for instance, with humans and moles. The genetic data that only the mouse can provide must be tested in species with clearly regionalised gonads, to gain a better understanding of how ovarian organogenesis occurs in these species.

The Interstitial Gland of the Ovary: A Cause of Much Inter-Species Variation in Mammals

Based on very early observations made by Leydig and Tourneaux in the mid-nineteenth century, Bouin and Limon established the term 'interstitial gland' (IG) to refer to the interstitial tissue of the ovary, which in many (but not all) instances represents a persistent medullary region [quoted in Popoff, 1911]. Fraenkel [1905] studied the ovary in adult females of 45 mammalian species and established that 24 of them, including the human female, lacked any significant amount of IG, whereas the other 21 species presented an IG that was more or less developed. The IG of the ovary may have variable aetiology, which Mossman and Duke [1973] classified into seven different types: foetal, thecal, stromal, gonadal-adrenal, adneural, medullary-cord and rete. This classification refers to the origin and nature but not to the morphology

of the interstitial tissue. In fact, the presence of a particular type of IG does not provide the ovary with any defined and characteristic morphology. The morphological manifestation of the IG may be very variable, from filling almost unnoticeably the interstitial spaces between the different components of the ovary (follicles, corpora lutea, hilus, rete ovarii), to forming a prominent large mass of interstitial tissue (the mole IG, for instance). Also, the amount of interstitial tissue may vary throughout the animal's life, according to the physiological status of the ovary, a fact which is evident in seasonally breeding mammals. Foetal, adrenal-gonadal, and medullary-cord types of IG have a medullary origin and are thus especially interesting in this review due to the developmental implications of this origin. The other IGs are less relevant here, as they appear to be the intrinsic consequence of ovarian function in adult females (thecal-, stromal- and rete-type of IGs are clear examples of this). The medullary-cord type of IG sometimes appears in the form of testicular cords.

From a total of 76 species reviewed in this respect by Mossman and Duke [1973], 19 showed an IG containing medullary cords, and 6 out of these were in turn classified as containing testicular cords, including particular species of moles, shrews, desmans, tupaiids (originally included in the order Primates instead in Scandentia in the Mossman and Duke book), and rodents. As indicated above, the number of species known to show this particular feature is currently higher. Hence, the existence and the type of interstitial gland is an important cause of inter-species variation in the mammalian ovary.

According to these reports, mammals can be classified at first into two categories, depending on whether or not they have a conspicuous IG of medullary origin in their female gonads. The group of species lacking this kind of IG includes, for instance, humans, pigs, goats, and sheep. The other group, those presenting a conspicuous interstitial gland, may in turn be divided into two subgroups: species that have an interstitial gland in both foetal and adult stages of life (cat, mole, desman, *Vesperugo pipistrellus*), and those that present an interstitial gland during foetal life, but lose it afterwards thus lacking it during adulthood [the horse, for instance; Aimé, 1907]. The interstitial gland in *Talpa* [MacLeod, 1880; Popoff, 1911; Matthews, 1935; Jiménez et al., 1993], *Vesperugo pipistrellus* [MacLeod, 1880] and the Pyrenean desman [*Galemys pyrenaicus*; Peyre 1962] is clearly more developed than in other mammals. Tourneaux [1904] considered the interstitial gland of the female mole as a rudimentary testis,

according to its ontogenetic origin. The application of modern cytological, histological, embryological and molecular genetic methods to study the development of these gonads has evidenced more recently that they contain a portion of testicular tissue, so that female moles are phenotypically true hermaphrodites [Barrionuevo et al., 2004; Zurita et al., 2007; Carmona et al., 2009b]. The ovary of adult mouse females develops an IG of thecal type (non-medullary).

Hence, it could be concluded that, although *Vesperugo*, *Galemys* and mainly *Talpa* have an abnormally well-developed IG of medullary origin, which can be considered an ovotestis at least in the mole, its existence is not exceptional as there are many species where it is present, too. The data reported by Mossman and Duke [1973] show that the species with an IG developed from the medullary region of the gonad belong to all mammalian orders, although Carnivora and Insectivora (the modern order Eulipotyphla) are more represented in this group.

Regionalisation: A Pivotal Issue in the Controversy on Gonad Ontogenesis

It is well known that in many animal taxa, including most vertebrates, the gonadal primordium is regionalised, containing both cortex and medulla, which Waldeyer [1870] proposed to be the precursors of the ovarian tissue in females and the testicular tissue in males, respectively. This is the case in groups as different as Aves, Amphibia and Reptilia [see Witschi, 1951; Merchant-Larios et al., 1997; Capel, 2000; DeFalco and Capel, 2009]. Even the genital disk of an insect such as *Drosophila*, whose origin is different, also contains two discrete cell populations, each with only one possible fate, testis or ovary [reviewed by Lauge, 1982]. The ontogenetic origin of the gonads in males and females has been the subject of scientific debate since the 19th century. Waldeyer [1870] assumed that the cortex derives from proliferation of the coelomic epithelium, whereas the medulla derives from the mesonephros. Janosik [1885] suggested that both medulla and cortex derive from the coelomic epithelium, the former resulting from an initial proliferation wave and the later from a second one. According to this author, the coelomic epithelium proliferates once in males, thus originating the primary (medullary) cords (precursors of the seminiferous tubules), and twice in females, which gives rise to the 'ovigerous' cords of the cortex. This hypothesis was accepted for decades and con-

sidered the classical theory for mammalian gonad ontogenesis [see Forbes, 1942], although Winiwarter and Sainmont [1909] proposed that the coelomic epithelium proliferates three times instead of twice, the second one resulting abortive as it does not originate the ovarian cortex. This would be formed after the third proliferation wave.

Based on his previous studies in amphibians and mammals, Witschi [1951] suggested that only the medulla has the potential to originate testicular tissue, and only the cortex may be the precursor of ovarian tissue. Following Waldeyer's original hypothesis, this author assumed that the medulla derives from the mesonephros. Witschi's hypothesis has been repeatedly discussed in contrast to that reported by Gillman [1948], who proposed the so-called 'common precursor' hypothesis, i.e. that the cellular components of the gonads are bipotential, having the ability to differentiate into either testicular- or ovarian-specific cell types.

It is widely accepted that the gonadal cortex derives from the coelomic epithelium, so that testing the above hypotheses was reduced to demonstrate whether or not testis-specific cells, Sertoli cells for instance, may also derive from the coelomic epithelium. Indirect support for the common-precursor hypothesis has come from in vitro experiments in which cultured mosaic XX-XY gonads were shown to contain XX Sertoli cells [Palmer and Burgoyne, 1991a] and XY granulosa cells [Burgoyne et al., 1988; Palmer and Burgoyne, 1991b], and from the molecular demonstration that both Sertoli and follicle cells share a common cell precursor [Albrecht and Eicher, 2001]. The lack of intensified apoptosis in both the cortex of testes and the medulla of ovaries has also been argued as additional support for this hypothesis [Capel, 2000]. However, apoptosis should not be expected to occur in these gonadal regions, because (1) the cortex in the testes rapidly differentiates to form the tunica albuginea, and (2) the medulla in ovaries frequently remains vestigial, not necessarily degenerated. Nevertheless, conclusive evidence was reported by Karl and Capel [1998], who used DiI lineage-tracing experiments to show that both testicular and ovarian cells may derive from the coelomic epithelium.

The fact that both Sertoli and follicle cells derive from the coelomic epithelium fits Gillman's hypothesis, mainly because all these experiments were performed in the mouse, an animal where gonad regionalisation is not obvious. But how must these results be interpreted in mammals that have a conspicuous medullary region? In this context, lineage-tracing experiments suggest that both

Fig. 4. Functional, non-morphological regionalisation of the undifferentiated gonad of the mole. **A** Haematoxylin-eosin stained transversal section of a mole gonad at the s4c stage (16 dpc) with no prior gonadal sex differentiation. No morphological regionalisation is visible. **B** Immunofluorescence staining of an s4c gonad using an anti-SOX9 antibody. Immunoreactive cells (bright red) occupy a gene-expression domain which does not reach the coelomic epithelium, thus defining a functional cortex with no morphological manifestation (area between dashed lines).

cortex and medulla contain cellular components derived from the coelomic epithelium, thus ruling out Waldayer's and Witschi's suggestions that the medulla derives from the mesonephros. However, the old axiom that the testicular tissue derives from the medulla whereas the ovarian tissue comes from the cortex remains undisputed. Thus, available data support the old hypothesis of Janosik [1885], which states that the medulla comes from an initial proliferation of the coelomic epithelium, and the cortex derives from a second proliferation round. The early development of human and mole gonads described above closely fits this model.

Nevertheless, it is known that the mesonephros participates actively in gonad organogenesis by providing the gonadal primordium with endothelial cells that migrate at the critical time period of testis differentiation, thus contributing to the formation of the testis-specific vasculature [Coveney et al., 2008]. Thus, the mouse testis does contain abundant mesonephros-derived cells, whereas the mouse ovary does not. By contrast, multiple indirect pieces of evidence suggest that mesonephric cell migration occurs within the gonadal primordium of both male and female moles, although conclusive evidence is not yet available [Barrionuevo et al., 2004; Carmona et al., 2009b]. Since this phenomenon has not been deeply investigated in other mammalian species, we do not know whether it is specific for species with females developing ovotestes, such as the mole, or whether it is more generalised among mammals.

Gonad regionalisation is usually said to occur when it is morphologically observable. However, gene-express-

sion studies in the mouse [Yao et al., 2004; Coveney et al., 2008] and in the mole [Carmona et al., 2009a and b] have shown that a functional regionalisation takes place before any morphological evidence appears in the developing gonad. The expression domains of particular genes in the undifferentiated gonad, which show well-established boundaries that have no correspondence with any morphological structure, define the existence of medullary and cortical regions in the gonad prior to any morphologically recognisable regionalisation. Figure 4 shows the expression domain of the SOX9 gene, a key element of the testis-determining pathway in most vertebrates, during the s4c stage of the mole-testis development (16 dpc), 1 day before regionalisation of the female gonad and 2 days before testis differentiation in males [Carmona et al., 2009a and b]. A thin layer of gonadal tissue, located just beneath the coelomic epithelium and quite constant in width, where this and other genes are not expressed, is the functional cortex at these early stages of gonad development. At later stages, this functional domain seems to be maintained in the female gonad and probably defines the boundary of the outer cortex, which is never reached by the projections of mesenchymal tissue that penetrates the inner cortex [Barrionuevo et al., 2004]. The cause of this non-morphological, early functional regionalisation of the gonad, which also occurs in the mouse (but without a conspicuous morphological manifestation), is currently unknown. A possibility is that the cells of the coelomic epithelium produce a paracrine signal that establishes the boundary of the expression domains of particular genes. Since morphological regionalisation is initiated by the formation of a septum of mesenchymal tissue that separates the two gonadal regions (the so-called primary tunica albuginea), the coelomic paracrine signal could also produce a negative-chemotaxis effect for mesenchymal cells. In the mole ovotestis, this effect could also be maintained later when a second septum of mesenchymal tissue separates the external cortex from the internal one.

In What Direction Is the Mammalian Ovary Evolving?

Gonad regionalisation is a primitive trait in vertebrates, being common in amphibians, reptiles, and birds. Contrarily, it is not evident in many mammalian species, thus suggesting a trend in this group to avoid regionalisation during gonad development. Several facts suggest that this process could be occurring in a progressive manner: (1) regionalisation in one of the sexes (males) is much less

evident than in the other; (2) regionalisation is less frequent in adult females than in foetal, infantile and juvenile individuals; (3) when morphological regionalisation of the gonad is not conspicuous, as in testis development, a functional regionalisation persists, nevertheless, in the form of defined gene-expression domains. In some species, regionalisation is almost completely lost (the mouse, for example), in others, it is present during development but disappears afterwards, being absent in the adult gonad (horse), while in some species ovaries are clearly regionalised throughout the whole life of the animal (mole). Considering (1) the absence of apparent regionalisation in the mammalian testis, in contrast with what occurs in the ovary, which develops in *SRY*-negative individuals (XX females), and (2) that *SRY* is a mammal-specific gene (not present in other vertebrates), Barrionuevo et al. [2004] suggested that this gene could have an anti-regionalisation effect in mammals.

It is noteworthy that mammalian variants in ovarian development cannot be associated with particular taxonomical groups. Different developmental models can be found inside the same group. This suggests that the cause for the maintenance of regionalised ovaries in a particular species is probably not phylogenetic but adaptive. In the case of moles, Carmona et al. [2008] have proposed that the testosterone produced by the testicular portion of the ovotestis during the non-breeding season could foment the aggressive behaviour of females, which have to defend their territories as males do. The mechanism by which testosterone induces aggressive behaviour, through the activation of the aromatase enzyme, which converts testosterone into estrogen in the brain, has been recently reported by Wu et al. [2009]. In the reproductive period, serum testosterone levels decrease in females [Jiménez et al., 1993; Whitworth et al., 1999], so that they would permit males to enter into their territories for breeding. This hypothesis is supported by the fact that female moles exhibit territorial behaviour, another exceptional feature in these animals [Gorman and Stone, 1990; R. Jiménez, unpublished data]. By contrast, it has been reported that some American mole species lack ovotestes, although they have a masculinised body including a penile clitoris, as described in moles with ovotestes [Rubenstein et al., 2003]. Similarly, not all Asian mole species have ovotestes [Carmona et al., 2008]. However, in most cases it is not known whether female moles without ovotestes are also territorial. In the case of *Urotrichus talpoides*, females lack ovotestes and are not territorial [Ishii, 1993; Carmona et al., 2008].

Carmona et al. [2008], mapping the presence or absence of ovotestes in different phylogenies recently proposed for moles of the family Talpidae, showed that at least one reversal (appearance and further disappearance) must have occurred in the evolution of this trait, indicating a high plasticity in mammalian gonad development. Such a developmental plasticity is necessary to explain how ovotestes may appear as a consequence of selective pressure in some species, but not in others of the same taxonomical group. Furthermore, developmental plasticity is not difficult to explain in genetic terms. The idea is being commonly accepted that developmental differences between species rely more on differences in the regulatory elements that control the expression pattern of master genes (promoters, enhancers, transcription factors, etc.), than on the existence of notable differences between the orthologue genes themselves, which are quite well conserved in most cases. A very clear example was recently reported by Cretekos et al. [2008], who replaced a limb-specific transcriptional enhancer of the mouse *Prx1* gene with the orthologous sequence from a bat species, and observed that the forelimbs of the transgenic mice mimicked those of the bat. Regarding gonad development, it is well known that very subtle genetic mutations may provoke developmental consequences as drastic as the complete sex reversal, and there are several genes susceptible to provoking such reactions [see Sekido and Lovell-Badge, 2009]. In the case of the Iberian mole, available data suggest that the presence of ovotestes may be simply due to a change of the WNT4-signalling pathway in the female gonad [Carmona et al., 2009b].

Hence, it seems possible that particular species may have returned to more primitive forms of ovarian development, probably in accordance with selective agents acting in each case. Once again, the gonads of female moles provide a clear example. The high similarity between prenatal development of the gonads in the Iberian mole *Talpa occidentalis* [Barrionuevo et al., 2004] and those of the sea turtle *Lepidochelys olivacea* [Merchant-Larios et al., 1997] is surprising. Similarities include: (1) an undifferentiated gonadal primordium with medullary cords in both sexes, (2) these cords are almost identical in shape in both species, zigzagging, with varying diameter, (3) the medullary cords develop into seminiferous tubules in males and become fractionated in females (in the turtle these further disappear), (4) the cortex originates the ovarian tissue in females and becomes the gonadal envelope in males.

In conclusion, mammals show a clear tendency to avoid regionalisation during gonad development, al-

though notable variation exists in ovarian organogenesis, probably driven by natural selection. The plasticity observed in the process of ovarian development, which may undergo noticeable changes in response to subtle genetic mutations, would make this variation possible.

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