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Editors

Gynecologic and Obstetric Pathology, Volume 1

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Foreword

The field of gynecologic and obstetric pathology is at a cross-roads. In the past decade we have begun to witness the departure of venerated contributors to this discipline and now we are being inundated with new information about pathogenesis that informs diagnostic expectations and patient outcome. The old model in which the next generation sits at the knee of the learned and patiently awaits their turn at the helm is rapidly fading. Succession is now not simply achieved by learning the old language but by speaking a new one.

The textbook *Gynecologic and Obstetrics Pathology*, edited by Drs. Zheng, Fadare, and Quick is emblematic of the sea change. We've all heard the joke about resorting to one's grandchild to solve a computer conundrum. How many of us turn to our younger colleagues to interpret emerging genomic information in the management of gynecologic cancer? An appreciation of such talent is crucial to our evolution as well as that of our discipline.

The senior editor in this project, Dr. Wenxin Zheng, has a long track record of innovation. With this has come a facility to recognize the most talented young clinician-investigators and to recruit them into this new textbook. Drs. Fadare and Quick as well as the younger chapter authors are well on their way, having already put us on notice that by their dedication, creativity and their role in discovery. Their input is what will keep this and subsequent editions at the forefront of pathology texts dedicated to women's health.

Energy and intellect drive discovery but experience is essential to provide a needed perspective when this information is transmitted to the practicing pathologist. The editors wisely balance the list of talented newcomers with recognized experts in the field. Together they provide the finer details of diagnosis and differential diagnosis while eliciting the nuances relevant to clinical management.

In the current world, where discoveries and their impact on practice can become global almost instantaneously, one does not need to travel far to realize that expertise in obstetric and gynecologic pathology is intercontinental. In recognizing this, Zheng et al. will also provide an edition written in Chinese, bringing this message to pathologists (and their patients) in countries where the language is read and spoken. To my knowledge, this book will be the first of its kind to accomplish this, creating a truly international presence that will place this first edition among the leading texts in the field. The editors and authors are to be commended for their contribution and I look forward to their success in opening a new chapter (and book!) in the history of the pathology of the female reproductive tract.

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Preface

Pathology of the female genital tract is complex, and encompasses a wide spectrum of neoplastic and non-neoplastic diseases of the gonads, reproductive ducts, secondary müllerian system, and external genitalia. Clinical practitioners in this discipline must therefore familiarize themselves with a broad spectrum of pathology, including skin-like diseases of the vulva, a myriad of peritoneal diseases, as well as conventional diseases of other female genital tract organs. This field progresses in a vibrant and dynamic academic environment in which diagnostic concepts continually evolve as our understanding of various disease processes improves over time. The contemporary gynecologic pathologist is in a unique position to recognize and define morphologic correlates to newly defined genomic profiles and individual gene mutations, assess whether they are likely to have diagnostic or prognostic significance for a given patient and/or her family, and broadly participate in the push towards increasingly personalized cancer care. These exciting trends notwithstanding, it remains true that definitive pathologic classification of gynecologic disease is still primarily based on the traditional pillars of surgical pathology, including gross pathology, morphologic assessment buttressed by immunophenotypic analysis where needed, and careful clinicopathologic correlation.

This book is envisioned as a "bridge" that acknowledges both of the aforementioned realities. It is designed to provide a broad coverage of diagnostic gynecologic and obstetric pathology, inclusive of both neoplastic and non-neoplastic diseases. The book is neither a dense and comprehensive treatise on every disease process nor is it a dry listing of relevant "facts" about each entity. Rather, it is best conceptualized as a large scale aggregation of the most up to date information in gynecologic pathology, all presented in a concise and narrative manner that is designed to be easily accessible to the general practitioner, specialist and student alike. An overt effort has been made to discuss each topic in a way that is maximally relevant to the diagnostic surgical pathologist, such that by reading any section should substantially increase the reader's confidence that the most germane clinicopathologic information on that entity has been reviewed before a diagnostic decision is made.

The material is organized into 36 chapters, representing the full spectrum of diagnostic gynecologic pathology. In addition to chapters on traditional topics in gynecologic pathology, there are individual chapters on site-specific carcinogenesis, gynecologic cytology, intraoperative consultation, endometriosis and development/maldevelopment of the female reproductive system, among others. Additionally, in a departure from most current texts, there are stand-alone chapters to provide intensive coverage of some traditionally under-covered topics, including melanocytic lesions of the female genital tract, non-neoplastic diseases of the endometrium, and vulvovaginal soft tissue lesions. Entities with a significant diagnostic component are presented, where feasible, divided into the following subsections: *Clinical features, Gross findings, Microscopic findings, Differential diagnosis, Biomarkers,* and *Genetic features.* As expected, not all entities or chapters lend themselves to this specific format, but most chapters are broadly structured based on these general themes. Microscopic findings are lavishly illustrated, and numerous tables help summarize pertinent points for easy reference. The overall objective of each chapter is to integrate traditional pathologic features, clinical features, where applicable, and current paradigms in disease classification into a format that can be readily applied in routine practice. These chapters are authored by over 50 physicians, most of whom

are experienced subspecialty practitioners of clinical gynecologic pathology from around the world, and without whose expertise, dedication, and diligence this work would not have been possible. It is the sincere hope of the editors that all of those who are interested in gynecologic pathology—diagnostic pathologists, students, residents and investigators—will find this book tremendously useful.

The understanding of gynecologic disease involves pathologists and researchers from across oceans and all over the world, and to that end an exciting feature of this book is that it is written with a direct linkage to the second edition of the book "Gynecologic and Obstetric Pathology". The latter book is in Chinese, and is published by Science Press, Beijing, China. The current text is published in both English and Chinese, representing a collaborative effort by both publishers: Springer and Science Press. Although the titles and the number of chapters are identical in the two books, the authors of the chapters are different. Additionally, while the chapter outlines and some of the contents overlap, the two books do not represent a direct translation from one to the other. Rather, they are best considered complementary "sister" books. This results from Dr. Wenxin Zheng serving as the first editor-in-chief for both books. Considering this special and close relationship, the co-editors for the Chinese book, Drs. Danhua Shen and Donghui Guo, are listed as co-editors of the English version of this book, while Drs. Fadare and Quick are also listed as co-editors on the second edition of the Chinese book.

Dallas, TX — Wenxin Zheng
San Diego, CA — Oluwole Fadare
Little Rock, AR — Charles Matthew Quick

Acknowledgments

To my dear wife Wenda and my beloved family (Yuxin, Genfu, and Deshun) for their constant love and endless support! In memory of my parents Maoguan Zheng and Jinxian Wang as well as my ultimate mentor Dr. Stuart C. Lauchlan.

Wenxin Zheng

To my wife Abby for her love, encouragement, and inspiration, and to our beloved children Nathaniel, Darrell and Olivia for (mostly) putting up with the occasional absences that were required to do this.

Oluwole Fadare

To my everything, Shelly, and my wonderful children Dexter, Bernice, and Alice.

Charles Matthew Quick

To my beloved family members for their continuous support and care, and to all my colleagues who participated in editing this outstanding book. I am honored to be an editor for this prestigious book.

Danhua Shen

To my beloved family members for their constant support and care, and to Drs. Zhaoai Kong and Song Lin whose past instruction and training have been and continues to be invaluable.

Donghui Guo

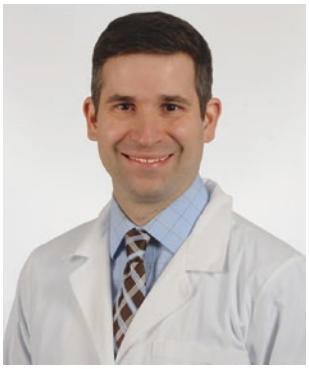
The Editors would like to sincerely thank Dr. Christopher P. Crum, M.D. for serving as a senior consulting adviser for this book. Dr. Crum has made significant contributions to the field of gynecologic pathology and has trained innumerable residents and fellows, including many who have served as an author for this book. The quality of this work is in part attributable to his years of dedicated teaching and research in the field of gynecologic pathology.

Contents

About the Editors

Oluwole Fadare Dr. Oluwole Fadare is a Professor of Pathology at the University of California San Diego School of Medicine (UCSD, San Diego, CA, USA), where he also serves as the Chief of Anatomic Pathology for the UCSD Health System and Director of the Gynecologic/Breast Pathology fellowship. Dr. Fadare completed a fellowship in breast and gynecologic pathology at the Yale University School of Medicine (New Haven, CT, USA) in 2005, and has spent his subsequent academic career focused on the pathologic aspects of women's health. Dr. Fadare has been the recipient of numerous prestigious awards, including most recently the 2018 Arthur Purdy Stout Prize from the Arthur Purdy Stout Society of Surgical Pathologists in recognition of "significant career achievements in Surgical Pathology by a Surgical Pathologist (less than 45 years old) whose publications have had a major impact on diagnostic pathology", a 2018 Stowell-Orbison Certificate of Merit from the United States and Canadian Academy of Pathologists (USCAP), and a 2017 Excellence in Mentoring Award from UCSD Health Sciences International "in recognition of a sustained commitment to helping create a cadre of global leaders in innovative academic medicine". Dr. Fadare has published more than 200 peer-reviewed articles in high impact scientific journals, predominantly centered on gynecologic pathology. Previous books edited or co-written include *Diagnosis of Neoplasia in Endometrial Biopsies: A Pattern-Based and Algorithmic Approach* (Cambridge University Press, 2014) and *Precancerous Lesions of the Gynecologic Tract: Diagnostic and Molecular Genetic Pathology* (Springer 2015). Dr. Fadare has served in various editorial capacities for over 80 journals, and is currently an editorial board member for the *International Journal of Gynecological Pathology, Human Pathology, Advances in Anatomic Pathology, Archives of Pathology and Laboratory Medicine, Archives of Medical Research*, and *Diagnostic Pathology*, among others. He is also active in various professional societies, and currently serves on the education committee for the International Society of Gynecologic Pathologists and on the membership committee for USCAP. Dr. Fadare's research has been clinical based, and has focused on integrating morphological, immunohistochemical and molecular aspects of gynecologic tract neoplasms to optimize diagnostic, prognostic and predictive patient care.

Donghui Guo Dr. Guo graduated from the Fourth Military Medical University of China in 1974, and is one of the top gynecologic pathologists in China. Mentored by Professor Song Lin in the early part of her career, Dr. Guo has practiced gynecologic pathology for more than 30 years. Dr. Guo served as Chairman in the Department of Pathology, Tianjin Central Hospital of Obstetrics and Gynecology for 10 years. She has mentored many graduate students, residents, and fellows with an interest in gynecologic pathology. Dr. Guo has served as a committee member as well as a well-recognized expert in gynecologic pathology for many professional societies in China. Dr. Guo has completed 4 major scientific achievements (please provide the details here) with multiple awards in Tianjin, China. Dr. Guo has authored and co-edited 5 pathology books and has published more than 30 peer-reviewed articles.



Charles Matthew Quick Dr. Charles "Matt"hew Quick is an associate professor and clinical educator in the Department of Pathology at UAMS in Little Rock, Arkansas. He completed fellowships in surgical pathology at UAMS and Women's & Perinatal pathology at Harvard Medical School, Brigham & Women's Hospital. Dr. Quick serves as the Director of Anatomic Pathology Sub-Specialty Practice, Gynecologic Pathology, and the Surgical Pathology Fellowship.

He has published numerous research publications and review articles, and has authored and co-edited multiple textbooks, including "High-Yield Pathology: Gynecologic and Obstetric Pathology." He loves all things teaching and gynecologic pathology related and has won numerous teaching awards for his medical student and resident education efforts, including UAMS's campus-wide "Chancellor's Award for Teaching Excellence." Dr. Quick is dedicated to expanding pathology education in medical school and has started numerous programs at UAMS to effect this change including the UAMS pathology interest group: SCOPE, a summer pathology preceptorship, and the integration of autopsy pathology into the first year gross anatomy course, earning him an Education Innovation award in 2015. His efforts have led to a dramatic increase in medical students choosing pathology as a career in the state of Arkansas.

Dr. Quick serves as an Ambassador for the United States and Canadian Academy of Pathology, and has taught numerous interactive microscopy courses for the USCAP at both annual meetings and the new teaching complex located in Palm Springs, California. He serves as the Gynecologic Pathology Course Director for the USCAP Interactive Microscopy Center. Dr. Quick's research interests include the study of endometrial precancers, vulvar squamous carcinogenesis and the impact of epithelial-mesenchymal transition on tumor behavior.

Danhua Shen Dr. Danhua Shen, Associate Professor of Pathology, is the Chairman of the Department of Pathology, People's Hospital of Peking University, China. Dr. Shen is one of the top gynecologic pathologists in China. In addition to her dedication to pathology diagnosis, Dr. Shen has participated in many research projects of *National Natural Science Foundation of China.* She is the head of the *Female Reproductive Diseases Group of the Pathology Branch of the Chinese Medical Association*, and serves as an executive committee member for many prestigious professional societies including the *Chinese Gynecologic Oncology Group, Chinese Society of Obstetrics and Gynecology*, and *Chinese Society of Colposcopy and Cervical Pathology*, etc. Dr. Shen is also an editorial board member for the *Journal of Chinese Pathology* and the *Chinese Journal of Obstetrics and Gynecology*, and the *Journal of Diagnostic Pathology*.

Dr. Shen has published more than 100 peer reviewed articles, and has been involved in seven clinical and pathological related monographs/books or book chapters either as an editor-in-chief or deputy editor-in-chief. Dr. Shen has also participated in many book translations in the field of Pathology and Obstetrics and Gynecology.

Wenxin Zheng Dr. Zheng is a tenured Professor in the Department of Pathology and the Department of Obstetrics and Gynecology at the University of Texas Southwestern Medical Center (UTSW). An internationally recognized gynecologic pathologist as well as active physician scientist, he specializes in all aspects of gynecologic pathology and holds the Mark and Jane Gibson Distinguished Professorship in Cancer Research. Dr. Zheng also serves as the Director of Gynecologic Pathology service and the Director of Gynecologic Pathology Fellowship at the UTSW Medical Center.

Dr. Zheng earned his medical degree at Shanghai Medical College Fudan University. He completed a residency in obstetrics and gynecology at the Hospital of Obstetrics and Gynecology in Shanghai and, later, a residency in anatomic and clinical pathology at New York Hospital-Cornell Medical Center. He received advanced training through a gynecologic pathology fellowship at Women & Infants Hospital of Rhode Island and a research fellowship in molecular reproductive medicine at Columbia University College of Physicians and Surgeons (New York).

Dr. Zheng runs an active consultation practice that receives material world wide. Dr. Zheng has published more than 180 peer-reviewed articles in high impact scientific journals, mostly in the field of gynecologic pathology. He has served in various editorial capacities for over 50 journals, and is currently an editorial board member for multiple journals in the biomedical sciences. His main research contributions include endometrial serous carcinogenesis and precancerous lesion endometrial glandular dysplasia, cell origin of low-grade

ovarian serous carcinoma, molecular mechanism of progestin resistance in endometrial cancer and its precancers, hormonal etiology of ovarian epithelial cancers, and tubal contribution of ovarian endometriosis and its associated ovarian cancers. In addition, Dr. Zheng has created a novel approach called one-stop cervical care (OSCC) to diagnose and treat cervical pre-cancers. Dr. Zheng Loves teaching gynecologic pathology to residents and fellows and have taught numerous gynecologic pathology courses nationally and internationally.

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Development and Maldevelopment of the Female Reproductive System

1

Diego H. Castrillon

Abstract

The female reproductive tract has its embryological origins in the paired Müllerian ducts and their fusion to each other and the urogenital sinus. The Müllerian ducts give rise to the oviducts, uterus, and cervix, while the urogenital sinus forms the vagina and external genitalia. Primordial germ cells formed near the yolk sac migrate to colonize the gonadal ridge, where in females interactions between germ and somatic cells coalesce to create the functional ovarian units known as follicles.

A classical morphologic understanding of the embryology of these structures has served as the foundation for a general understanding of myriad conditions due to maldevelopment or malignant transformation. Increasingly, the molecular underpinnings of these complex underlying developmental transformations are being revealed, yielding deeper insights into the biological basis of female reproductive tract disease pathophysiology and also providing many useful markers, such as Sall4, Foxl2, Wt1, and Pax8, routinely used in clinical practice. In some cases, Müllerian maldevelopment syndromes such as Müllerian agenesis are now known to be caused by mutations in the genes encoding factors required for Müllerian duct development.

The once far-fetched idea that epithelial cells of the oviduct—not the ovary itself—are the origin of most "ovarian" carcinomas now has universal acceptance. Female reproductive tract malignancies of the cervix, uterus, and ovary recently believed to have disparate cellular/embryologic origins are now understood to have a shared origin in the epithelial lining of the Müllerian ducts. This insight rationalizes many prior observations, for example, that the diverse tumor histotypes common to the cervix, endometrium, or tubo-ovarian complex are encountered across each site. This chapter summarizes our understanding of female gonadal and reproductive tract development, with an emphasis on morphologic and molecular aspects that currently appear most relevant to disease pathophysiology.

Keywords

Primordial germ cells · Gonadal ridge · Sex determination · Müllerian duct · Maldevelopment · Female reproductive tract

1.1 Primordial Germ Cells: Formation and Migration

Four embryologically and anatomically distinct primordia form the female genital tract: (1) primordial germ cells, (2) the gonadal ridge, (3) the paired Müllerian ducts, and (4) the urogenital sinus. The initial formation of these primordia and subsequent developmental processes occur in coordinated steps throughout gestation (summarized in Table 1.1).

Germ cells, which ultimately give rise to gametes, are responsible for the transmission of genetic information and the propagation of species. Because of their relatively small numbers, their formation and preservation is of utmost biological importance. The initially formed germ cells are termed primordial germ cells (PGCs). In invertebrates such as the common fruit fly *Drosophila melanogaster*, PGCs are specified by cytoplasmic components known as the germplasm allocated to the posterior of the egg during oogenesis, and PGCs are the first cells formed in the embryo (preformation) [1]. In mice, humans, and other mammals, PGCs are formed much later in development by inductive processes requiring cell-cell interactions and external signals (epigenesis) [2]. Thus in mammals, the germ cell lineage (the "germline") is discontinuous.

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Table 1.1 Development of the female reproductive tract

Weeks gestation (from ovulation)	Crown-rump length (mm)	Heel-toe length (mm)	Developmental event
3 weeks	2.5		Germline specification begins
4 weeks	3–5		Primordial germ cells are formed
5 weeks	7		Gonadal ridge formation
6 weeks	11		Müllerian ducts appear as funnel-shaped opening of coelomic epithelium (initiation phase)
7 weeks	18		Müllerian ducts migrate to about half distance to urogenital sinus
	23		Müllerian ducts extend caudally to near the urogenital sinus
8 weeks	30		Primordial germ cells colonize gonadal ridge
			Müllerian ducts begin midline fusion and make contact with urogenital sinus at the Müllerian tubercle
9 weeks	50		Müllerian ducts fuse (septum disappears); epithelium lining uterovaginal canal stratifies (1–2 cells layers thick)
10 weeks	60	2–5	Oogonia in leptotene prophase of meiosis I (initiation of meiosis in some oogonia continues to at least 16 weeks)
11 weeks	71	5–8	Bilateral sinovaginal bulbs (evagination of UGS) appear
	75		Vaginal plate first seen distinctly at 75 mm (complete at 140 mm; week 17)
14 weeks	110–140	15–17	Marked growth of caudal vagina
			Vaginal rudiment reaches level of vestibular glands; uterovaginal canal (15 mm total length) divisible into vagina (one-half), cervix (one-third), and corpus (one-sixth); boundaries ill-defined
			Endometrial and myometrial layers of uterus become apparent
			Solid epithelial precursors of anterior and posterior fornices appear
			Vagina shows epithelial squamous differentiation
15 weeks	130–140	18–21	Primordial follicle individualization begins and continues through first postnatal months
			Cervix about 5 mm long
			Fallopian tube begins active growth phase
			Vaginal plate extends to endocervical canal
16 weeks	142	21–24	Uterine/cervical glands begin as outpouchings of simple columnar epithelium
			Vaginal plate longest and begins to canalize
			Solid epithelial projections of anterior and posterior fornices demarcate cranial end of vagina
17 weeks	153–162	24–27	Palmate folds of cervix appear (forerunner adult cervix)
			Mucoid development of cervix begins. Estrogen-induced thickening of vaginal epithelium
19 weeks	177	31–33	Canalization of vaginal plate completed
			Uterine tube growth marked (~3 mm/week to week 34)
			Cervix about 10 mm long
22 weeks	208	40–43	Vagina completely formed
			Differentiation of uterine myometrium complete
24 weeks	215–295	47–49	Uterine fundus well defined, uterus assumes adult form, uterine body about 10 mm long
38 weeks	362		Birth
Postnatal			Meiosis and primordial follicle individualization continue and are completed within a few months to establish primordial follicle reserve

Landmarks in Müllerian tract adapted from Robboy SJ et al. 97:9 (2017) [80] with permission (Elsevier). Other landmarks from references as described in text

1.1.1 Germline Specification and Migration

Germline specification in humans begins at around the time of gastrulation, at the 3rd week of development, and PGCs are first observed during the 4th week of development in the extraembryonic yolk sac wall near the allantois (Fig. 1.1) [3]. PGCs are cytologically distinctive and significantly larger than the surrounding somatic cells, with a diameter of 25–30 μm. The initial complement of PGCs may be as few as 50–100 individual cells [3]. PGCs express alkaline phosphatase, which can be detected via a histochemical reaction [4]. They also express the cell surface receptor tyrosine kinase Kit (CD117) and other germ cell-specific markers [5]. Kit is required for PGC migration and survival [6]. PGCs can be identified in human embryos by immunostaining for Kit or the pan-germ cell marker Vasa (Fig. 1.2) [5]. Human PGCs also express pluripotency genes first turned on in embryonic stem cells, such as Oct4 [7] and Sall4 [8], which are sup-

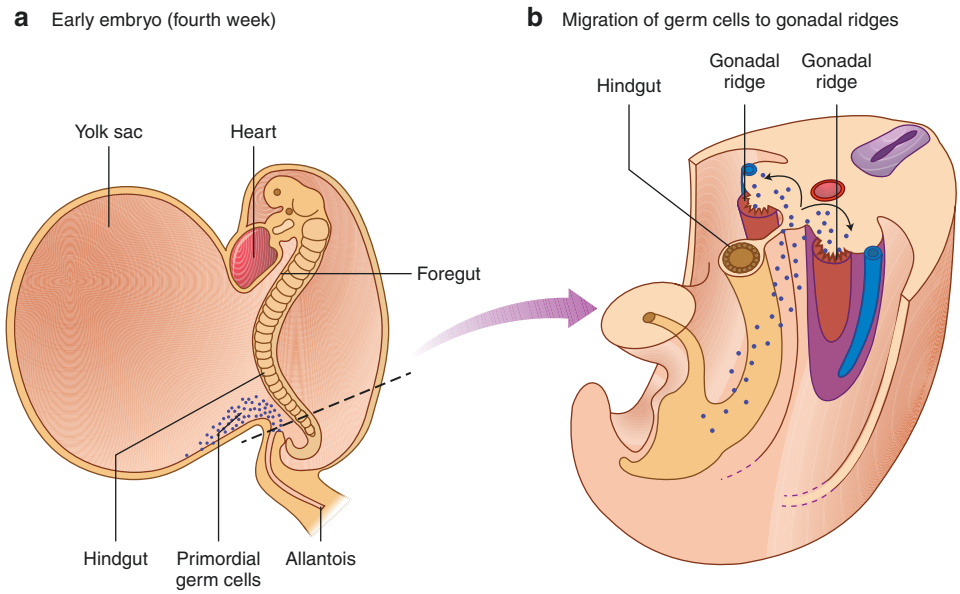

Fig. 1.1 Formation and migration of primordial germ cells (PGCs). (**a**) PGC formation in early embryo. (**b**) Migration through embryonic structures to eventual destination in bilateral gonadal ridges. Redrawn with permission from Mesiano S and Jones EE, Medical Physiology 3rd edition, Chapter 53 (2017) (Elsevier)

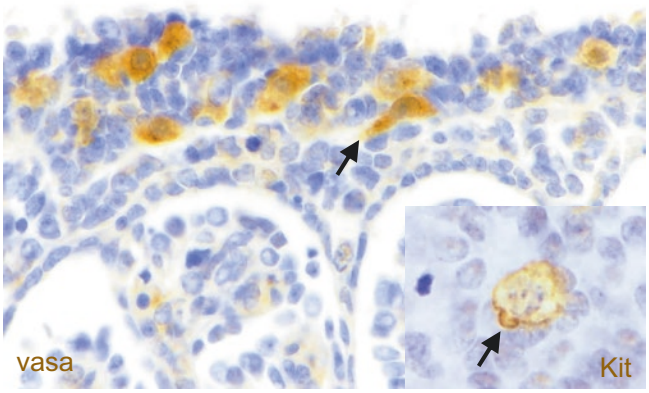

Fig. 1.2 Primordial germ cells migrating into gonadal ridge, human embryo at 8 weeks estimated gestational age. The top cell layers are part of the gonadal ridge; the subjacent tubules are part of the mesonephros (early excretory system that will degenerate). Inset: Kit immunostaining shows membrane localization in migratory PGC near gonadal ridge. Note pseudopodia (arrows)

pressed in somatic lineages. Therefore, Oct4 and Sall4 are useful as male and female germ cell markers throughout life, although they do exhibit some differences. In the neonatal ovary, Oct4 is expressed in oogonia but not in oocytes (i.e., in individualized primordial follicles, see discussion below), whereas Sall4 exhibits the opposite pattern (Fig. 1.3).

After their formation, PGCs begin a remarkable long-range migration from the yolk sac through various embryonic structures including the gut to eventually reach and colonize the gonadal primordium (the gonadal ridge) by 8 weeks gestational age (Fig. 1.1). During migration, PGC numbers expand mitotically, and the cells adopt an amoeboid shape with pseudopodia [3]. In females, PGCs that have colonized the gonadal ridge become round and are termed oogonia. The external signals and homing mechanisms guiding PGCs to the gonadal ridge are poorly understood but involve Kit ligand (aka stem cell factor) acting through the Kit cell surface receptor and cell adhesion molecules [9–11]. The existence of extragonadal germ cell tumors (i.e., sacrococcygeal or within the mediastinum or cranium) has been attributed to mismigration of PGCs during embryogenesis (see e.g., [12, 13]), but this intriguing notion remains unproven.

Due to the impracticability of studying early postimplantation human embryos, the molecular specification of PGCs has been most extensively explored in mice, although there has been an increasing focus on human studies. In mice, Bmp signals act through downstream Smad proteins to induce the expression of the transcriptional repressor Prdm1 in PGCs. Prdm1 robustly suppresses somatic gene expression (i.e., the expression of non-germline genes) and is critically required for mouse PGC development [14, 15]. Prdm1 is also induced in human PGCs and is required for their development. The secreted factor Wnt3 acting through the downstream transducer β-catenin is also involved in mouse and human PGC specification. However, while human and mouse PGC specification share many similarities, there are also salient molecular differences. For example, the lineage-specifying transcription factor Sox2 is required for PGC proliferation in mice, whereas human PGCs lack Sox2 but require Sox17 for their specification [16].

1.1.2 Epigenetic Reprogramming of the Germline

DNA methylation at cytosine residues in CpG dinucleotides (producing 5-methylcytosine or 5mC) is an important repressor of transcription in the genome. During mammalian development, epigenetic reprogramming occurs via global

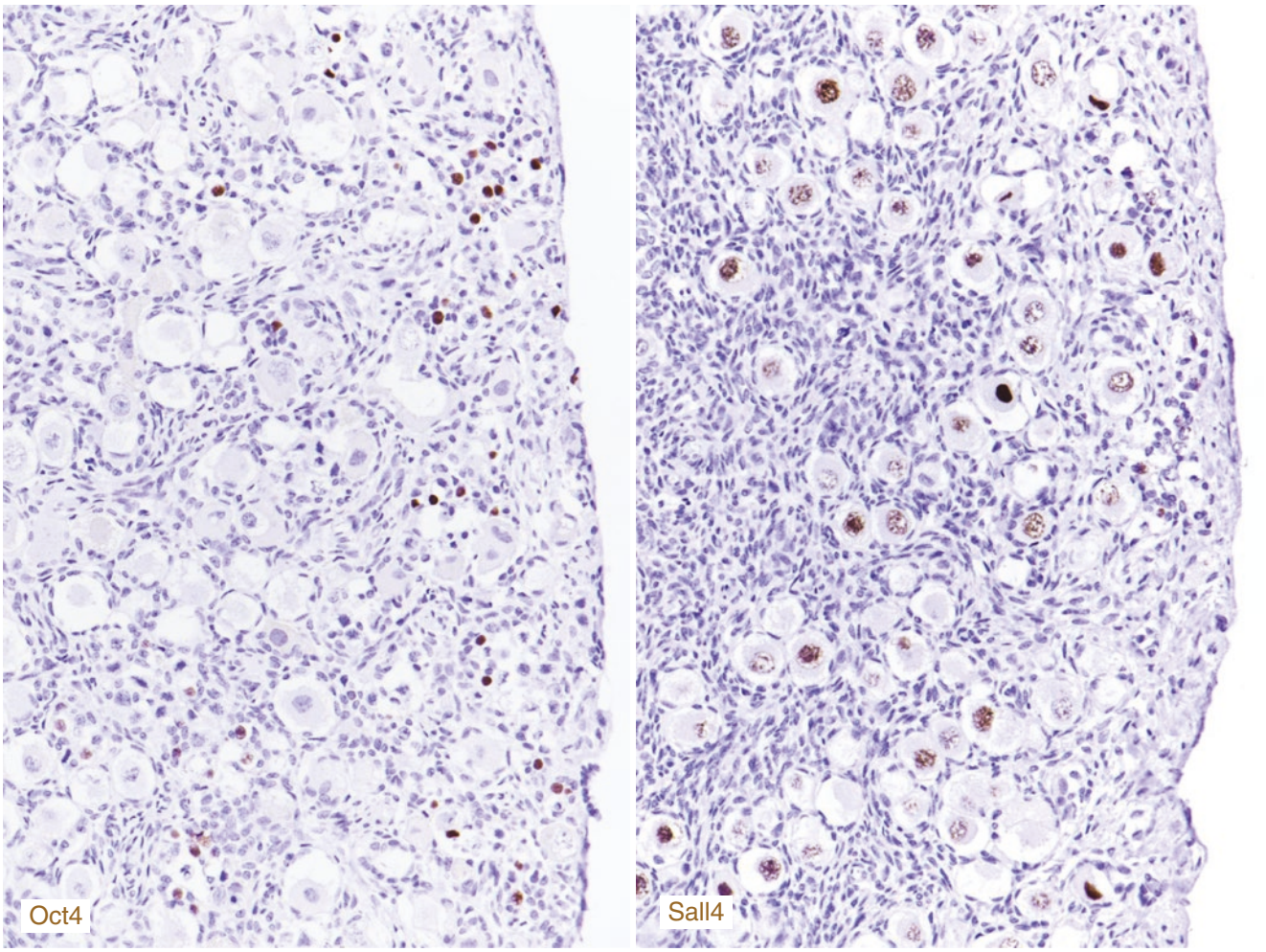

Fig. 1.3 Expression of Oct4 and Sall4 in the neonatal human ovary. Oct4 is highly expressed in oogonial clusters that have not yet individualized but not in individualized primordial oocytes, whereas Sall4 is expressed in the oocytes in fully individualized primordial follicles but not in the oogonia. Note nuclear localization for both markers

DNA demethylation, both (1) after fertilization in the zygote and (2) during early germline (i.e., PGC) development. DNA methylation, which is the basis of genomic imprinting (differential expression of alleles based on parent-of-origin), is generally stably inherited through cell divisions. Reprogramming in the zygote serves to erase epigenetic marks acquired from the gametes (except the marks on imprinted loci such as *CDKN1C*, which encodes p57KIP2) permitting the acquisition of totipotency. A key aspect of the developing germline is the resetting of the epigenome (i.e., genomic imprints) [17]. Such imprints are erased in PGCs of both sexes as they undergo genome-wide demethylation, X chromosome reactivation, and chromatin reorganization during migration and colonization of the gonadal ridge. This hypomethylation is believed to occur through a passive mechanism whereby Prdm1 and other factors repress the expression of the DNA methylases Dnmt3a and b. Consequently, both de novo and maintenance methylation is repressed, resulting in passive demethylation during DNA synthesis as PGCs proliferate. CpG methylation levels drop dramatically, and because of this hypomethylation, parental epigenetic memories are erased through secondary chromatin modifications including depletion of H3K9me2 repressive histone marks (Fig. 1.4). Other mechanisms also contribute to DNA demethylation in PGCs [16].

1.2 The Gonadal Ridges as the Origin of the Bipotential Gonads

The gonadal ridges (aka genital ridges) are the paired precursors (anlagen) of the gonads in both sexes. At around the 5th week of gestation, two small bulges form on the dorsal coelomic wall, lateral to the aorta and medial to the mesonephric duct (Fig. 1.1). The bulges consist of overlying coelomic epithelium (i.e., mesothelium) and the underlying mesenchyme

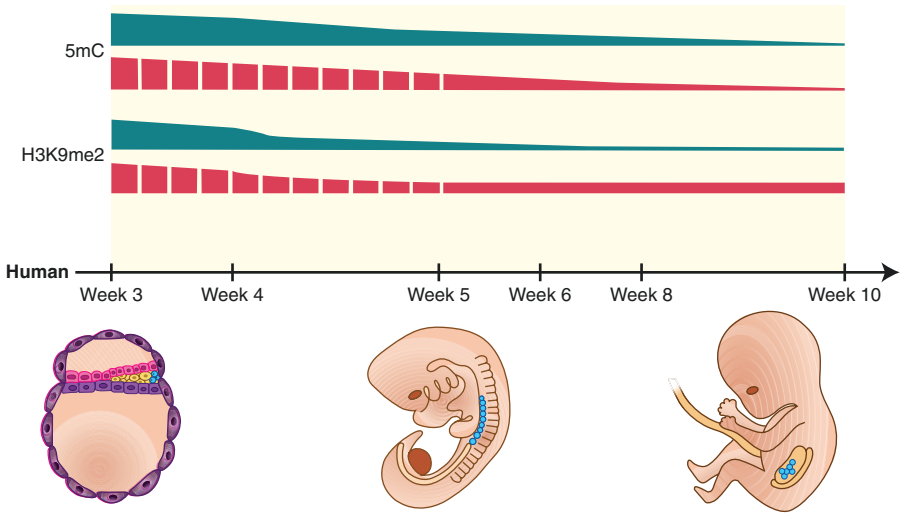

Fig. 1.4 PGCs are epigenetically programmed to reset genomic imprints during their development. Levels of DNA methylation (5-methylcytosine, 5mC) decrease due to a number of mechanisms. This alters various chromatin/histone marks such as H3K9me2 as well as others not shown. Redrawn with permission from Tang WW et al., Nature Review Genetics 17:585 (2016) [16] (Springer Nature)

[18]. The coelomic epithelium proliferates, and the basement membrane becomes fragmented, allowing the epithelial cells to migrate inward, an example of epithelial-mesenchymal transition (see section below on this subject). Prior to the arrival of PGCs, the gonad is histologically identical in both sexes (bipotential gonad), and the cells therein have the unique ability to differentiate into one of two functionally and morphologically distinct organs, an ovary or testis.

The embryologic cells of origin of the adult ovarian somatic cell types (granulosa and theca cells) remain the subject of some debate, and they may have more than one source [19, 20]. The signals that trigger and direct coelomic epithelium to proliferate and differentiate are unknown, but several transcription factors that are highly expressed in the bipotential gonad (Wt1, Sf1, Emx2, Lhx9, and Gata4) are required for formation of the gonadal ridge, among other embryological functions. Mice genetically engineered to be deficient for these factors fail to form gonadal ridges [21–24].

1.3 Sex Determination, Meiosis, and Early Gonadal Development

1.3.1 The Bipotential Gonads

Remarkably, the gonad is the only tissue in the mammalian body plan with two different potential developmental outcomes. In contrast, the development of other sexually dimorphic organs such as the male vs. female reproductive tract (discussed below) depends on the differentiation vs. regression of two entirely distinct precursor structures (the Müllerian vs. Wolffian ducts). Following the arrival of the PGCs, the bipotential gonad adopts two very different fates—testis or ovary—based on chromosomal sex (XX or XY). Subsequent sexual differentiation, including the acquisition of secondary sexual characteristics such as the development of external genitalia, is governed by what type of gonad develops in the embryo.

1.3.2 Sex Determination: Sry, Sox9, and the Male Pathway

The sex-determining region Y protein (encoded by the *Sry* gene on the Y chromosome) is a Sox (Sry-related HMG box) family transcription factor that is both necessary and sufficient to induce testis development [25]. The key and apparently only function of Sry is to induce expression of a second Sox family member gene, Sox9, which is autosomal (human chromosome 17). In XY gonads, Sox9 expression is increased, whereas the opposite occurs in XX gonads. The Sry-positive cells in XY gonads differentiate into Sertoli cells. It is currently thought that a precursor cell in the bipotential gonad differentiates into either Sertoli or granulosa cells dependent on the induction of Sox9 within these cells. Sox9 continues to be highly expressed in adult Sertoli cells and is a useful marker for Sertoli cells within the gonad. It is also believed that a second distinct type of precursor cell differentiates into either testicular Leydig or ovarian theca cells. The transcription factor Wt1, which also functions in MD development, promotes Sertoli cell differentiation and participates in the activation of Sry [26]. Once its expression is established, Sox9 initiates expression of downstream genes including anti-Müllerian hormone (AMH) (Fig. 1.5) [25]. Sox9 also antagonizes β-catenin, a key component of the ovary-determining pathway (see below) by transcriptional and other mechanisms [27, 28].

Deletion of Sox9 in the gonads of XY mice leads to the development of ovaries, and conversely, overexpression

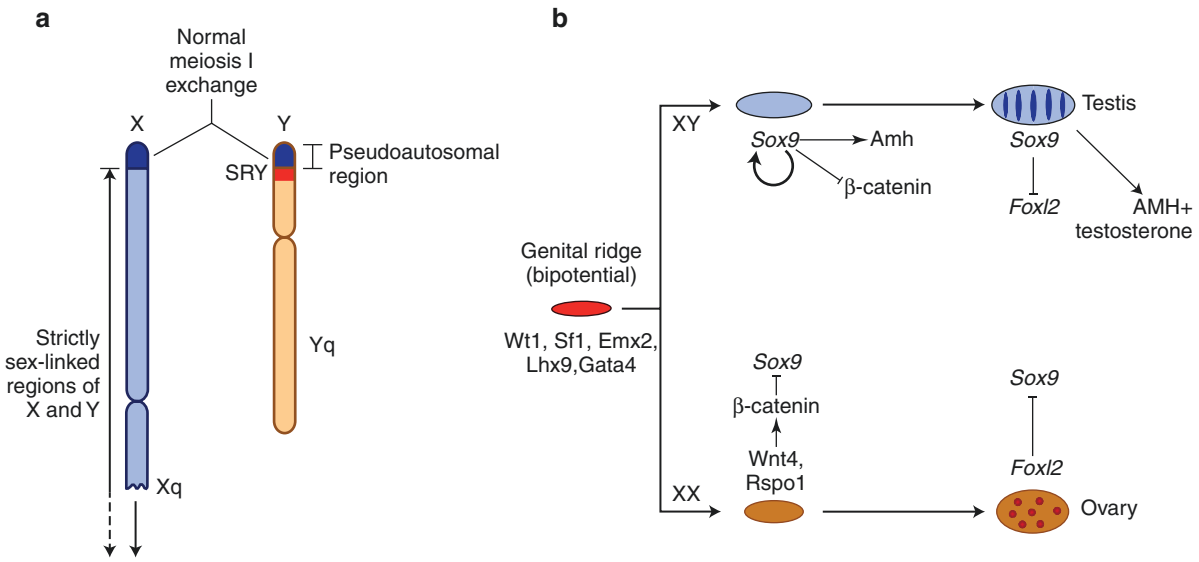

Fig. 1.5 The Y chromosome/SRY/Sox9 pathway of gonadal sex determination. (**a**) The Sry gene is Y-linked (exists only on Y chromosome) and resides on the p arm outside of the pseudoautosomal region. The pseudoautosomal regions are regions of homology on the X and Y chromosomes needed for pairing and crossing-over during meiosis. (**b**) Molecular pathway of gonadal sex determination. Factors required for genital (gonadal) ridge formation are on the left. Simplified male (XY) and female (XX) gonadal pathways are schematized. Redrawn with permission from Nussbaum Rl et al., Chapter 6, Genetics of Medicine, 8th edition (2016) (Elsevier); and Sekido R and Lovell-Badge R, Trends in Genetics, 25:19 (2009) [31] (Elsevier)

of Sox9 in the gonads of XX mice leads to the formation of testes. Thus, Sox9, like Sry, is both necessary and sufficient for testis differentiation. It is interesting to consider why Sox9 evolved as a necessary intermediate step versus a simpler mechanism requiring only Sry as the sole driver of sex determination. One attractive explanation is that Sox9 serves to amplify feeble signals from Sry. Due to the Y chromosome's lack of a homologous partner for crossing-over, the Y undergoes meiotic recombination with the X only in its small pseudoautosomal region, which is needed for X chromosome pairing during meiosis. This makes the Y vulnerable to accumulation of mutations at a much faster rate than the other chromosomes, leading to its biological and functional "enfeeblement" in the non-pseudoautosomal loci including the *Sry* locus over evolutionary timescales [29].

Once Sox9 expression is induced, Sox9 protein maintains its own expression in a robust feed-forward loop that no longer requires a sustained Sry signal. This secures an "all-or-none" binary decision for the appropriate gonadal cells to adopt a Sertoli vs. granulosa cell fate, thus ensuring either completely male or female sexual differentiation. The situation has been compared to "a short pulse from a relatively flimsy starter motor [Sry] which awakens a powerful engine that keeps itself running [Sox9]" [30]. Acquisition of secondary sex differentiation including the development of a male vs. female reproductive tract thus ultimately depends on the formation of Sertoli vs. granulosa cells. If Sertoli cells develop, they secrete factors that induce testicular differentiation, and the Sertoli cells together with Leydig cells produce factors that dictate all subsequent sex determination [31]. These factors are further discussed in the section below on Müllerian duct development.

As mentioned above, at around the time of PGC colonization of the gonadal ridge, the bipotential gonads begin to differentiate into testes in XY embryos and ovaries in XX embryos.

By mechanisms that are not well understood but that depend on a variety of cues including the vasculature, the Sertoli cells from discrete cords, or tubules that can easily be observed grossly by transillumination and are the precursors to the seminiferous tubules. The germ cells, now subjected to the sex-specific cues of their surroundings, adopt very different fates. The male germ cells become embedded within the tubules and enter a state of mitotic arrest; those that fail to become incorporated into the tubules are eliminated. In contrast, the ovaries remain relatively unstructured, containing interspersed somatic cells and oogonia [32].

1.3.3 Initiation of Meiosis in the Female Germline

While germ cells in the testis become mitotically arrested and do not initiate meiosis until puberty, the female germ cells initiate meiosis. In human oogonia, as in other mammals, the initiation of meiosis proceeds rather asynchronously; that is to say, meiosis is not triggered simultaneously

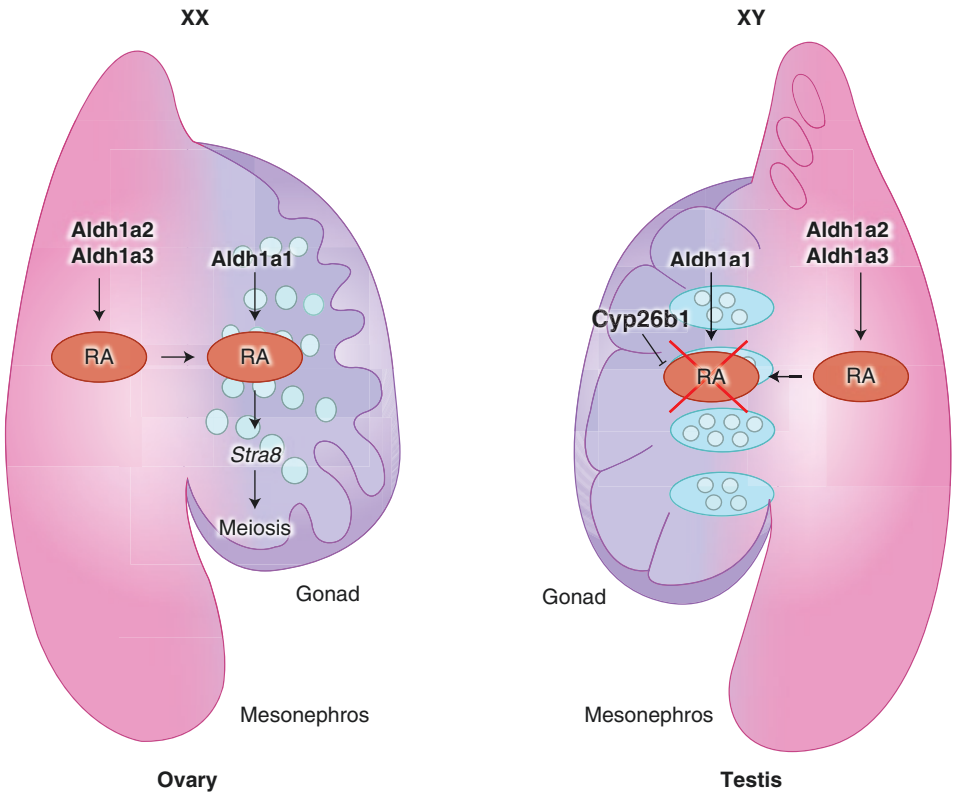

Fig. 1.6 Pathways of retinoic acid (RA) metabolism driving the initiation of meiosis in the XX (female) gonad. Expression of Cyp26b1 in the male gonad metabolizes RA, inhibiting the initiation of meiosis

in all oogonia. Oogonia in the initial leptotene prophase of meiosis I are first observed in embryos at 10–11 weeks gestation [33], but other oogonia continue proliferating and do not initiate meiosis until at least 16 weeks. Oogonia remain arrested in prophase I of meiosis for years or decades, until meiosis resumes following fertilization.

Entry into meiosis is the first outward sign that germ cells have initiated female development. This initiation of meiosis in females (and its absence in males) depends upon retinoic acid (RA) signaling. RA is a diffusible signal and the essential metabolite of vitamin A (retinol). RA acts through binding the retinoic acid receptor (RAR), leading to the control (repression or activation) of downstream genes whose regulatory control regions contain RAR binding sites [32]. Genes encoding the RA synthesis enzymes Aldh1a1, Aldh1a2, and Aldh1a3 are expressed in the gonad and developing mesonephros (part of the developing renal system) adjacent to the gonad, exposing the gonad to relatively high levels of RA. In the XX gonad, these high levels of RA induce the expression within germ cells of downstream RA target genes such as Stra8, leading to progression through meiosis. However, in the XY gonad, the expression within somatic cells of Cyp26b1, a RA-degrading enzyme of the P450 family, effectively degrades the RA signal, and meiosis is not initiated. Cyp26b1 is upregulated by Sox9, linking the sex determination pathway to the initiation of meiosis in germ cells (Fig. 1.6) [25, 32, 34].

1.3.4 Somatic Gonadal Differentiation: Foxl2, Granulosa Cells, and the Ovary-Determining Pathway

Another salient difference between the male and female germline is that while spermatogonia in early cords are individualized (i.e., physically separate from one another), the oogonial mitotic divisions preceding the initiation of meiosis occur with incomplete cytokinesis, leading to syncytial nests of female germ cells interconnected by intercellular bridges (ring canals). These oogonial nests, also known as cysts, are invested in somatic cells destined to become granulosa cells.

In humans, *Wnt4* and *Rspo1* loss-of-function mutations result in sex reversal in XX females, and both of these factors function by stabilizing β-catenin. Consistent with this idea, XY mice engineered to express a constitutively active form of β-catenin in the somatic cells of the gonad undergo ovarian development (sex reversal). This does not affect the initial upregulation of Sox9 in the gonad but interferes with maintenance of Sox9 expression. Thus, Wnt4, Rspo1, and

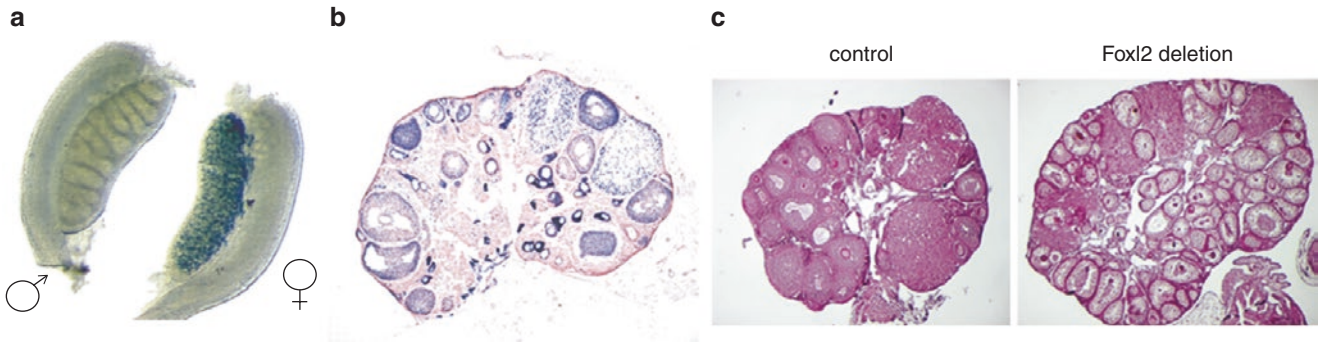

Fig. 1.7 Foxl2 is a key factor in the ovary-determining pathway. (**a**) Foxl2 is the earliest known marker of granulosa cell differentiation: mouse testis (left) and ovary (right) at 12.5 days postfertilization. These male and female gonads are from a mouse engineered to express β-galactosidase in Foxl2$^+$ cells, permitting their visualization. No expression is seen in the testis (note presence of cords in testis but not ovary). (**b**) Foxl2 expression persists in granulosa cells in adult mouse ovary (blue stain) and is required for their function. (**c**) Deletion of Foxl2 in adult mice (after normal ovarian development has taken place) results in sex reversal phenotype where ovarian follicles are transformed into tubular structures resembling seminiferous tubules and comprised of Sox9$^+$ Sertoli-like cells. This striking sex reversal shows that Foxl2 function is required in the somatic cells of the ovary to maintain female gonadal differentiation. (**a**, **b**) Reproduced with permission from Schmidt D et al., Development, 131:933 (2004) (Company of Biologists) [36]. (**c**) Reproduced with permission from Uhlenhaut NH et al., Cell 139:1130 (2009) (Elsevier) [41]

β-catenin function together within the gonad as "anti-testis" factors (Fig. 1.5) [25].

The forkhead transcription factor Foxl2 also serves an essential and evolutionarily conserved role in the establishment and continuation of ovarian fate [35]. Foxl2 is among the earliest genes induced in the developing ovary and is the earliest established marker of granulosa cell fate. In mouse XX gonads, Foxl2 is first expressed in the somatic cells surrounding germ cell nests of XX gonads at the time that cord-like structures are first formed in XY gonads (Fig. 1.7) [36]. The mechanisms underlying the induction of Foxl2 induction specifically within XX gonads and its lack thereof in XY gonads are not well understood. Later in development and in early postnatal mouse ovaries, Foxl2 expression occurs in both granulosa and interstitial cells, but decreases in the latter population with age (Fig. 1.7) [37]. This expression in both granulosa and interstitial ovarian cells also occurs in the human ovary (Fig. 1.8) and rationalizes the fact that Foxl2 is a sensitive and specific immunohistochemical marker of sex-cord stromal tumors in general, but is not specific for granulosa cell tumors [38].

Foxl2 is not required for the formation of the ovary in mice, as Foxl2-deficient mice develop ovaries. However, Foxl2-deficient female mice exhibit abnormal postnatal granulosa cell/follicular development, leading to defective follicles, massive follicular atresia, and premature ovarian failure and sterility [36, 39]. Interestingly, genes involved in the testis pathway are abnormally activated in Foxl2-deficient ovaries, indicating that Foxl2 represses the testicular gene expression program [40]. Foxl2 is also essential for the continued maintenance of granulosa cell fate in adult life. Bypassing this embryonic requirement for Foxl2 function in follicle formation through postnatal deletion of the *Foxl2* gene in adult mouse ovaries revealed a striking phenotype

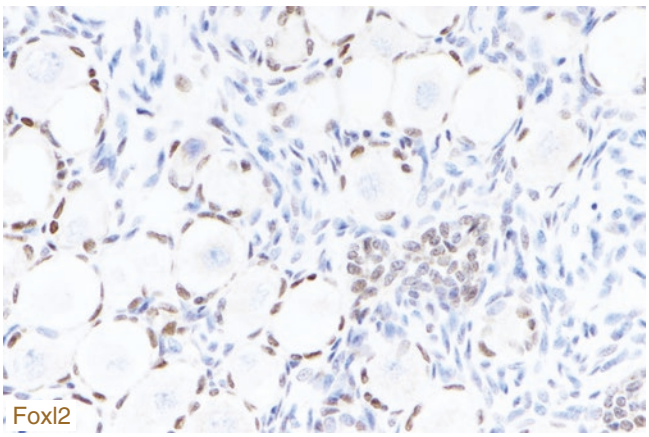

Fig. 1.8 Expression of Foxl2 in the human neonatal ovary. Expression is most prominent in the granulosa cells (at this stage almost all follicles are primordial and thus have a single layer of flattened granulosa cells) but there is also scattered expression in other ovarian mesenchymal cells

whereby all ovarian granulosa cells transdifferentiated into Sertoli-like cells (Fig. 1.7), with abnormal expression of Sox9 and AMH. This leads to conversion of the ovarian follicles into cord-like structures resembling testicular cords/seminiferous tubules, and testosterone levels similar to those of XY littermates. These results demonstrate that the ovarian vs. testicular somatic phenotype is actively maintained throughout female life by Foxl2 [41].

In humans, inherited loss-of-function mutations in the *Foxl2* gene do not lead to sex reversal, but rather to the autosomal dominant blepharophimosis-ptosis-epicanthus inversus syndrome (BPES), which is associated with eyelid malformations and premature ovarian failure (OMIM: 110100). In humans, as in mice, Foxl2 is expressed in the mesenchyme around the developing eyelid as well as