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Sameh Helmi Edward Doss
Editors

Clinical Embryology

An Atlas of Congenital Malformations

Springer

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Cover illustration: Cartoon by Leonardo Da Vinci(1452-1519) Depicting Studies of the foetus in the womb.

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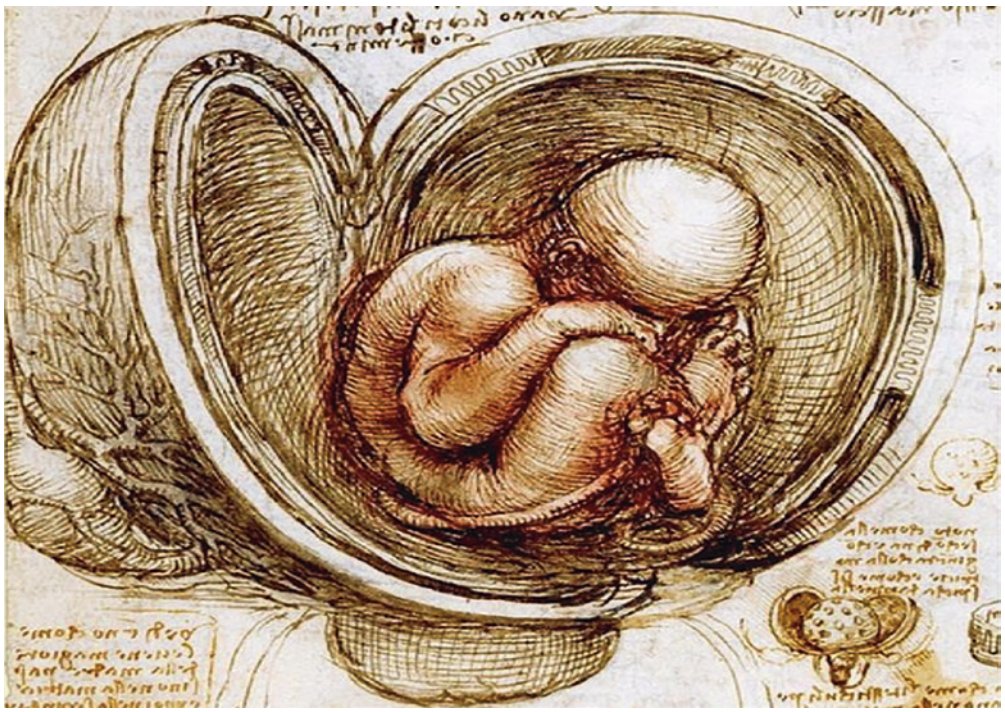
Cartoon by Leonardo Da Vinci (1452–1519) Depicting Studies of the foetus in the womb. Permission From The Royal Windsor Collection of Her Majesty the Queen



Preface

This book is a unique compilation of notes first documented and written in 1996 by Dr. S. Doss, one of the co-editors who is an anatomist and embryologist at Cairo University. The clinical photographs are a unique compilation of three generations of paediatric surgeons, a collection of over 40,000 images, the lifetime work of the late Professor D. G. Young, University of Glasgow. This book is dedicated to him.

Clinical embryology is defined as the study of the embryo and its development into the human form, first as a foetus until birth. It explores the relationship to clinical malformations that present at birth.

Congenital malformations affect an estimated 3.2 million birth defect-related disabilities each year worldwide (WHO statistics 2014). Three children out of every hundred born are affected by a congenital malformation and an estimated 270,000 newborn die within the first month each year. The ones who survive from the medical and surgical care they receive often result in long-term disabilities. These have significant impact on the patients, their families and carers, the health systems and society as a whole. The most common severe congenital malformations affect the heart, the nervous system, spine and Downs syndrome. In 2010 the World Health Assembly adopted a resolution calling all member states to promote primary prevention and the health of children with congenital malformations by:

1. Developing and strengthening registration and surveillance systems
2. Developing expertise and building capacity
3. Strengthening research and studies on aetiology, diagnosis and prevention
4. Promoting international co-operation

The problem with teaching embryology as an undergraduate subject is that all too often it is done in isolation as a science and part of the anatomy teaching. Since anatomy teaching has been dropped by many medical schools worldwide, the teaching of embryology has also been neglected. A recent survey of the whole of the medical undergraduate school of one of the largest universities in the UK revealed that only 17% of students were confident in their embryology knowledge and that 80% declared that it should definitely be included in the medical school curriculum. Eighty per cent found it a difficult subject to understand and only 36% were satisfied with the teaching of embryology. As a subject embryology is difficult to conceptualise, complex to understand and difficult to teach. The clinical abnormalities encountered in most conditions can be explained by a failure of this developmental process.

This book is unique because it combines the embryological development of the human and the malformations encountered in clinical practice.

Undergraduate medical students as well as nurses in training will find it helpful in their studies. Postgraduate trainees in paediatric surgery, paediatrics and neonatology will find it easy to follow, and it enables them to explain the mystery of congenital malformations to relatives and patients. General practitioners who will encounter more and more of these patients

with their disabilities will be able to understand how these developed. It is hoped that people who read this book will find it helpful in their work.

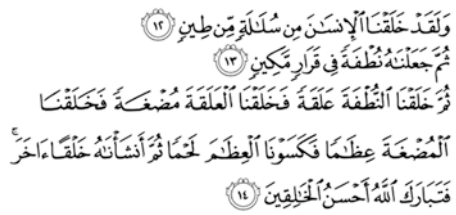
We would like to thank Ms. Suzanne McMahon and Dr. Sharon F. Sneddon as subeditors for all of their hard work in seeing this book to completion.

I would like to thank my wife Annette for all the work she has helped me over the years to see this to completion.

Glasgow, UK — Robert Carachi
Cairo, Egypt — Sameh Helmi Edward Doss

You created my inmost self,
knit me together in my mother's womb.
For so many marvels I thank you;
A wonder am I, and all your works are wonders.
You knew me through and through

Psalm 139 verses 13, 14 Jerusalem Bible


وَلَقَدْ خَلَقْنَا ٱلْإِنسَٰنَ مِن سُلَٰلَةٍ مِّن طِينٍ ﴿١٢﴾
ثُمَّ جَعَلْنَٰهُ نُطْفَةً فِى قَرَارٍ مَّكِينٍ ﴿١٣﴾
ثُمَّ خَلَقْنَا ٱلنُّطْفَةَ عَلَقَةً فَخَلَقْنَا ٱلْعَلَقَةَ مُضْغَةً فَخَلَقْنَا
ٱلْمُضْغَةَ عِظَٰمًا فَكَسَوْنَا ٱلْعِظَٰمَ لَحْمًا ثُمَّ أَنشَأْنَٰهُ خَلْقًا ءَاخَرَ ۚ
فَتَبَارَكَ ٱللَّهُ أَحْسَنُ ٱلْخَٰلِقِينَ ﴿١٤﴾

These verses appear in Surah Al Mu'minūn.

A simple translation of these verses (12–14) is as follows:

We created man from an essence of clay (12),

then We placed him as a drop of fluid (nutfah) in a safe place (13),

then We made that drop into a clinging form (alaqah), and We made that form into a lump of flesh (mudghah), and We made that lump into bones (idhaam), and We clothed those bones with flesh (lahm), and later We made him into other forms (nash'ah)—glory be to God, the best of creators (14)!

Contents

A Brief History of Embryology: Historical Vignettes in Embryology

1

Basith Amjad

What is embryology? It is perhaps an arbitrary term to describe a vast and fascinating science. Most embryologists consider their discipline as the study of "developmental biology", essentially encompassing biological development from a fertilized egg to a multicellular organism.

Human embryology considers the developmental aspects of life as a whole and not just the first 8 weeks of the embryo. The appreciation of human embryology is important, not just to give us an understanding of how we come into our adult form but also to explain the biological, chemical and physical forces, which shape the growing embryo. These very forces then continue to define and sustain the individual throughout their lifetime.

Within the last couple of decades, the science of embryology has taken tremendous strides providing us with the knowledge and understanding to bring about significant improvement in both maternal and child health. We are now equipped with techniques for improving fertility, diagnosing and managing prenatal conditions, sustaining the pregnancy and the foetus and preventing birth anomalies.

These breakthroughs in modern medicine have made a vast difference in human history and continue to do so. Thus embryology has brought about long-term social, cultural, religious, political and financial shifts.

Apart from the obvious better birth outcome, as an estimated 276,000 babies still die within 4 weeks of birth every year worldwide, the science of applied embryology has long-term effects on the physical and mental wellbeing of an individual.

The study of embryology, which is the formation and development of the embryo and the foetus, can be traced back to ancient Egypt. Around 1400 BC, the ancient Egyptians made references to the placenta and its importance as the seat of the external soul. However they did not consider the embryo to be alive until the baby was born. They also discovered that chicken eggs could be incubated in ovens, a finding later used for studying embryos during different periods of development (Fig. 1.1).

The science of embryology as an entity first appears in works by early Greek philosophers such as Empedocles, Anaxagoras and Diogenes. They were interested in the study of reproduction, development, differentiation and regeneration of body parts. They believed that new organisms could arise through sexual or asexual reproduction or spontaneous generation. A strongly held view was of a fire inside the embryo, which set the parts in order as the foetus developed.

Joseph Needham, one of the great historians of embryology, called Hippocrates (c460 BC–c370 BC) the first true embryologist. Hippocrates believed in what came to be known as preformationism, a concept that all organisms were fully formed in miniature within the womb before birth. He also felt that the embryo derived its blood supply from the placenta and developed by extracting moisture and breath from the mother. He then went on to identify what he called

Fig. 1.1 An Egyptian sculpture shows a pharaoh, and attendants in a ceremonial procession carry the pharaoh's soul, his placenta with the umbilical cord

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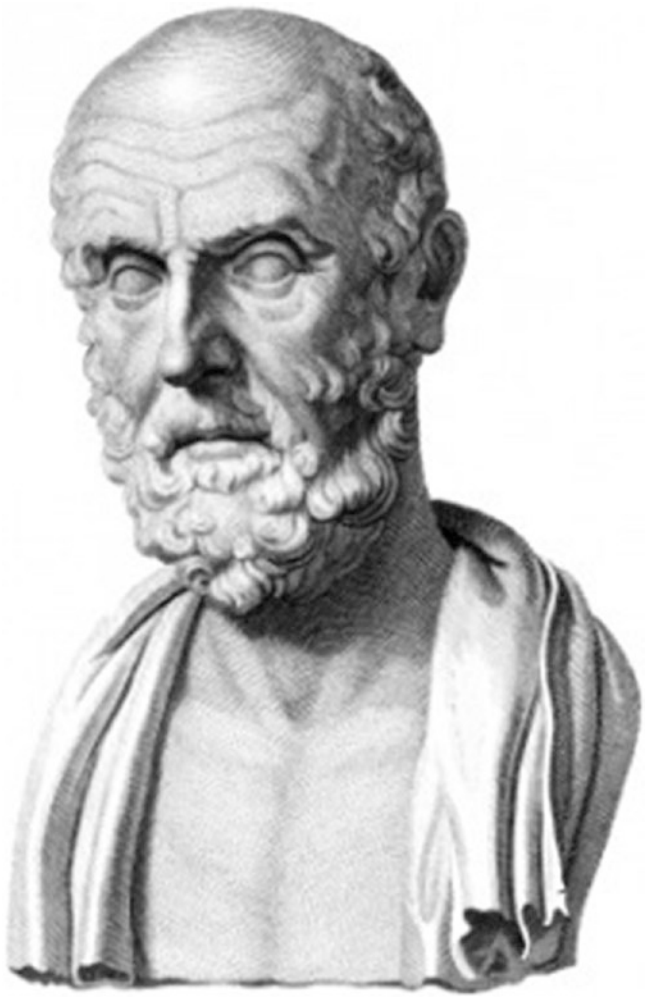

Fig. 1.2 A bust of Hippocrates: the first true embryologist

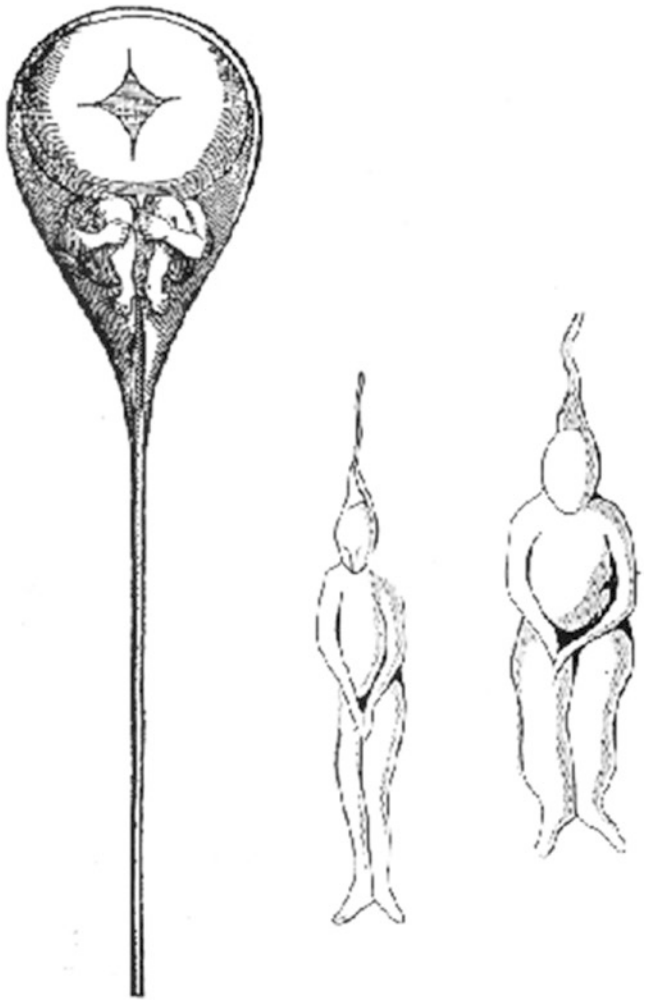

Fig. 1.3 "Preformation" drawn by Nicolaas Hartsoeker in *Essai de Dioptrique*, 1694

a number of condensations and fires that led to the formation and development of bones, flesh, gut and circulation within the embryo and foetus (Fig. 1.2).

Even though Plato deals with natural phenomenon in Timaeus (Dialogues 360 BC), the next major progress in embryology came about under his pupil Aristotle (384 BC–322 BC). His main embryological book was titled *On the Generation of Animals*, but most of his well-known observational science on embryology is found in the four compendiums, the *History of Animals*, the *Parts of Animals*, *On Respiration* and *On the Motion of Animals*. His writings confirm that he studied embryos of different organisms by not only opening up bird eggs at different stages of development but also dissecting mammalian embryos. He may have also observed human embryos, an almost impossible task in antiquity. Aristotle believed that the semen supplied a substance to give form to the embryo and then the mothers supplied another substance, which aided development (Fig. 1.3). He also believed that the menstrual blood had some part to play in the formation of the embryo. Aristotle also believed that all young embryos were similar with universal characteristics, but as the embryo grew, it started to show differentiation (Fig. 1.4).

Following the death of Aristotle, there was not much progress in the science of embryology till the time of Galen of Pergamon (129 AD–216 AD). Even though Galen did not give much attention to embryology, he wrote and propagated the strong belief that the umbilical cord was necessary for foetal respiration. Ali ibn Sahl Al-Tabari of Baghdad (808–864) wrote a seven-part system of medicine called *Firdous al-Hikmah* in which an entire part was devoted to embryology, a mixture of Greek and Arabic thinking at that time. The great Avicenna or to give him his proper name Abu Ali al-Hasan ibn Sina (980–1037) devoted certain chapters of his *Canon Medicinae* to the development of the foetus but added nothing new to the science past Galen (Fig. 1.5).

Fig. 1.4 Plato and Aristotle: *The School of Athens* by Raphael

Fig. 1.5 Avicenna at work on the *Canon of Medicine*

Again quoting Joseph Needham, the credit for helping to bring embryology back into the scientific realm belongs to Albertus Magnus of Cologne (c1200 AD–1280 AD). Albertus read extensively and then interpreted almost all the works of Aristotle and the Arabic commentaries that accompanied them and was therefore in the position of replacing speculative and theological ideas with observational techniques and attention to detail. Albertus believed that women had seeds and that female seeds congealed after coming into contact with male seeds, and once this egg came into contact with menstrual blood, there was nutrition available for the egg to develop into an embryo and then a foetus. A large portion of Albertus's knowledge came from studying chick and fish embryos.

Leonardo da Vinci (15 April 1452–2 May 1519) has been described by art historian Helen Gardner as "an individual of unquenchable curiosity and feverishly inventive imagination, the very epitome of renaissance humanist ideals". He was a polymath whose interests included sculpture, painting, architecture, music, engineering, cartography, literature, botany, anatomy and embryology. Among the artists of the Renaissance, he was not alone in his interest in human anatomy. But unlike Michelangelo, Raphael and Durer among others who undertook human dissection to increase their understanding of the human body, Leonardo was actually interested in biology. Leonardo's embryology is contained in the third volume of his notebooks called Quaderni d Anatomia. His drawings show the dissection of the pregnant uterus along with the amniotic and chorionic membranes. He also undertook dissection of the human embryo at various stages and produced quantitative measurements of the growth of the embryo. He was therefore the first to show that embryos can be measured chronologically and that they change in size, shape and weight as they grew. Leonardo's era also saw the emergence of midwifery and gynaecology as a field of science and practice, and this had direct implications on the emergence of modern embryology (Fig. 1.6a–c).

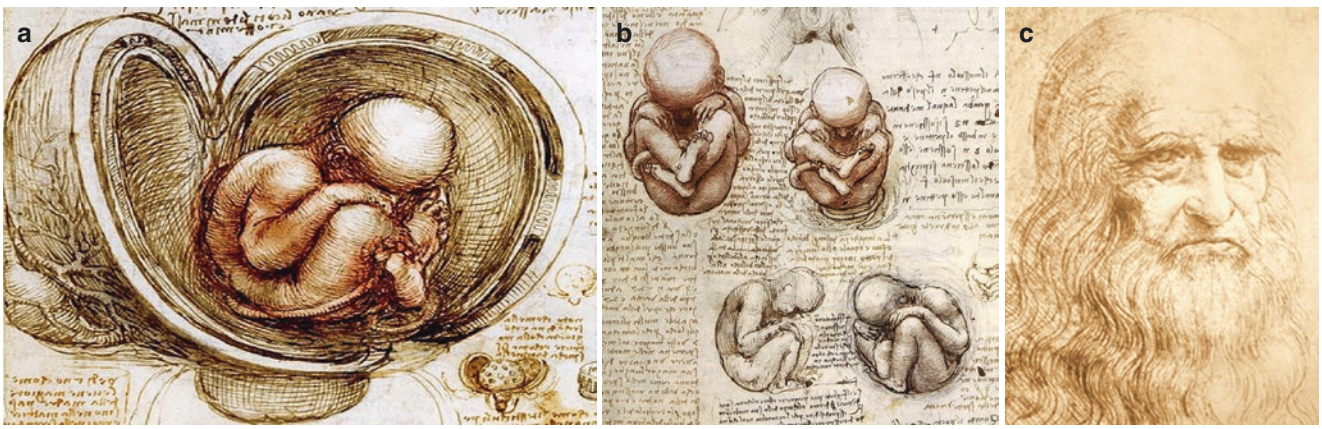

Fig. 1.6 (**a**) From *Studies of the Foetus in the Womb* by Leonardo da Vinci. (**b**) Views of a foetus in the womb by Leonardo da Vinci. (**c**) Self Portrait: Leonardo da Vinci

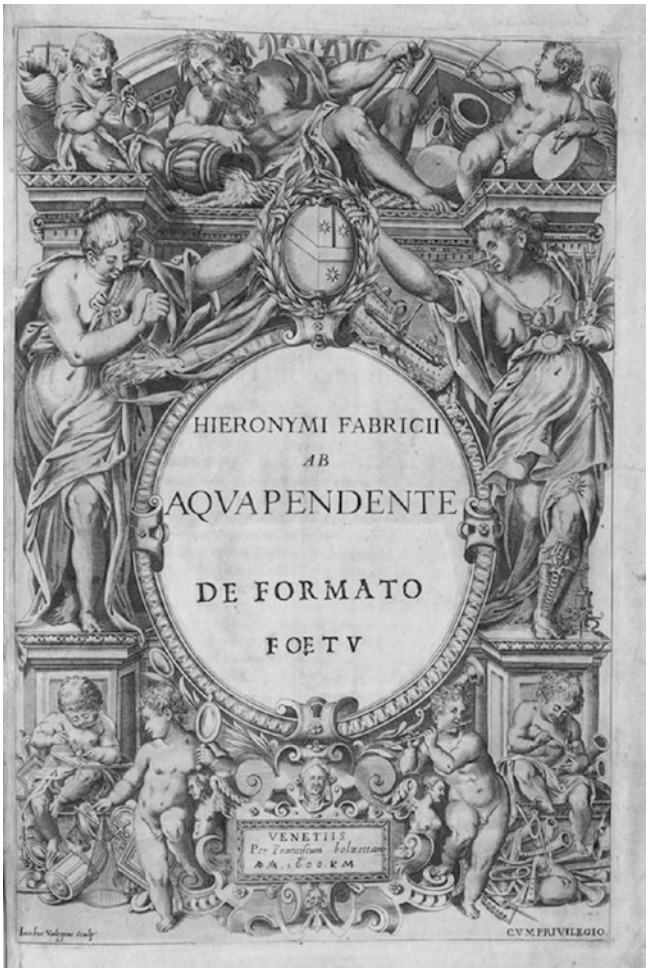

Fig. 1.7 First edition of Fabricius's treatise on human embryology Padua1600 AD

Hieronymus Fabricius (20 May 1537–21 May 1619) of Latium was a student of Falloppio and succeeded him as the professor of surgery and anatomy at the University of Padua. He is considered by many to be the Father of Embryology. In reality his work in comparative anatomy is where his legacy lies as he set up the first permanent theatre for public dissection. He dissected embryos of man, rabbit, guinea pig, dog, cat, sheep, ox, deer and viper, among others, a feat never before accomplished. This allowed him to investigate the formation of the foetus, the oesophagus, the stomach and the intestines. He also studied the function of the eye, the ear and the larynx (Fig. 1.7). The drawings and illustrations of Fabricius' anatomical works are incredibly accurate and beautiful and a testament to his genius. Unfortunately his works in embryology which included the belief that the chalaza found inside reptile and bird eggs and now known to suspend the yolk was the precursor of the brain, heart and liver and that the heart and other organs of the foetus had no proper function were erroneous and subsequently forced William Harvey to spend a considerable amount of his time challenging and refuting them (Fig. 1.8).

Fig. 1.8 Robert Hannah: William Harvey 1848

American historian Arthur M. Schlesinger Jr. has placed William Harvey (1 April 1578–3 June 1657) among the ten most influential people of the second millennium. A physician by training, Harvey's most defining contribution in human physiology and anatomy was to describe in complete detail the circulation of blood. Published in 1628, his work called *On the Motion of the Heart and Blood* gave a clear and detailed account of the function of the heart and the movement of blood around the body in a circuit. Less well known are Harvey's contributions to the study of embryology. Using low-powered lenses, Harvey had carried out extensive dissection work in deer and chicken embryos, described the blastoderm as the site of origin of the embryonic body, explained the importance of the amniotic fluid and, having shown that even the lowest organisms arise from eggs, finally laid the theory of spontaneous generation to rest.

Another unique individual who added to the advancement of embryology in the seventeenth century was the Italian physician Marcello Malpighi (10 March 1628–29 November 1694). Malpighi had an illustrious career as an academic professor of medicine and anatomy at Pisa but decided to retire from academic pursuit and return home to Bologna and dedicate his life to anatomical studies. Although some of his work involved gross anatomy, his most significant work appears to be based on the use of the microscope. Because of this many microscopic structures are named after him including the Malpighi layer in the skin, Malpighi corpuscles in the spleen and kidneys, etc. Needham credits him as the person responsible for the doctrine of preformation, metamorphosis and the development of the embryo as a simple unfolding of an already miniature adult organism.

Two rival schools of thought dominated the historical narrative of embryology in the eighteenth century. The preformationists, steeped in the writings of Malpighi, Swammerdam

Fig. 1.9 Albrecht von Haller: Swiss physiologist and naturalist

Fig. 1.10 Caspar Friedrich Wolff: German physiologist and embryologist

and Bonnet, believed that the embryo pre-existed in some form in either the maternal egg or the male sperm. They also advocated that all embryos had been formed by God at creation and encased within one another to await their future appointed time of development. Epigeneticists on the other hand, influenced by the legacy of Aristotle and Harvey, argued that each egg was newly produced through progressive development from unorganized material and proposed various theories to explain how this gradual formation occurred.

This discourse and dispute between the preformationists and the epigeneticists continued throughout the age of enlightenment and is best showcased by the debate that took place between Albrecht von Haller (Fig. 1.9) (16 October 1708–12 December 1777), a Swiss anatomist, physiologist and naturalist, and Caspar Friedrich Wolff (18 January 1733–22 February 1794), a German physiologist who is considered one of the founders of modern embryology (Fig. 1.10). Haller had been an ardent supporter of preformation since 1758, a year before the publication of Wolff's dissertation, which endorsed epigenesis. The tussle between the two men lasted over a decade and came to define and symbolize the key questions faced by biological sciences in that era, the idea of God in relation to the biology of creation, the question of spontaneous generation, the role of mechanism in developmental embryology, the issue of regeneration and the dilemma of "monstrous births". Haller, a "Newtonian mechanist" and a deeply religious man, held beliefs about the nature of the world and scientific views that were fundamentally very different to those of Wolff, whose own world views and scientific outlook derived largely from the tradition of German rationalism. Wolff's research work covered both the fields of anatomy and microscopic embryology. In a series of ground-breaking scientific papers, he laid the foundation of modern embryology. He was the first to describe the primitive kidneys or mesonephros or the "Wolffian bodies" and its excretory ducts as laid out in his dissertation entitled "Theoria Generationis". In 1768 he published *De formatione intestinorum* in which he explained the development of the intestine and foreshadows the idea of germ layers in the embryo. He demonstrated that the chick intestine was formed by the folding of tissue that detaches from the embryo's ventral surface. The folds eventually transform into a closed tube. He then argued that this observation proved that the intestine was not preformed and that the organs appeared gradually. Wolff's observations are now recognized as the most fundamental conception in structural embryology. Wolff also examined and dissected the so-called embryonic monsters and assessed correctly that they were

Fig. 1.11 Herman Boerhaave: Dutch pioneer of chemical embryology

formed by the mechanics of nature and thus were examples of epigenesis rather than preformationism. In spite of all this work by Wolff, Haller's reputation was such that his assertions continued to cast a powerful influence within the scientific community even though ultimately Wolff was vindicated by posterity.

Another embryological puzzlement that the embryologist wrestled with was the issue of foetal nutrition. The ideas put forward regarding the source of nutrition included amniotic fluid ingested by the foetus, a wholesome fluid made available to the foetus called uterine milk, nutrition circulating within the menstrual blood and passing via the umbilical cord. Without clear evidence backed up by experimental techniques, these ideas remained within the sphere of theory and conjecture.

Another remarkable individual was the Dutch physician and anatomist Herman Boerhaave (31 December 1668–23 September 1738). Boerhaave separated egg white from the yolk and conducted various chemical and physical experimentations including adding various acids and bases and shaking, heating and boiling the components to see the effects produced. He thereafter published his results in a first detailed account of chemical embryology. This work in turn led to the science of experimental work in the field of biology (Fig. 1.11).

The brothers William (23 May 1718–30 March 1783) and John Hunter (13 February 1728–16 October 1793), anatomists and surgeons, are both giants of modern medicine who have greatly advanced the scientific method in medicine. One of their most significant discoveries, published in their

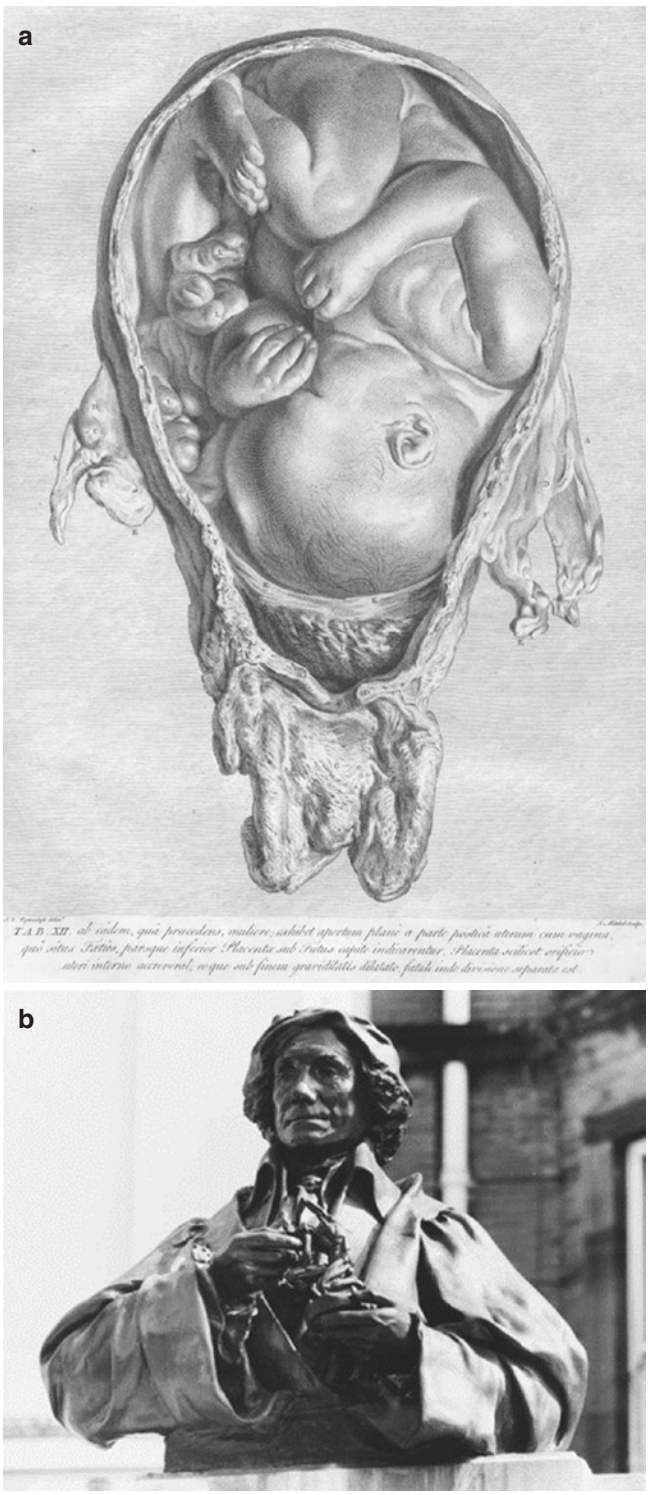

Fig. 1.12 (**a**) Page from the *Anatomy of the Human Gravid Uterus* by William Hunter. (**b**) Statue of John Hunter outside St George's Hospital in London

work on the anatomy of the gravid uterus, was to show clearly that maternal and foetal circulations were two distinct physiological systems (Fig. 1.12a, b).

The nineteenth century saw one of the great advancements in modern biology when cell theory came to be

Fig. 1.13 Matthias Schleiden: German botanist and proponent of cell theory

Fig. 1.14 Theodor Schwann: German biologist and physiologist

accepted. It was the work of two remarkable German scientists: Matthias Schleiden (Fig. 1.13) (5 April 1804–23 June 1881) and Theodor Schwann (Fig. 1.14) (7 December 1810–11 January 1882). While holding the chair of Botany at the University of Jena, Schleiden studied plant structure under the microscope and authored *Contributions to Phytogenesis* in which he stated that each and every part of a plant is made up of cells. He also mentions the cell nucleus and its role in cell division. Theodor Schwann working at the University of Berlin in Physiology was undertaking research in animal tissue. A meeting with Schleiden, where they talked about plant cells, made him realize that he had observed similar cells in animal notochord. This similarity was confirmed by both scientists working together, and the results appeared in Schwann's famous "Microscopic Investigations on the Accordance in the Structure and Growth of Plants and Animals", in which he declared that "All living things are composed of cells and cell products". This has now come to be known as cell theory or cell doctrine. In the course of this work, he went on to prove the cellular origin of highly differentiated tissues including nails and tooth enamel. Vitally he also studied the ovum and established that it is a single cell that eventually develops into a complete organism, thus confirming a basic principle of embryology. Schwann is also remembered for the discovery of Schwann cells in the peripheral nervous system and his discovery and study of pepsin.

One of the founders of modern embryology, Karl Ernst von Baer (17 February 1792–16 November 1876), also belonged to the nineteenth century and hailed from Estonia. He spent most of his productive years at the St. Petersburg Academy of Sciences, studying the embryonic development of animals (Fig. 1.15). His many achievements included the discovery of the mammalian ovum, the blastula stage of development and the notochord. Building on the work of Wolff, he described the germ layer theory of development and established that mammals developed from eggs. His book *Uber Entwickelungsgeschichte der Thiere* established the foundation of comparative embryology and laid down what have come to be known as Baer's laws of embryology. Another leading light in nineteenth-century embryology was the German zoologist and experimental embryologist Wilhelm Roux (9 June 1850–15 September 1924) who worked mostly with chicken embryos and frog's eggs to study developmental embryology. Maintaining his embryonic material in warm saline, he was the first to establish the idea of tissue culture and helps establish the mosaic theory of epigenesis.

By the middle of the last century, a sound body of basic knowledge was finally established which could describe the events of development within the embryo. Thereafter two themes have dominated the progress and development

Fig. 1.15 Karl Ernst von Baer: established the foundations of modern embryology

of embryology as a science. One of these themes, chemical embryology, coming into its own between the 1940s and the 1970s and using experimental techniques, has tried to explain the nature of embryonic induction or embryogenesis. This process of induction helps to define and then direct the development of a group of cells into particular tissues and organisms. The disciples of this field have tried to do this by seeking out and characterizing these inducing signals. A concurrent theme has been modern molecular embryology, which beginning in the 1980s has led to the current revolution in biological sciences. It seeks to explain at the genomic level the differentiation of cells into specific tissues and structure within the same organisms. Thus embryology is now concerned with the development of the organism from the telescope of activation and transcription of the DNA, thus allowing us to understand the genetic mechanisms of development and its consequences.

Hans Spemann (27 June 1869–9 September 1941), a German embryologist and Nobel laureate in Medicine in 1935, spent a lifetime of work in embryonic induction (Fig. 1.16). After qualifying as a doctor of medicine, he was initially intent on a career in medicine, but fate had other plans for him. He contracted tuberculosis and was confined to a sanatorium in 1896 and there, while recuperating, read *The Germ-Plasm: A Theory of Heredity* by August Weismann. Weismann, considered one of the great biologists of all times, is today best remembered for his germ plasm theory which states that in a multicellular organism, inheritance

Fig. 1.16 Hans Spemann: German embryologist and Nobel laureate in Medicine

takes place only by means of the germ cells, that is, the egg cells and the sperm cells, and other cells of the body, that is, the somatic cells, do not play any part in this process. After reading Weismann, Spemann switched to the field of zoology and embryology and embarked on a career that eventually led to his appointment at the Institute of Biology in Berlin. Working with a protégé named Hilde Mangold, he used microsurgical techniques to transplant a specific group of cells (which he called the primitive knot and now named Spemann's organizer) from one embryo to another. This organizer upon transplantation was then able to induce secondary embryonic primordial regardless of its location. He then went on to show how different parts of this organizer would produce different parts of the embryo. Thereafter the work of Johannes Holtfreter (9 January 1901–13 November 1992), Joseph Needham (9 December 1900–24 March 1995) and Conrad Waddington (8 November 1905–26 September 1975) showed that even if these foci of cells were killed, by either fixing, boiling or freezing them, they would continue to cause induction within the embryo. Their conclusion that these were inert molecules was better understood by the turn of the century when there was an appreciation of signalling within cells and the embryo.

Fig. 1.17 Gerald Edelman: American biologist and Nobel laureate

One of the giants of molecular embryology would surely be Gerald Edelman (1 July 1929–17 May 2014), an American biologist who won the Nobel Prize in Medicine for his work on the molecular structure of antibodies (Fig. 1.17).

He was initially intent on a career as a concert violinist but decided that he did not have the inner drive and willpower to succeed in that enterprise and switched, by a gift of providence, to medical research. In his most seminal work called *Topobiology: An Introduction to Molecular Embryology*, Edelman presented a theory that "morphogenesis is driven by differential adhesive interactions among heterogeneous cell populations and it explains how a single cell can give rise to a complex multicellular organism". Topobiology therefore as proposed by Edelman is a biological process that creates and maintains differentiated tissues and is acquired by segregation of cells through heterologous cellular interactions.

The advent of the twenty-first century saw the mapping of the human genome, and it appears likely that this will be the epoch of biological sciences and that it will play a key role in tackling global challenges. Embryology has not yet exhausted all its fascinating and mysterious possibilities.

Suggested Reading

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General Embryology

2

Sameh H. Doss and Sharon F. Sneddon

Female Reproductive Tract

Ovary

The female reproductive system shows a monthly cycle of growth and development throughout adult life. In the female, the reproductive tract comprises of a gland producing both gametes and steroid hormones and a duct system for transport of the gametes. The ducts develop from two sets of precursors found in both sexes. In the female, it is the paramesonephric ducts that are maintained and develop into the uterine tubes and uterus while the mesonephric ducts (of the male tract) degenerate. The anatomy of the female reproductive tract and structure of the ovary containing ovarian follicles at various stages of development can be seen in Fig. 2.1.

Genital Ducts

Body: the main part of the uterus.

Ovaries (Fig. 2.2)

The ovary is composed of an outer cortex (the functional zone of the organ), which function to produce gametes and endocrine secretion (oestradiol and progesterone), and an inner medulla where vessels and nerves predominate. The mesovarium, a fold of peritoneum carrying blood vessels, nerves and lymphatics to the ovary, is attached to the ovary at the hilum. The stroma of the cortex is richly cellular, and is looser than that of the medulla, to accommodate the growth of blood vessels that occurs when the ovary is active. Usually, only one of the two ovaries is active in each cycle. A cohort of follicles begin to develop together, but only the secondary oocyte at the metaphase of the second meiotic division from the dominant Graafian follicle is ovulated.

After ovulation, the follicle collapses and forms the corpus luteum. The basement membrane between the granulosa and thecal layers breaks down, and blood vessels from the theca interna can invade the granulosa. Granulosa cells develop into lutein cells, seen in cordlike associations separated by blood vessels. This sort of structural arrangement is typical of endocrine glands: each secreting cell is close to a blood vessel. Lutein cells are large and contain lipid inclusions and produce oestrogens and progesterone. Unless there is a pregnancy, the corpus luteum will degenerate after a fortnight. If there is a pregnancy, the embryo is able to signal its presence and the corpus luteum is saved, developing into a corpus luteum of pregnancy which maintains the pregnancy until placental hormones take over.

Uterine Tubes

The uterine tube is a duct for the passage of the oocyte. It has no direct continuity with the ovary, opening instead into the peritoneal cavity. One of the problems with the female system is that this provides a potential route of infection from the external environment to the peritoneal cavity (vagina to uterus to uterine tube to peritoneal cavity). The uterine tube has four constituent parts:

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R. Carachi, S. H. E. Doss (eds.), *Clinical Embryology*, https://doi.org/10.1007/978-3-319-26158-4_2

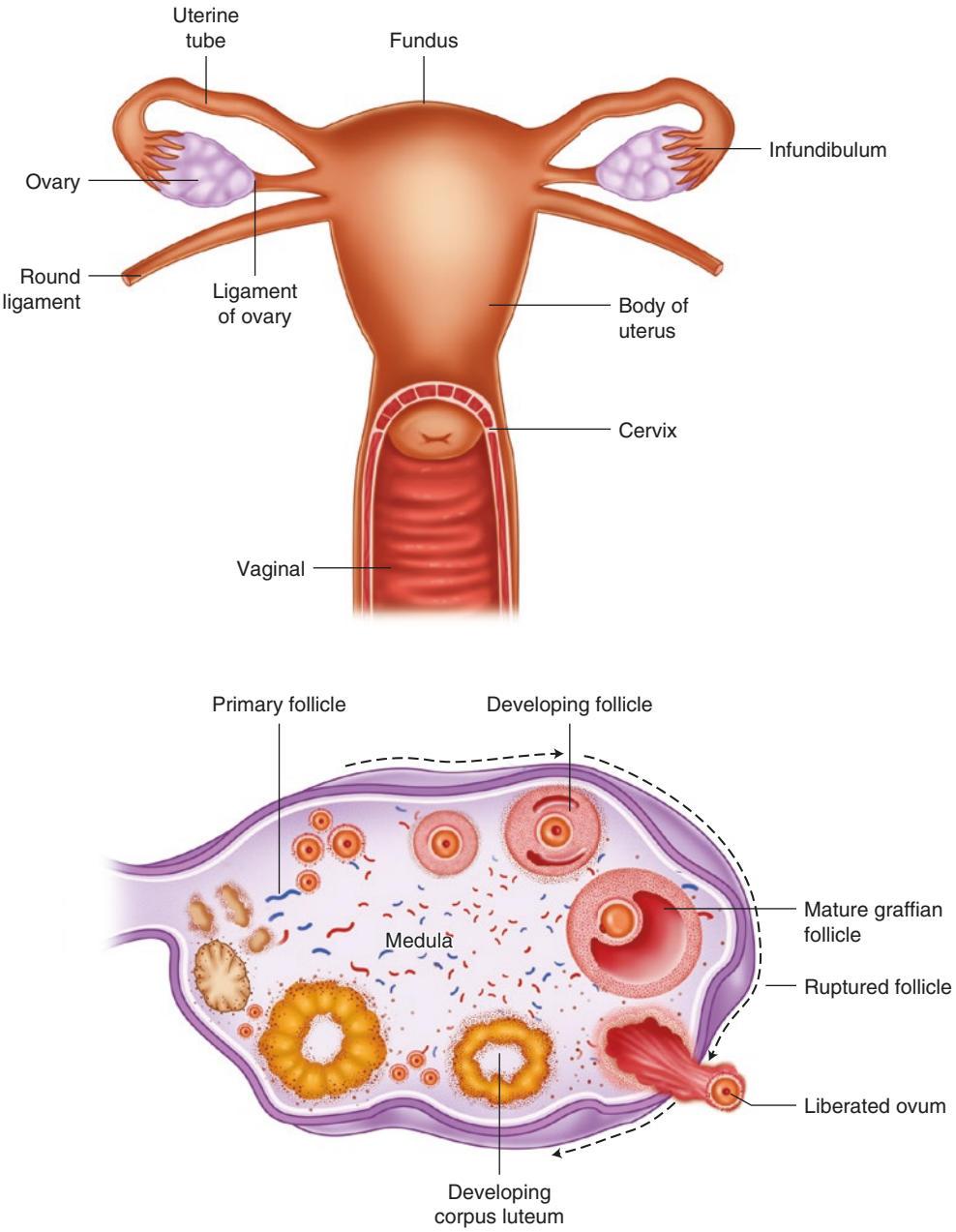

Fig. 2.1 Anatomy of the female reproductive tract and structure of the ovary containing ovarian follicles at various stages of development

- Interstitial part—through the wall of the uterus.
- Isthmus—medial third of tube, next to interstitial part, narrow.
- Ampulla—occupies more than half the length of the tube; fertilisation occurs here.
- Infundibulum—funnel-shaped end, wafts over ovary at ovulation and has folds on free margin—fimbriae.

The mucosa of the fallopian tube is composed of a simple columnar epithelium and a supporting connective tissue lamina propria. The epithelium has both ciliated and secretory cells. These may be variations of a single cell type as the ciliated cells are oestrogen-dependent. After the menopause, the epithelium becomes low cuboidal, but on administration of oestrogens, cells become ciliated. Cilia mainly beat towards the uterus – aiding the passage of the oocyte and zygote and beating back infection. The lamina propria has reticular fibres and fusiform cells, similar to cells of the uterine stroma. External to the mucosa is a smooth muscle coat, the muscularis, whose waves of contraction aid the rapid transport of the oocyte – which takes only 10 min after ovulation to reach the ampulla. Movement of the zygote, which must be slower to allow the uterus time to prepare, depends more on ciliary beating. The uterine tube has an outer covering of peritoneum, the serosa.

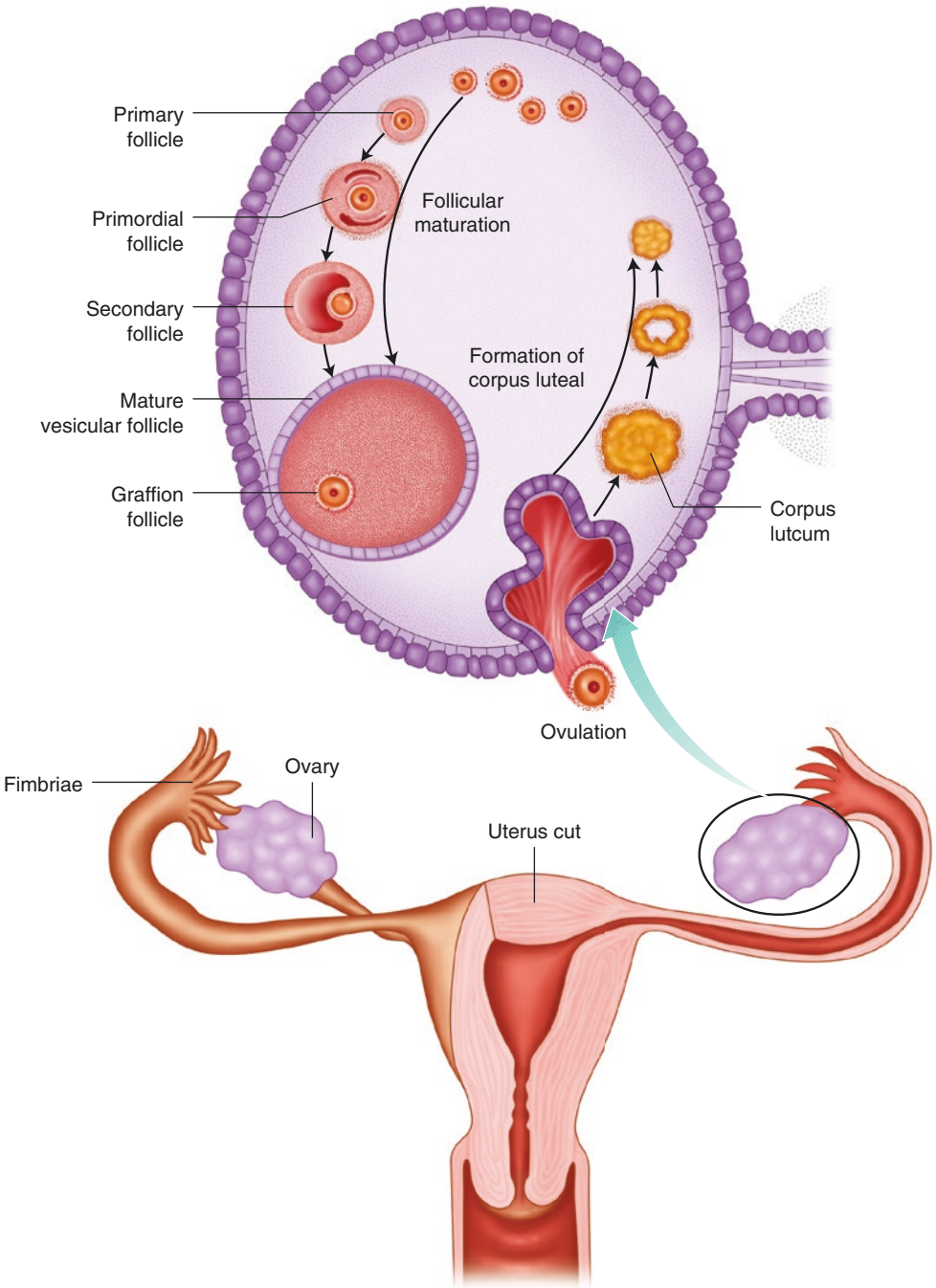

Fig. 2.2 Follicular maturation and ovulation within the ovary

Uterus

The uterus is composed of:

- *A body (main part)*
- *Fundus (part that extends above level of uterine tube entry)*
- *Cervix (neck)*

The uterine wall consists of:

- *A mucosa (secretory layer), known as the endometrium*
- *A muscle layer or myometrium*
- *An outer serosa or perimetrium*

Like the ovary, the uterus undergoes cyclical changes, which are seen in the endometrium. This can be divided into

an upper functional layer and a lower basal layer, which is adjacent to the myometrium. The basal layer contains the bases of the endometrial glands, which are simple tubular glands. The functional layer can itself be subdivided into an upper stratum compactum, where the necks of the glands are, and a stratum spongiosum beneath it. The stratum spongiosum is named for its appearance in the latter half of the cycle, when the glands are coiled and distended with secretion. The myometrium has smooth muscle arranged in three layers.

Male Reproductive Tract

In the male, the reproductive tract comprises a gland producing both gametes and steroid hormones and a duct system for transport of the gametes. In the male, each mesonephric duct develops into the ductus (or 'vas') deferens, whereas the paramesonephric ducts of the female tract degenerate.

Testes

Testes are found in the scrotum, partially covered by a serous membrane derived from the peritoneum, the tunica vaginalis. Deep to this is a connective tissue capsule, the tunica albuginea, which sends septa into the gland dividing it into 200–300 lobules. Each lobule of the testis contains from 1 to 4 seminiferous tubules; each tubule is 30–70 cm long and coiled on itself, the two ends being joined together where they open into the straight tubules. Straight tubules connect to an anastomosing network, the rete testis. Fifteen to twenty efferent ductules leave the rete. Up to this point, movement of sperm is brought about by the flow of luminal fluid produced by the Sertoli cells; from now on, muscle is responsible.

Epididymis

The ductules join to the epididymis, a single convoluted tube about 4–6 m in length. The tube is so convoluted that gross anatomical parts of the epididymis can be distinguished: the head or caput, body or corpus and the tail or cauda. The lining epithelium of the epididymis is pseudostratified columnar epithelium bearing long (15 μm) microvilli, termed stereocilia. In the caput region, the epithelium is absorptive, resorbing fluid from the testis and probably modifying the composition of the seminal fluid. Although the functions of the epididymis remain to be fully elucidated, it is thought to be very important, since it is while in the epididymis that the spermatozoa mature; this process is androgen-dependent.

Vas (Ductus) Deferens

The epididymis is continuous with the vas or ductus deferens, the main duct of the testis. This tube is found in the spermatic cord, a collection of structures, blood vessels, nerves, ductus and striated cremaster muscle, running from the anterior abdominal wall to the scrotum. The ductus has a very thick wall. Its mucosa forms longitudinal folds; the pseudostratified columnar epithelium bears stereocilia and is supported by a lamina propria rich in elastic fibres. The muscularis is very well developed and moves sperm by peristalsis. At its distal end, the ductus is dilated to form the ampulla – here the mucosa is folded to provide recesses for sperm storage, though most sperm are stored in the epididymis. On each side, the seminal vesicle joins the end of the ampulla to form an ejaculatory duct, which opens into the prostatic urethra.

Accessory Glands

Accessory glands associated with the male reproductive tract include the seminal vesicles and the prostate gland. Each seminal vesicle is a tortuous tube coiled upon itself. The folded mucosa is composed of a pseudostratified columnar epithelium whose cells show ultrastructural characteristics of protein-secreting cells. The alkaline secretion which accumulates forms the main bulk of the fluid which is ejaculated during copulation. It contains fructose as an energy source for sperm, globulin and vitamin C for nutrition and motility of sperm. Functional activity of the seminal vesicles is testosterone-dependent.

The prostate gland surrounds the first part of the urethra, into which its ducts empty. It is composed of 30–50 branched tubulo-alveolar glands surrounded by a fibroelastic capsule rich in smooth muscle and supported by a fibromuscular stroma. Glands fall into three types: mucosal, submucosal and main glands. The latter contribute most to the prostatic secretion which contains acid phosphatase, citric acid and fibrinolysin, which has a role in the liquefaction of semen. The lumen of the glands often contains pink circular or oval structures – prostatic concretions or corpora amylacea.

Penis

The penis contains three masses of erectile tissue: there is a corpus cavernosum on each side, each containing a deep artery, and below them is the corpus spongiosum bearing the penile urethra. The erectile tissue is composed of endothelial-lined vascular spaces with fibrous tissue between the vascular spaces. It can be inflated with blood.

The finer mesh of the fibrous tissue in the corpus spongiosum does not prevent distension of the urethra when the erectile tissue is filled with blood. In the male, the urethra forms the final duct for both the reproductive and the urinary systems.

Gametogenesis

Gametogenesis is the process of production of mature gametes in the testis and ovary. The process results in a reduction in the halving of chromosome content by meiotic division to allow fertilisation to occur.

In the female, by the time of birth, oogonia have all differentiated into primary oocytes, arrested in the dictyotene stage in the prophase of the first meiotic division. This stock will be steadily depleted throughout the woman's reproductive life. Spontaneous degeneration of oocytes (atresia) occurs leading to the theory that dictyotene is unstable. There are approximately 6.8 million oocytes at the peak in the 5th month of intrauterine life; 2 million remain at birth and only 40,000 survive until puberty. However, a total of only about 480 will be ovulated in a woman's reproductive life (1 a month for 12 months × 40 years of reproductive life).

Oogenesis

The production of a mature ovum ready for fertilisation by sperm.

Oogenesis occurs in the cortex of the ovary. The aim of the process is:

1. A reduction in the number of chromosomes from the diploid number to the haploid number
2. Increase in the size of the ovum from 30 μm to 120 μm

Oogenesis occurs during the fertile period of the female, starting at puberty (11–14 years) and ending at menopause (45–55 years). During this fertile period, one mature ovum develops in the ovary every cycle.

The oogonia (primitive germ cells) lie in the cortex of the ovary. At puberty, each ovary contains about 40,000 oogonia. Each oogonium is surrounded by a layer of flat epithelial cells called follicular cells to become the primary oocyte.

Oogenesis includes two processes:

1. Maturation of the primary oocyte
   (a) To become a mature ovum containing the haploid number of chromosomes
2. Maturation of the follicular cells
   (a) Around the oocyte to become a mature follicle for protection of the ovum and production of hormones

The female gamete is the major source of cytoplasm for the new individual after fertilisation; meiosis in the female is geared towards producing a gamete with a lot of cytoplasm. Thus cytoplasmic organelles are inherited through the maternal line; of particular significance, mitochondria and hence mitochondrial genes are inherited via the mother.

The oogonium contains a diploid number of chromosomes (44 + X). It grows and becomes surrounded by a single layer of follicular cells to become the primary oocytes. The primary oocytes and the follicular cells are together known as the primary follicle (Fig. 2.3).

The primary oocytes undergo meiosis to give:

(a) The secondary oocytes— a large cell containing 23 chromosomes (22 + X)
(b) The first polar body— a small cell with a very small amount of cytoplasm (22 + X)

The secondary oocytes undergo mitosis to give rise to the mature ovum and a second polar body. The first polar body divides into two by mitosis, and then all the polar bodies disappear.

The simple flat epithelial cells which surround the primary oocytes enlarge and become cuboidal and then columnar which divide to form many layers around the oocyte (Fig. 2.4).

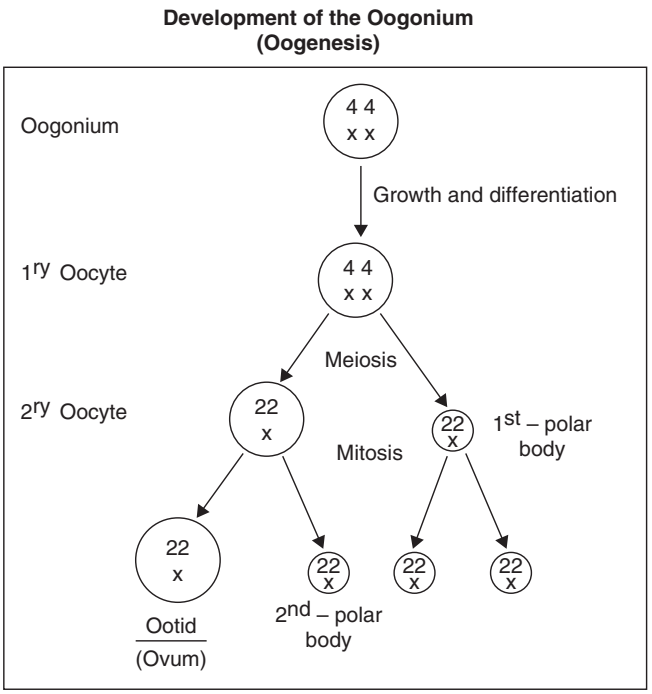

Fig. 2.3 Development of the oogonium during oogenesis

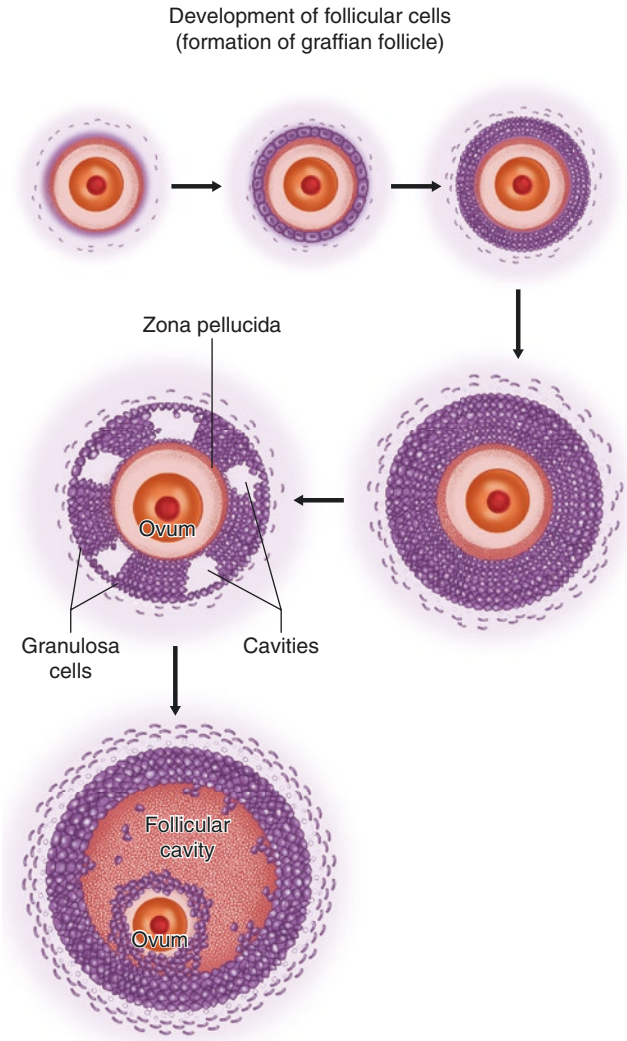

Fig. 2.4 Development of the follicular cells

The follicular cells deposit a glycoprotein coat around the oocyte known as the zona pellucida, and the follicular cells are now called granulosa cells.

Formation of the Graafian Follicle

Small irregular spaces appear between the granulosa cells and later join each other to form one large follicular cavity. This fills with follicular fluid from the granulosa cells.

The follicular wall is formed of two layers, an outer fibrous layer, the theca externa, and an inner vascular theca interna (Fig. 2.5).

The ruptured follicle in the ovary undergoes morphological change to become the corpus luteum, the fate of which depends on whether fertilisation takes place. If no fertilisation occurs, the oocyte will degenerate after 24–36 h, and the corpus luteum will collapse and progesterone levels will fall (Fig. 2.6).

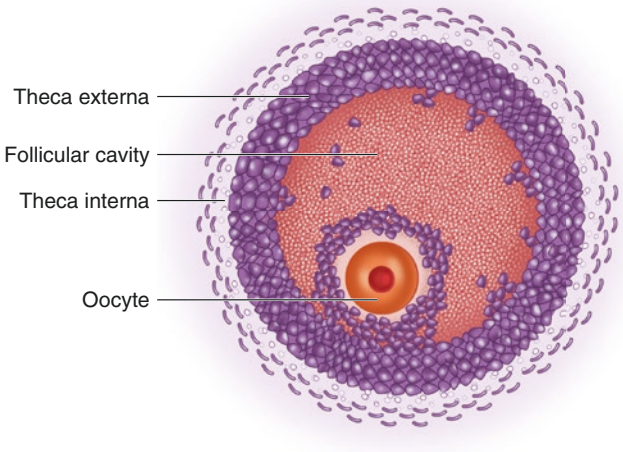

Fig. 2.5 Formation of the follicular wall

Fate of the Graafian Follicle and the Ovum

The mature Graafian follicle ruptures at the time of ovulation, releasing the mature ovum. This enters the uterine tube where it awaits fertilisation. Within the follicle, the oocyte is surrounded by follicular cells known as cumulus oophorus; at ovulation, some of these follicular cells will be released along with the ovum, now termed the corona radiata.

If no fertilisation occurs, the ovum degenerates after 24–36 h. The ruptured Graafian follicle is transformed into the corpus luteum, a yellow-bodied structure which produces hormones.

Spermatogenesis and Spermiogenesis

Spermatogenesis is the process of sperm formation in the seminiferous tubules of the testis (shown in Fig 2.7). Its aim is to

1. Reduce the number of chromosomes from diploid (46) to haploid (23) by meiosis.
2. Change the shape of the male germ cells to produce a highly motile sperm ready for fertilisation of the ovum.
3. Increase the number of cells.

Spermatogenesis occurs continuously in the seminiferous tubules of the testis from puberty and continues throughout life.

The process begins with the differentiation of spermatogonia into spermatids.

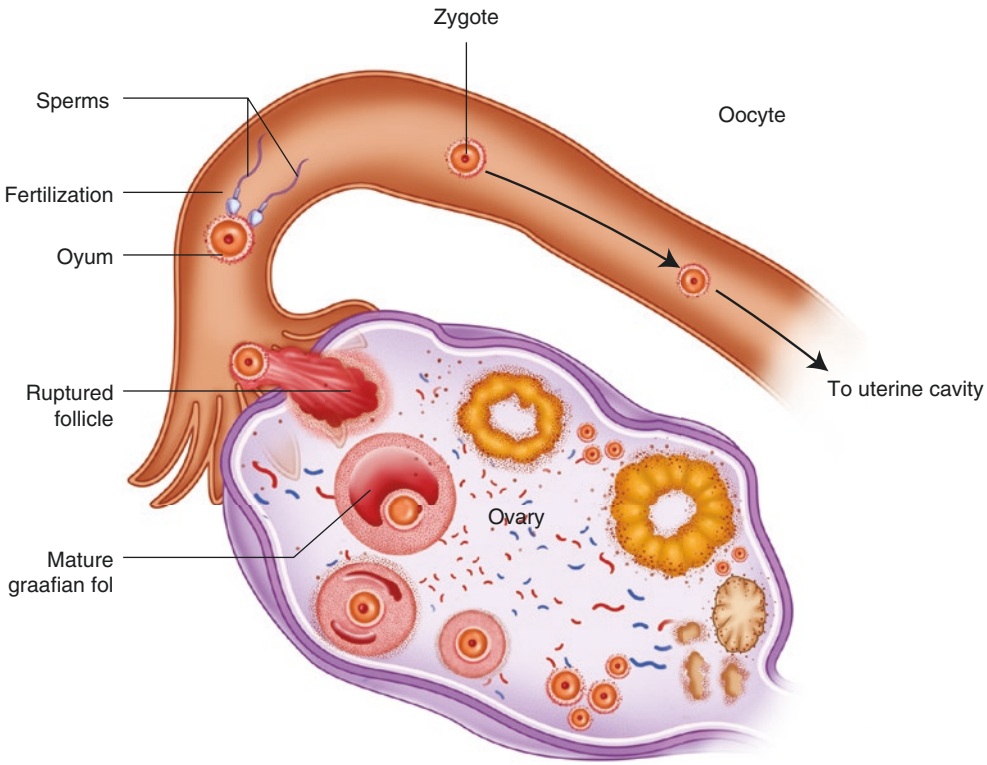

Fig. 2.6 Ovulation of the mature ovum from the Graffian follicle

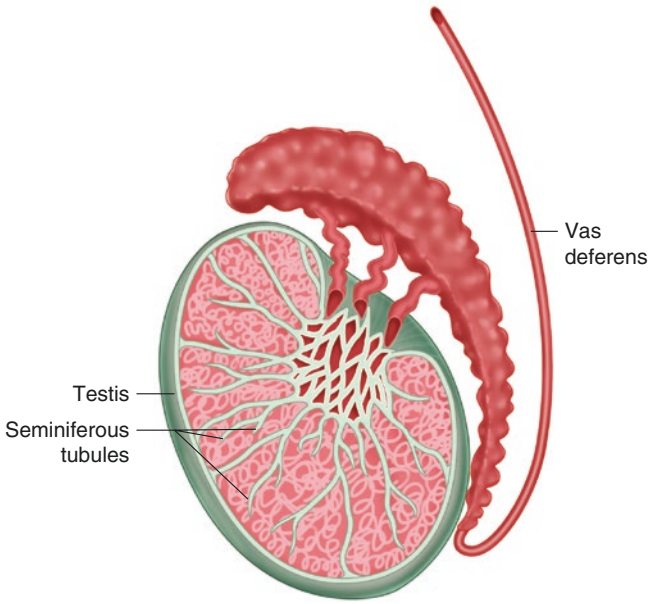

Fig. 2.7 Structure of the testis and vas deferens

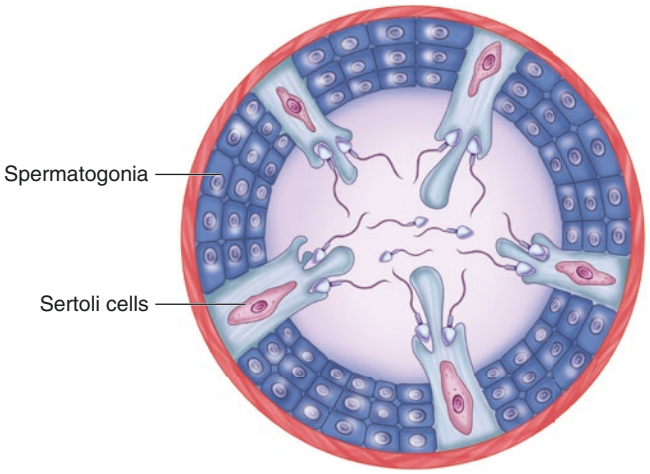

Fig. 2.8 Spermatogenesis

Spermatogonia are the most primitive male germ cells. They contain a diploid number of chromosomes (44 autosomes and 2 sex chromosomes). They lie on the wall of the seminiferous tubules and are supported by Sertoli cells.

Each spermatogonium undergoes mitotic division to give two daughter cells. Each of these grows to produce a primary spermatocyte (Fig. 2.8).

Primary spermatocytes undergo meiotic division, giving rise to two secondary spermatocytes, each of which contains a haploid number of chromosomes

(22 + X or 22 + Y).

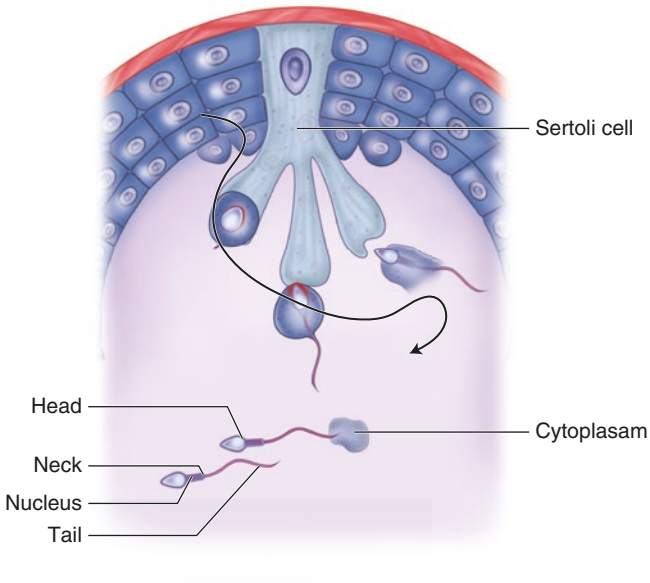

Fig. 2.9 Arrangement of cells within the seminiferous tubule

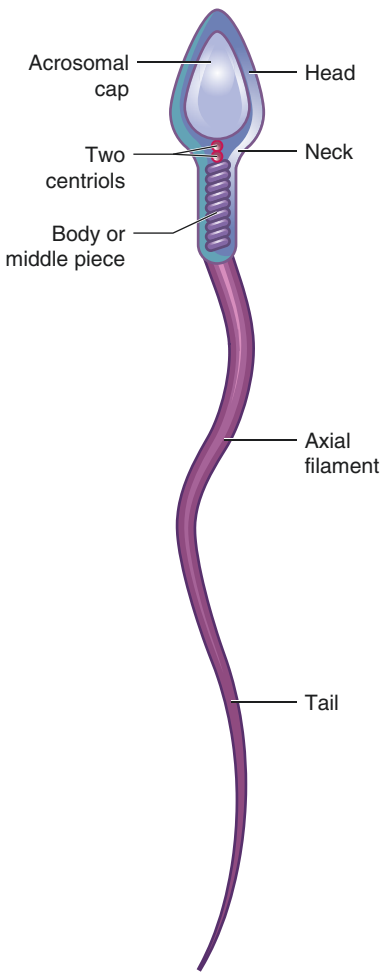

Fig. 2.10 The structure of the mature spermatozoa

Each secondary spermatocyte undergoes meiosis to give two spermatids.

Spermiogenesis

The spermatid which is rounded undergoes morphological and structural changes to become transformed into a mature sperm as follows (Figs. 2.9 and 2.10):

1. The nucleus becomes condensed and forms most of the sperm head.
2. Golgi apparatus forms the head cap covering the anterior half of the nucleus.
3. The centriole elongates to form the axial filament which traverses the neck, mid piece and tail of the sperm.
4. The mitochondria form a spiral sheath around the mid piece.
5. The remainder of the cytoplasm is shed.

The mature sperm is 60 μm in length and is formed from the following parts:

Head: 5 μm, formed mainly by the nucleus of the spermatid and carries the genetic information. It is partly covered by the acrosomal cap which contains enzymes to aid in penetration of the ovum.

Neck: Very short, contains two centrioles.

Mid piece: 5 μm, formed by the axial filaments surrounded by a mitochondrial sheath and concerned with energy production.

Tail: 50 μm, formed of axial filaments covered by a thin protoplasmic membrane and is concerned with motility.

Each spermatogonium divides by mitosis to produce two different daughter cells—one replaces the stem cell population, one divides again by mitosis and its progeny becomes the primary spermatocytes. These divide by meiosis to produce secondary spermatocytes which are very short lived and quickly enter the second meiotic division to produce spermatids which then undergo a maturation process, spermiogenesis, to form spermatozoa. In the human, the cycle of development from stem cell to sperm takes 64 days.

The mature male gamete or sperm is essentially a nucleus with a flagellum for motility; most of its cytoplasm has been shed during spermiogenesis when the mature spermatozoon develops from the spermatid, which itself is the end product of meiosis.

In the male, testosterone, produced by the Leydig cells in the testis, is essential for spermatogenesis because of its local effect on the Sertoli cells. The Leydig cells in turn depend on the secretion of LH by the gonadotrophs of the anterior pituitary. FSH is also essential for spermatogenesis because of its direct action on the Sertoli cells. Prolactin, from the anterior

pituitary, potentiates the effects of testosterone and LH. Secretion of GnRH from the hypothalamus and LH and FSH from the anterior pituitary is controlled by negative feedback, as in the female. The normal functioning of the feedback control is essential for fertility. Testosterone in the plasma reduces the frequency of the pulses of GnRH released and also the quantity of LH released per pulse by the anterior pituitary. In females progesterone acts this way, rather than testosterone. Inhibin, secreted by the Sertoli cells, suppresses the secretion of FSH.

Fertilisation

Human development begins at fertilisation with the fusion of sperm with a newly ovulated Graafian follicle released from the ovary. Ovulation occurs at mid-cycle under the influence of LH released from the anterior pituitary at mid-cycle. The follicle ruptures releasing the secondary oocyte which is ovulated at metaphase II of the second meiotic division. The movement of the fimbriae of the uterine tube pulls the oocyte towards the uterine tube where fertilisation can occur.

The site of fertilisation is the ampullary region of the uterine tube, the oocyte moves to the site of fertilisation by ciliary action, and sperm reach the oocyte aided by contraction of tubal musculature. Fertilisation is a multistep process occurring 12–24 h after ovulation:

1. Capacitation of sperm. This takes place once the sperm enters the female reproductive tract. Taking approximately 7 h, this process involved the removal of the glycoprotein coat and seminal plasma proteins.
2. Penetration of the corona radiata and cumulus oophorus. At ovulation, the oocyte is surrounded by a cellular layer known as the corona radiata. The cellular layer is composed of protein and hyaluronic acid, and cumulus oophorus cells released by the follicle at ovulation may also surround the oocyte complex penetration driven by enzymatic degradation of the cellular layer by hyaluronidase produced in the sperm head. Active swimming of the sperm also plays a role in penetration of the corona radiata.
3. Penetration of the zona pellucida. 13um thick in the human, the zona pellucida is composed of four glycoproteins, ZP 1–4.
4. Acrosome reaction. The acrosome is derived from the Golgi apparatus and contains its own membrane. Fusion occurs between the acrosome membrane and the sperm membrane.
5. Sperm contacts the oocyte cell membrane. At this point, polyspermy must be prevented.
   (a) Fast block to polyspermy – rapid electrical depolarisation of oocyte plasma membrane.
   (b) Slow block to polyspermy – wave of Ca2+ ions from the site of sperm fusion acts on the cortical granules. These fuse with the plasma membrane, and this releases enzymes which break down the sperm receptors in the zona.

Fusion of the oocyte and the sperm occurs when the nuclear membranes around the male and female pronuclei break down nucleus allowing fusion of chromosomes resulting in formation of a single-celled diploid zygote.

Once fertilisation is complete, this leads to resumption of the oocytes' second meiotic division. In the adult human, there are 10^{14} cells, taking 45 generations of mitoses to produce these cells from a single fertilised egg, and cell division, growth, morphogenetic movement, differentiation and cell death are all required to shape the developing human.

Results of Fertilisation

Fertilisation results in the formation of the zygote which then divides to form a blastocyst, as seen in Fig. 2.11.

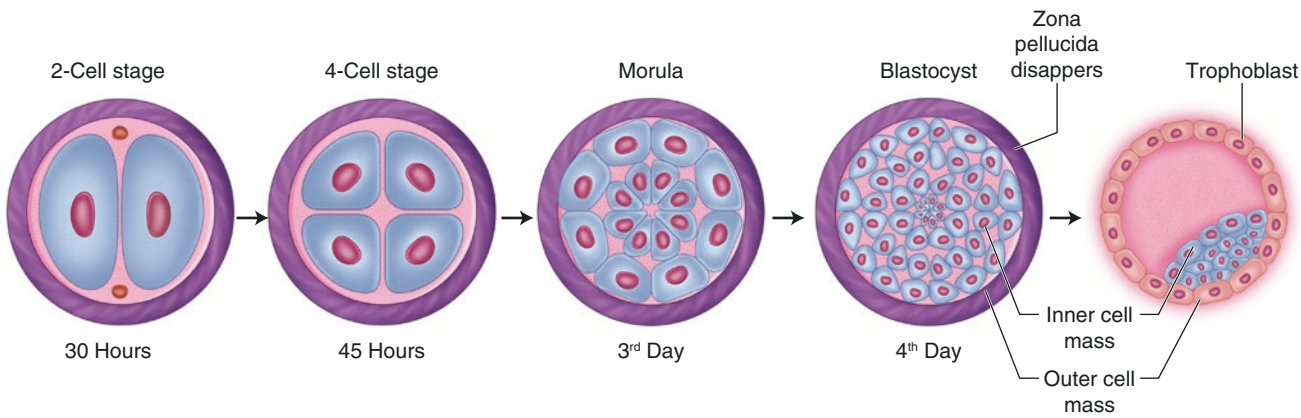

Fig. 2.11 Division of the zygote to produce a blastocyst stage embryo

1. Diploid number of chromosomes is restored.
2. Sex of embryo is determined.
3. Activation of the zygote occurs structural and molecular.
4. Cleavage commences.

The fertilised egg passes along the uterine tube, undergoing a series of mitotic divisions. There is no increase in size, so that component cells become smaller with each division. This is known as cleavage and allows the conceptus to pass through the narrow tubal isthmus. Early cleavage-stage embryos are totipotent. The zona pellucida is still intact preventing premature implantation in ectopic site.

The two-cell stage appears around 30 h after fertilisation, and by 45 h post fertilisation, the four-cell stage should have been reached. By day 3, the developing embryo has reached the 16-cell stage and is termed a morula, and the process of compaction occurs. During compaction, individual cells flatten against each other and form cell junctions. Cells of the morula show the first sign of cellular differentiation, cells flatten against each other, form junctions and two populations of different cells arranged into an inner cell mass population and an outer layer of cells, the trophectoderm, in the periphery. Post compaction, the blastomeres continue to divide, and fluid enters between spaces in the cells forming a blastocoel cavity marking the formation of the blastocyst which enters the uterine cavity by the 5th day.

Allocation of cells to an inner cell mass which will form the embryo or trophectoderm lineage depends on relevant positions of cells during cleavage. Segregation of cells to different positions is guided by cell-cell interactions. This may begin as early as the two-cell stage. Control of differentiation is exerted by micro environmental factors and development of polarity within the cells.

Implantation

Once in the uterine cavity, the blastocyst penetrates the superficial compact layer of the uterine endometrium. This begins around day 6 or 7 and is complete by around day 12 after fertilisation. The normal site of implantation is the endometrium of the posterior wall of the fundus of the uterus and can be seen in Fig. 2.12.

Implantation Is a Multistep Process and can be summarised as shown in (Fig. 2.13).

1. The blastocyst becomes attached to the endometrium.
2. The trophoblast cells lying over the inner cell mass begin to erode the endometrium by enzymatic action.
3. The blastocyst burrows into the endometrium.
4. After complete embedding of the blastocyst, the endometrium wall is closed by a fibrin clot. Implantation is completed by the growth of epithelium to cover the entry site.

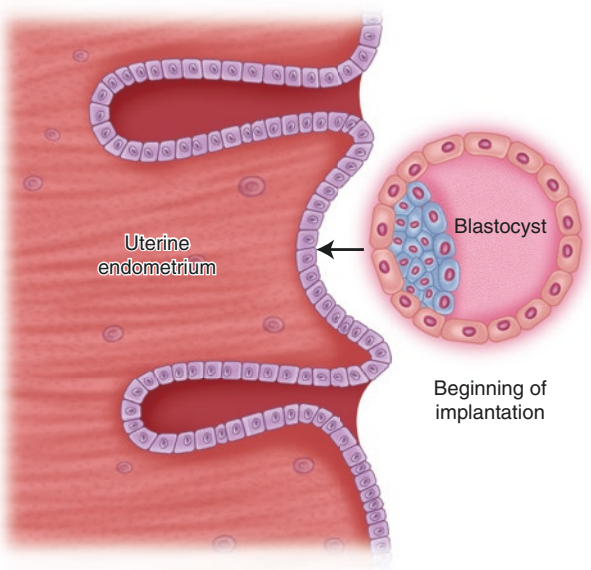

Fig. 2.12 Beginning of implantation

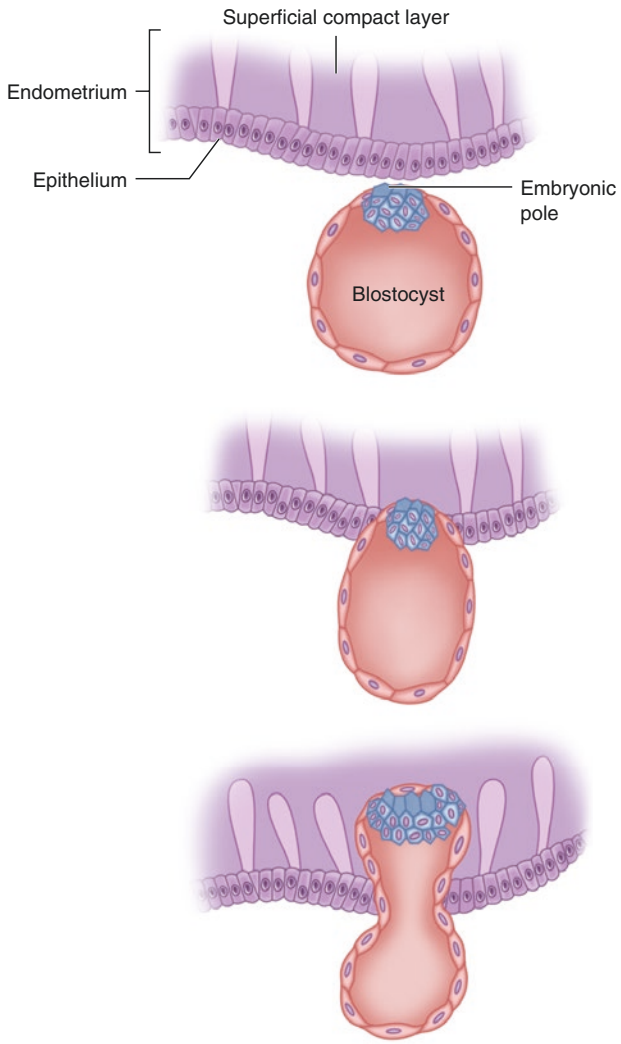

Fig. 2.13 Attachment of the blastocyst to the endometrium followed by enzymatic invasion

Week 2 of Pregnancy

During week 2, the major events result in the development of two trophoblast layers, two embryonic layers and two cavities.

Trophoblast

During week 2, the trophoblast, seen in Fig. 2.14 shows a rapid rate of growth compared to the growth of the embryonic disc.

Entry to the uterine cavity occurs 4–5 days after fertilisation. The blastocyst can remain free in the uterine lumen for several days before implanting, but demand for space and nutrients is a driver for implantation. Pressure in the blastocyst cavity allows the cells of the blastocyst to 'hatch' from the zona pellucida, and the trophoblast immediately over the inner cell mass, and invade the maternal tissue allowing attachment in the posterior wall of the uterus, near the midsagittal plane. Cells push between the uterine epithelial cells and through their basement membrane. Stromal cells of the uterine stroma undergo decidualisation, filling with glycogen and lipids which act as a source of nourishment for the invading embryo, as well as creating an immunologically privileged site for the embryo. Implantation is complete by day 12 in the human. It is estimated that up to 50% of all pregnancies are aborted around this time by a natural screening process for chromosomal abnormalities.

The trophoblast over the inner cell mass is the first to implant and differentiates as it erodes the endometrial stroma, forming:

1. An inner mitotic layer, the cytotrophoblast
2. An outer syncytial (multinucleate) layer, the syncytiotrophoblast

Spaces called lacunae begin to appear in the syncytiotrophoblast to allow exchange of foetal and maternal blood.

Embryonic Layers and Cavities

At day 8, the inner cell mass rearranges to give two distinct cell layers as seen in Fig. 2.15:

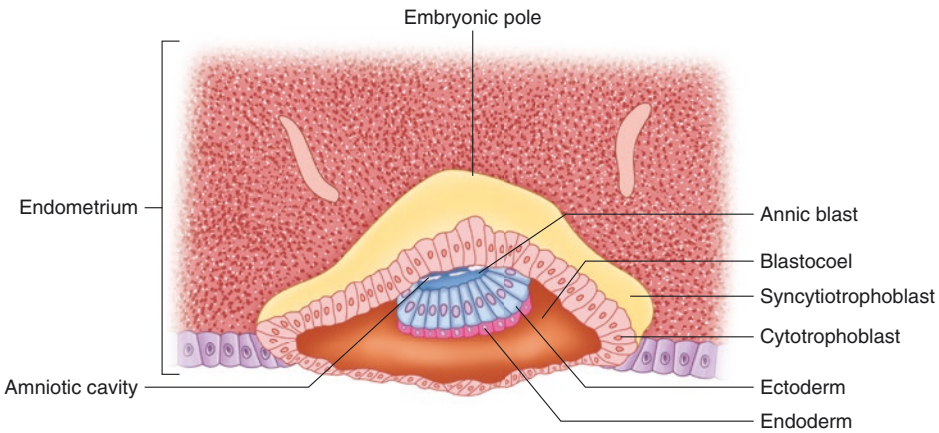

Fig. 2.14 Early stages of implantation

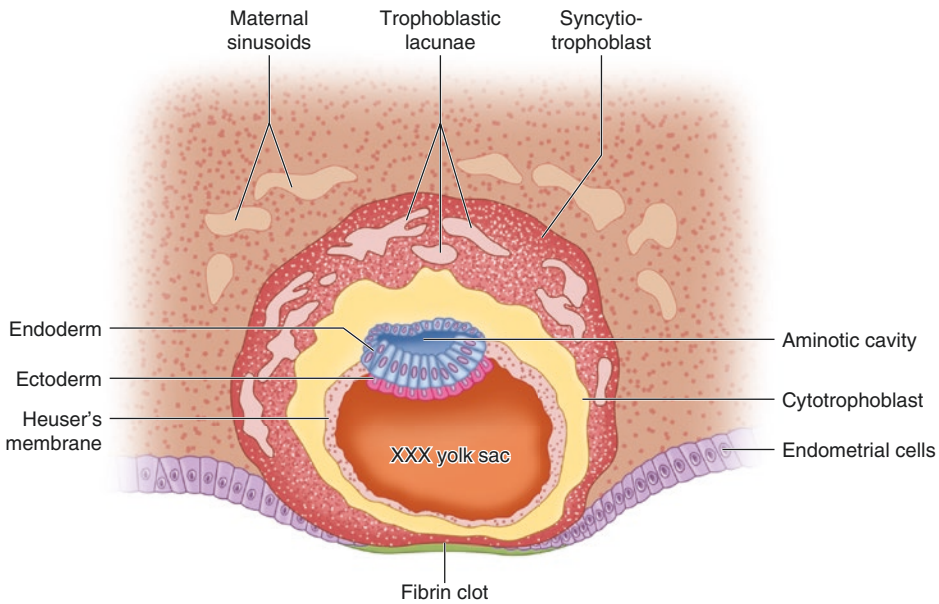

Fig. 2.15 Establishment of the embryonic layers and extra-embryonic membranes and cavities

1. Epiblast – high columnar cells adjacent to the cytotrophoblast layer
2. Hypoblast – small cuboidal cells nearest to the blastocyst cavity

The embryo is now in the form of a bilaminar germ disc.

The epiblast layer will go on to form the three germ layers of the embryo. The hypoblast is concerned with the derivation of the extraembryonic membranes. Each of these layers becomes continuous at its margins with an extraembryonic membrane which develops in association – the epiblast with the amnion and the hypoblast with the yolk sac. Each layer associated with a membrane forms a vesicle enclosing a cavity: the amniotic cavity and the yolk sac cavity.

Amniotic Cavity

Small clefts begin to appear between the ectodermal cells and the trophoblast. These clefts join each other forming the amniotic cavity. The cytotrophoblast develops a layer of flat cells called amnioblasts which form the roof of the amniotic cavity while its floor is formed by the ectodermal layer.

Yolk Sac

About 12 days after fertilisation, extraembryonic mesoderm appears outside these two vesicles in the blastocyst cavity. It is of great significance in the development of the placenta. Almost as soon as it appears, a cavity, the extraembryonic or chorionic cavity, forms within it. The trophoblast and its lining of extraembryonic mesoderm are referred to as the chorion and can be seen in Fig. 2.16.

The yolk sac forms on the ventral aspect of the embryonic disc. Cells of the endoderm layer grow and line the inner surface of the cytotrophoblast forming Heuser's membrane. The yolk sac replaces the cavity of the blastocyst.

Both cavities are fluid filled which facilitate diffusion of nutrients at this early stage before the placenta and its circulation are established. The amniotic cavity persists throughout development allowing symmetrical growth and protection for the foetus.

At the end of week 2, cavities are formed inside the extraembryonic mesoderm. These cavities then fuse together forming the extraembryonic coelom. This coelom divides the mesoderm incompletely into somatopleuric mesoderm which lines the cytotrophoblast and covers the amniotic cavity and the splanchnopleuric mesoderm which covers the yolk sac. The roof of the amniotic cavity is connected to the trophoblast by the body or connecting stalk.

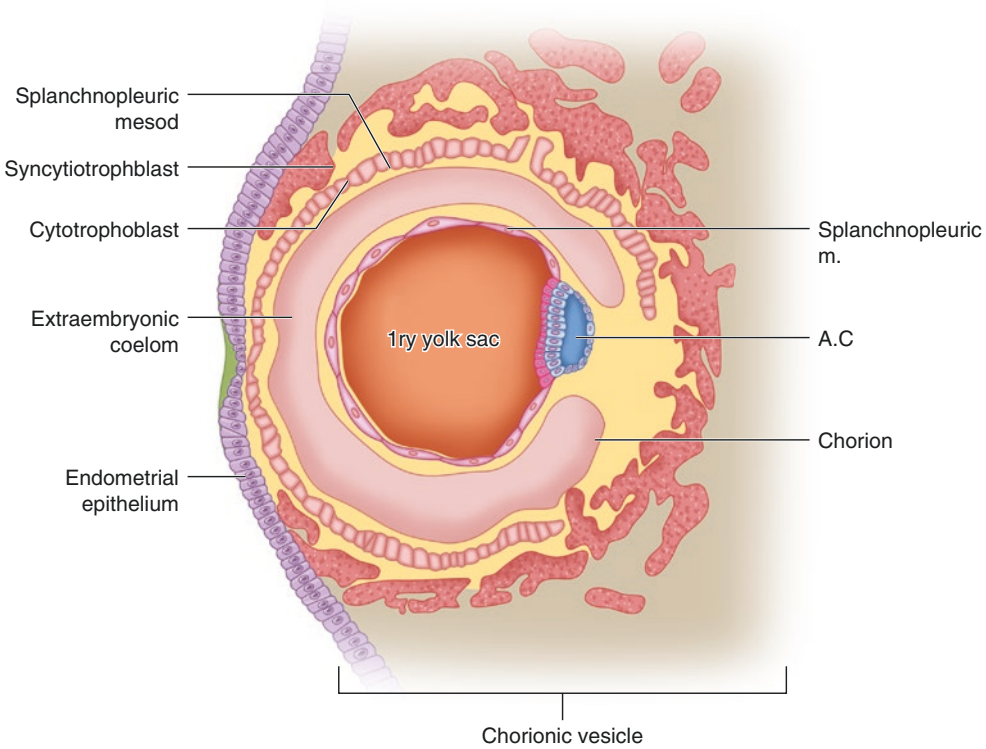

Fig. 2.16 Development of the embryonic coelom and chorionic vesicle at the end of week 2 of development

Week 3

In the third week of life, the three definitive germ layers, ectoderm, mesoderm and endoderm, are formed, transforming the bilaminar germ disc into a trilaminar structure (Fig. 2.17).

The three germ layers arise from the epiblast by a process of cell proliferation and migration known as gastrulation. Three important structures are involved in gastrulation, all of which make an appearance in week 3, the primitive streak, the notochord and the neural plate.

Ectodermal cells in the caudal part of the bilaminar disc migrate to the midline forming the primitive streak. Cells of the primitive streak proliferate and invaginate through the streak. A population of cells replace the hypoblast cells, which are pushed out laterally into the yolk sac to form the endoderm. Others form a new middle layer between the epiblast and hypoblast/endoderm called the mesoderm. At the end of gastrulation, the remaining epiblast is termed the ectoderm.

At the head end of the streak, a thickened area of ectoderm appears; this primitive node consists of a small central depression called the primitive pit. Cells of the primitive node proliferate to form a solid rod of cells, the notochord, a mesodermal structure which grows in a cephalic direction along the midline between the ectoderm and endoderm. This is an important structure as it forms a primitive skeletal axis and plays an important role in the development of the central nervous system.

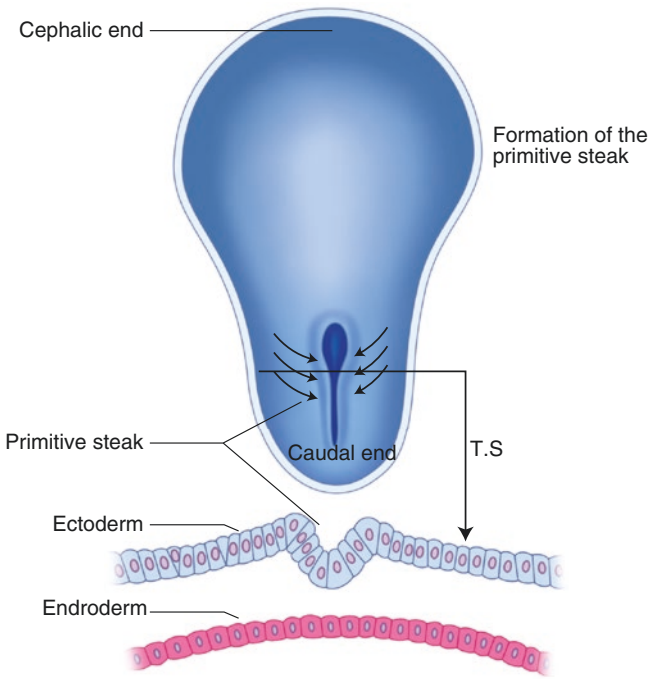

Fig. 2.17 Formation of the primitive streak signalling the start of gastrulation

The notochord induces changes in the overlying ectoderm, which thickens to form the neural plate. The edges of the plate fold and eventually meet in the midline to form the neural tube, the forerunner of the central nervous system which sinks inwards as the gap in the surface ectoderm is repaired (Fig. 2.18).

A population of tissue at the summit of the neural fold is excluded from the tube and pinched off separately to lie alongside the tube on each side. This is the neural crest tissue. Neural crest cells are highly migratory; they give rise to many derivatives including cells of the spinal ganglia, autonomic ganglia, pigmented cells, Schwann cells, mesoderm in the head, adrenal medulla and leptomeninges.

Closure of the neural tube begins in the future cervical region of the spinal cord; for a while openings are present at the head and tail ends – the anterior and posterior neuropores. These will eventually close in week 4. Failure of their closure leads to defects such as anencephaly and spina bifida.

Two areas are present which do not contain mesodermal cells, just ectoderm and endoderm cells. The fate of these areas is to break down and form the two openings to the gut, the oral cavity and the anus.

Differentiation of the germ layers during the embryonic period (Table 2.1).

Ectoderm

At first, the ectoderm forms the dorsal layer of the embryonic disc and makes the floor of the amniotic cavity. After the embryo begins the process of folding, the ectoderm becomes the outer layer of the body of the embryo. The ectoderm differentiates into the following:

1. Epidermis of the skin, including hair, nails and skin glands
2. Neural tube, which will form the nervous system
3. Sensory epithelium of the sense organs
4. Pituitary gland

The Neural Tube

Development of the neural tube in week 3 of development happens with the following steps: (Fig. 2.19a)

1. Formation of the neural plate

   At the beginning of week 3, the ectoderm over the notochord thickens, forming a median band called the neural plate. This is neuroectoderm in origin and extends

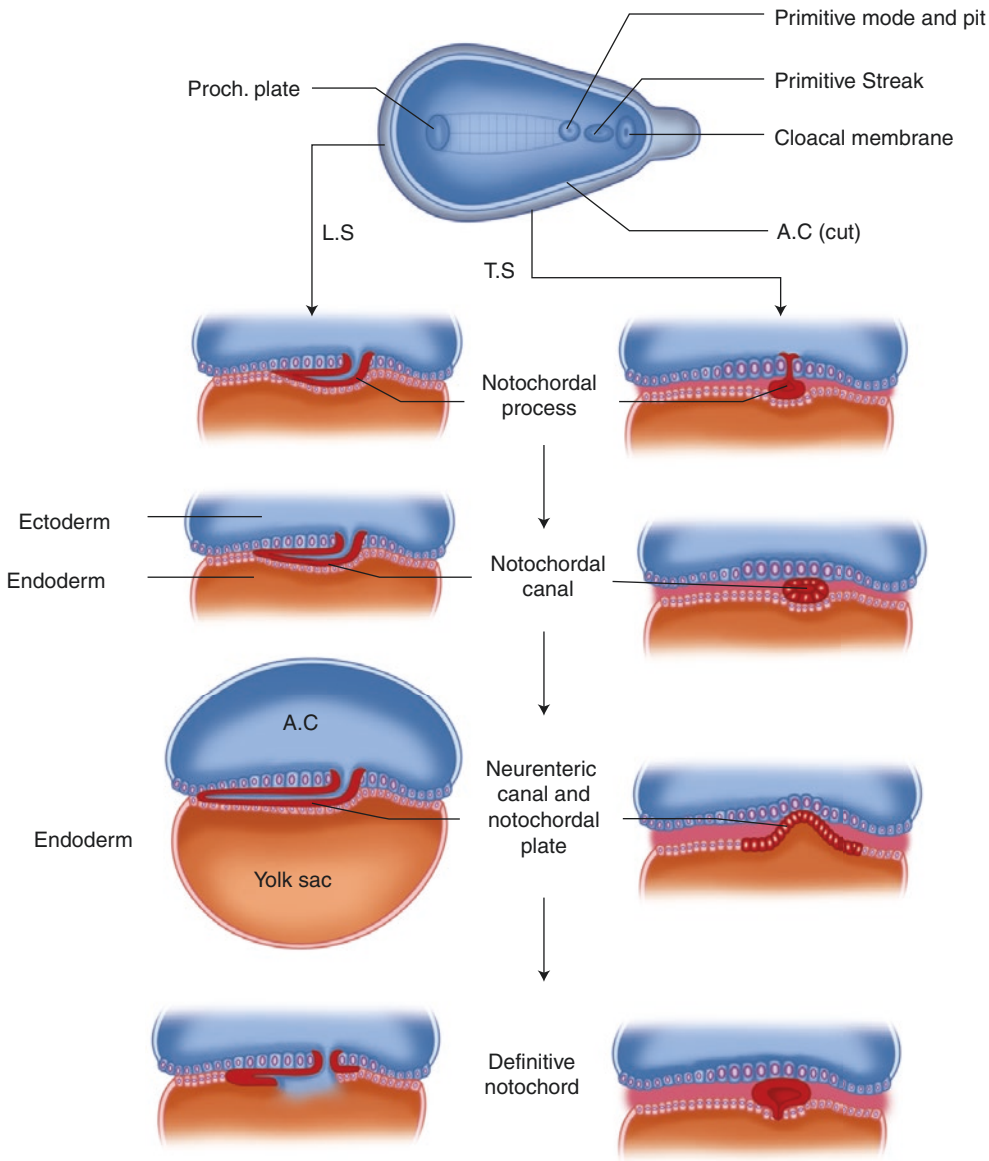

Fig. 2.18 Formation of the notochord

Table 2.1 The significance of the germ layers and their derivatives

Germ Layer	Derivative
Ectoderm	Forms epidermis of skin and nervous tissue (through the neural tube and neural crest)
Mesoderm	Forms cardiovascular system, urogenital system, muscle and connective tissue
Endoderm	Forms epithelial linings of the gut and respiratory systems

from the primitive node to the buccopharyngeal membrane (Fig. 2.19b).

2. Formation of the neural groove

   The edges of the neural plate become elevated forming right and left neural folds, and as a result, the neural plate is transformed into the neural groove.

3. Formation of the neural tube

   As the neural groove deepens, the right and left neural folds approach each other in the midline and fuse at the region of the fourth somite. The fusion of the two folds then proceeds in both a cranial and caudal direction, transforming the neural groove into a tube structure which is buried under the surface ectoderm.

4. Closure of the anterior and posterior neuropores

   As the closure of the tube proceeds in a cranial and caudal direction, the anterior and posterior ends remain open and are connected to the amniotic cavity. These are the anterior and posterior neuropores. The anterior neuropore closes at the 20 somite stage, while the posterior neuropore closes at the 25 somite stage.

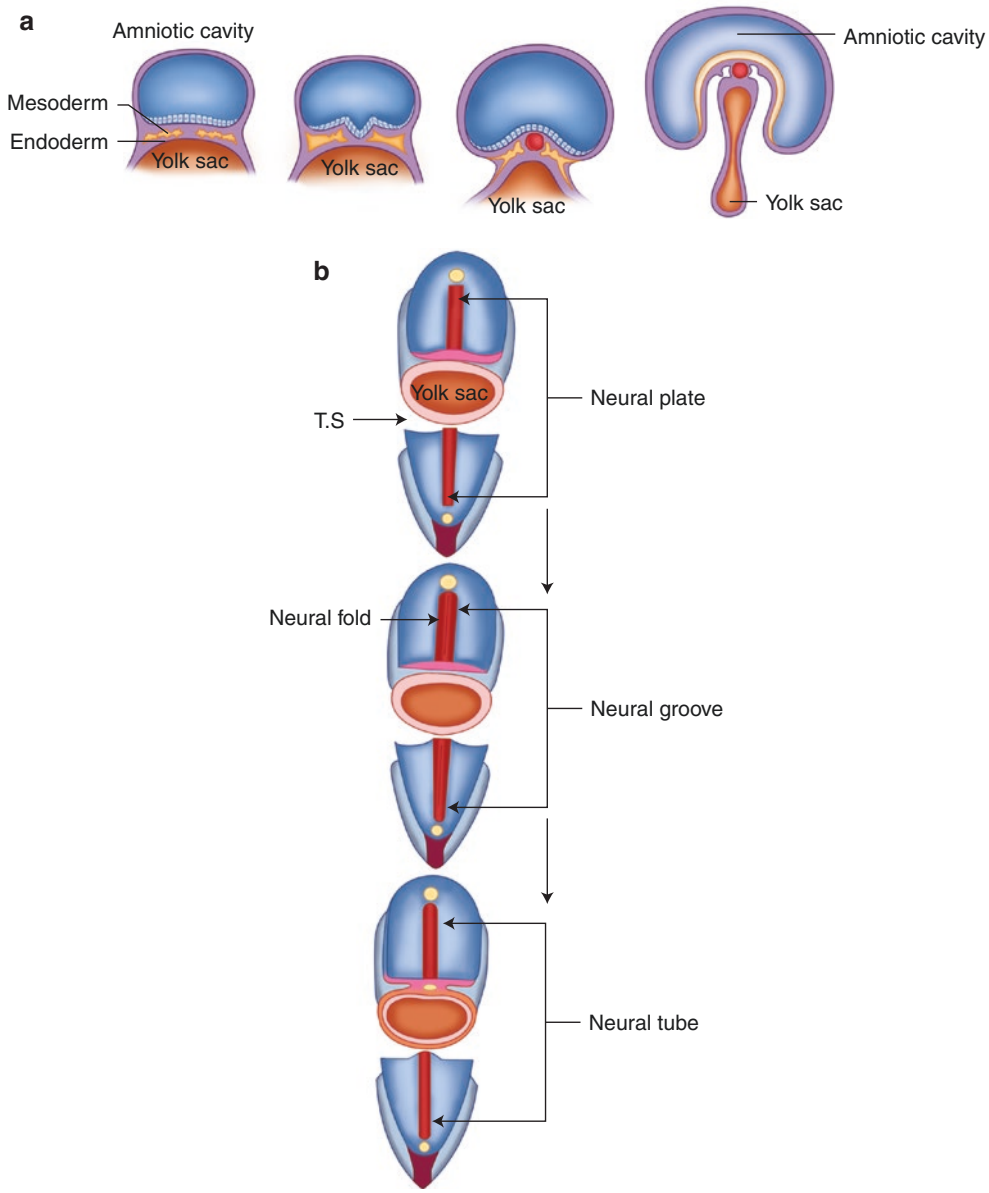

Fig. 2.19 (**a**) Establishment of the ectoderm layer from the embryonic disc. (**b**) Establishment of the neural plate from the ectoderm layer

Differentiation of the Neural Tube

1. The broad cranial part of the neural tube will become the brain, while the narrower caudal portion of the tube will form the spinal cord (Fig. 2.20).

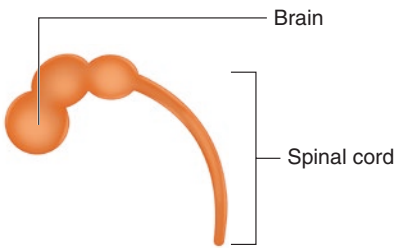

Fig. 2.20 Differentiation of the neural tube

Mesoderm (Fig. 2.21)

Initially, the mesoderm is a sheet of loose tissue between the ectoderm and the endoderm on either side of the notochord (Fig. 2.19b).

As development proceeds, two longitudinal grooves appear in the mesoderm on either side of the notochord dividing it into three parts: