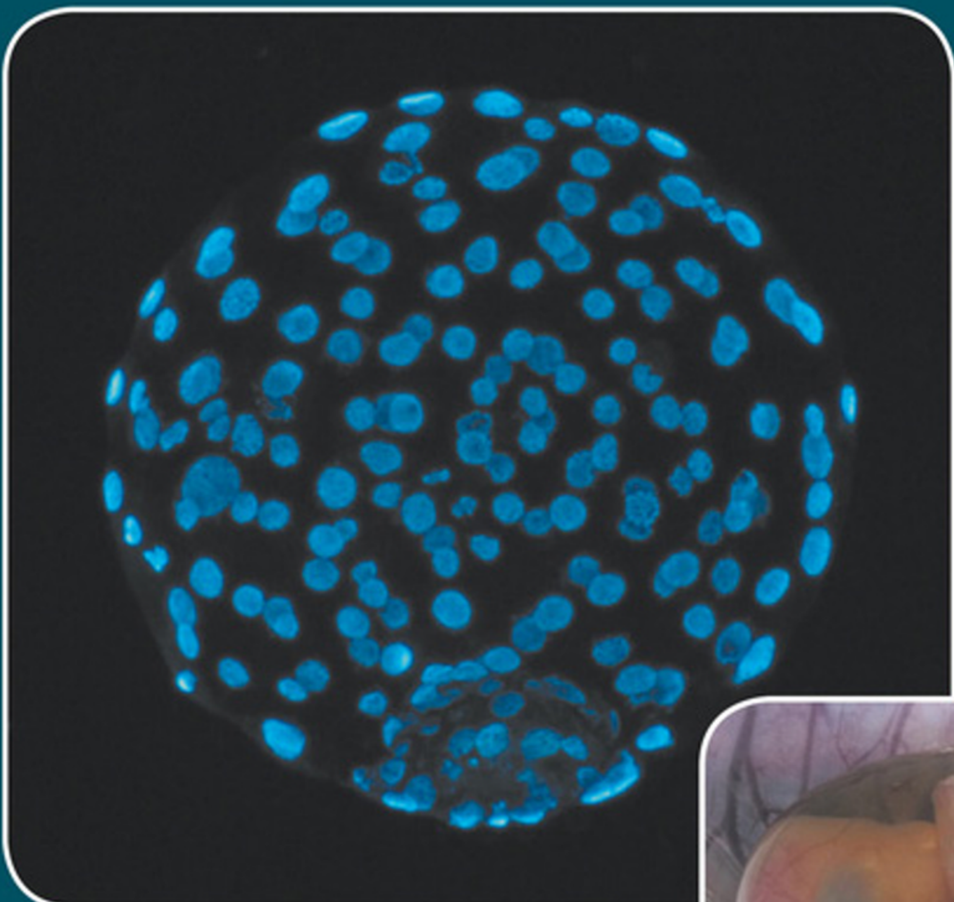
VETERINARY EMBRYOLOGY

SECOND EDITION

T.A. MCGEADY, P.J. QUINN, E.S. FITZPATRICK, M.T. RYAN, D. KILROY AND P. LONERGAN



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Veterinary Embryology

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Second edition

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Cover images: Bovine blastocyst stained to count the nuclei (courtesy of P. Lonergan); Feline foetus of approximately 40 days' gestation (courtesy of F. Randi).

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P.J. Quinn, MVB, PhD, MRCVS was Professor of Veterinary Microbiology and Parasitology and head of the department in the School of Veterinary Medicine, University College Dublin, from 1985 to 2002. After graduating from UCD with a degree in veterinary medicine in 1965, he spent some time in practice before enrolling as a postgraduate student in Ontario Veterinary College, University of Guelph, Canada. In 1970 he was awarded a PhD for research in veterinary immunology and he remained on the staff of Ontario Veterinary College until his return to the School of Veterinary Medicine, UCD in 1973.

His research interests have included allergic skin reactions in the horse to biting insects, the epidemiology of toxoplasmosis in wildlife in Canada and sheep in Ireland, leptospirosis in dairy cattle, mechanisms of immunity in the respiratory tract of cats, botulism in gulls around the Irish coastline, factors affecting the tuberculin test in cattle and evaluation of the efficiency of chemical disinfectants against *Brucella abortus* and *Mycobacterium bovis*.

In addition to numerous refereed publications in journals and chapters in books, he edited *Cell-Mediated Immunity* (1984), is senior co-author of *Animal Diseases Exotic to Ireland* (1992), *Clinical Veterinary Microbiology* (1994), *Microbial and Parasitic Diseases of the Dog and Cat* (1997), *Veterinary Microbiology and Microbial Disease* (first edition, 2002, second edition, 2011), *Concise Review of Veterinary Microbiology* (first edition, 2003, second edition, 2016) and is co-author of *Veterinary Embryology* (2006).

He was awarded the title Professor Emeritus by University College Dublin in 2002. In 2006, he was recipient of the Association of Veterinary Teachers and Research Workers outstanding teaching award.

Eamonn S. FitzPatrick, FIBMS, FRMS, was awarded a Fellowship by the Institute of Biomedical Science in 1978 and was appointed Principal Technician in the Department of Veterinary Anatomy, University College Dublin. He was appointed to the Histopathology Advisory Committee of the Irish Academy of Medical Laboratory Sciences in 1979. From 1987 to 1989 he was external examiner for the Diploma in Medical Laboratory Science — Histopathology, at the Dublin Institute of Technology, where he also lectured for many years on electron microscopy in the medical laboratory sciences degree course. He was appointed Chief Technical Officer in the Veterinary Science Unit of the School of Veterinary Medicine, University College Dublin, in 2006. He taught veterinary anatomy and histology for over 25 years.

Recent published work includes papers on hormone receptors in the bovine reproductive tract and the effect of dietary supplements on the alimentary tracts of weanling pigs. His research interests are centred mainly on mucins, mucus gels and the interaction of microbial pathogens with epithelial surfaces, especially of the bovine and equine reproductive tracts. He is co-author of *Veterinary Microbiology and Microbial Disease*, second edition (2011), *Concise Review of Veterinary Microbiology*, second edition (2016) and *Veterinary Embryology* (2006).

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Preface

The first edition of *Veterinary Embryology* introduced undergraduate students to topics which explain the sequential stages of embryonic and foetal development. Since its publication in 2006, many changes have taken place in veterinary embryology, some related to an improved understanding of molecular features of embryology and others to rapid advances in the manipulation of embryonic cells, particularly stem cells. Embryology provides students with information on the development, structure, final form and relationships of tissues and organs. Congenital anomalies and their associated clinical conditions can be more completely understood if the underlying factors regulating development are related to the pathological changes caused by genetic or chromosomal defects, by infectious agents or by environmental teratogens.

This book is concerned with developmental aspects of cells, tissues, organs and body systems of animals, principally mammals and avian species. Comparative aspects of human embryology are included in particular chapters. Colour has been used to enhance the quality of the illustrations and to facilitate the interpretation of complex diagrams.

The 28 chapters in the second edition of *Veterinary Embryology* include four new chapters, namely historical aspects of embryology, stem cells, embryo mortality in domestic species and assisted reproductive technologies used in domestic animals. The first chapter presents a brief review of historical aspects of developments related to early concepts on the origins of mammalian life, conception and subsequent embryonic development. The contributions of Greek philosophers, early scholars and scientists whose concepts, observations and experimental methods laid the foundations of the principles of mammalian embryology are reviewed. Cell division, gametogenesis, fertilisation, cleavage and gastrulation are presented in sequential chapters. Succeeding chapters are concerned with cell signalling, stem cells, establishment of the body plan, foetal membranes, placentation and factors associated with embryonic mortality. Body systems are considered in separate chapters and embryological aspects of structures associated with special senses are reviewed. Age determination, assisted reproductive technologies used in domestic animals and aspects of mutagenesis and teratogenesis are briefly reviewed in final chapters.

Although intended primarily as a textbook for undergraduate veterinary students, this book may be of value to colleagues engaged in teaching embryology either as part of a veterinary curriculum or in courses related to animal science or developmental biology. Research scientists engaged in projects on toxicology, animal reproduction or allied topics may find particular chapters relevant to their fields of investigation.

In sequential chapters, emphasis is placed on the origin and maturation of tissues and organs and their relationship to each other. This logical approach provides a basis for developing an understanding of the form and relationships of cells, tissues, organs and structures in defined regions of the body. It also offers students a greater appreciation of topographical anatomy, which is a prerequisite for the acquisition of clinical skills, interpretation of diagnostic imaging data and procedures which may be appropriate for surgical intervention at defined anatomical sites. Molecular aspects of embryology introduce students to the role of genes and transcription factors in the orderly development of the embryo and foetus. The classification used throughout the book generally conforms to the systems adopted by the *Nomina Embryologica Veterinaria* (2006) and *Nomina Anatomica Veterinaria* (2012).

Relevant review articles and textbooks are listed in each chapter as additional sources of information. International websites providing educational resources in veterinary embryology and related topics are listed at the end of the book.

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Dublin, September 2016

About the companion website

This book is accompanied by a companion website:

www.wiley.com/go/mcgeady/veterinary-embryology

This website hosts all the figures from the book as PowerPoint slides, for you to download.

How to access the website

1 The password is the last word of the Glossary.
2 Go to **www.wiley.com/go/mcgeady/veterinary-embryology** to enter the password and access the site.

Chapter 1

Historical aspects of embryology

Key Points

- Up to the eighteenth century, the prevailing view of many scientists and scholars interested in embryology was that of *preformation*, namely that organisms develop from miniatures of themselves.
- An alternative hypothesis of embryonic development, referred to as *epigenesis*, proposed that the structure of an animal emerges gradually from a relatively formless egg. The epigenesis theory, first proposed by the Greek philosopher Aristotle, preceded the preformation theory by two millennia.
- Major advances in reproductive and developmental biology took place in the seventeenth century. Until that time, early civilisations held the view that a foetus resulted from the mixing of two parental 'seeds'.
- In human embryology, *ovists* believed in generation from oocytes while *spermists* believed that males contributed the essential characteristics of their offspring with females contributing only a material substrate. This theory was the dominant view of embryonic development until the late seventeenth century.
- As microscopy improved during the eighteenth century, biologists observed that embryos developed in a series of progressive steps and epigenesis displaced preformation as the basis of embryological development.
- Progress in understanding and manipulation of reproductive biology from a point in the past when the origins of human life were not understood to a point where early embryos can be generated *in vitro* represents a phenomenal scientific achievement.

Introduction

Embryology, as it relates to domestic animals, is concerned with the sequential stages of embryonic and foetal development, beginning with fertilisation. This dynamic science utilises cell biology, genetics and biochemistry to explain the complexities of development.

All mammals begin life as embryos. Despite the steadily increasing understanding of embryonic development and its underlying regulatory mechanisms, much remains to be discovered. For students of animal biology, veterinary medicine and related health sciences, embryology offers an insight into the development of the mammalian body at both the microscopic and anatomical levels. It also provides an important introduction to animal genetics, organ systems and reproductive biology.

At a superficial level, the basis of human reproduction is widely understood in most modern societies. In previous centuries, however, biological aspects of reproduction in the human population and among animal populations were a cause of considerable debate and much uncertainty prevailed. In the seventeenth and eighteenth centuries, the issue of 'generation', as the formation of new life was called, evoked strong religious and philosophical responses on the part of theologians and scholars, generating more heat than light. Indeed, the term 'reproduction' was not used until the eighteenth century. Prior to that time, there was no understanding that an organism was being copied, as the term implied.

Dominant theories of generation in the seventeenth and eighteenth centuries

In the history of embryology, *preformationism* was a theory of generation widely accepted from the late seventeenth to the end of the eighteenth century. This concept proposed that organisms develop from miniature versions of themselves, already fully formed in the eggs or sperm of their parents prior to conception. *Epigenesis*, the alternative theory to preformationism, contended that through a series of stages each embryo or organism was gradually produced from an undifferentiated mass.

Ovism, which held that the maternal egg was the location of the preformed embryo, was one of two models of preformationism. The other model, known as *spermism*, contended that offspring develop from a tiny, fully formed, embryo contained within the head of a sperm. The origin of spermism derived

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from the microscopic demonstration of the existence of sperm in the late 1670s. Support for ovism peaked in the mid to late eighteenth century but, by the turn of the nineteenth century, it had declined. While spermism was never as dominant as ovist preformationism, it had ardent followers whose work and writings greatly influenced the development of embryology during this period.

The origins of life

The art forms which were a feature of Stone Age civilisations conveyed the thinking of the time in relation to generation. Some of the earliest images created by humans are Venus figurines carved from soft stone, bone or ivory, or made of fired clay, most of which date from the Gravettian period, 28,000 to 22,000 years ago. In some of these figurines, certain parts of the female anatomy including the abdomen, hips, breasts, thighs and vulva were exaggerated. Archaeologists speculate that these figurines may be fertility symbols and may represent the earliest images of humans endeavouring to understand their own biological origins.

Prior to the seventeenth century, assumptions relating to the origin of life varied. It was generally believed that in mammals, including humans, 'like bred like', although it was not certain that this always occurred. Some believed, for example, that women could give birth to other species; claims that an English woman, Mary Toft from Godalming, Surrey, gave birth to rabbits in 1726 were widely accepted before she confessed that her story was untrue.

As recently as the beginning of the last century, the Polish anthropologist **Bronisław Kasper Malinowski** (1884 to 1942) claimed that the inhabitants of the Trobriand Islands in the South Pacific were unaware that babies resulted from sexual intercourse. In their native language, the word for 'father' literally means 'my mother's husband', suggesting a social rather than biological relationship. While perhaps surprising at first, there are good reasons why a link between the sexual act and the birth of a child may not have been obvious, since women can have sex without becoming pregnant. Furthermore, even when conception did occur, the two events, sex and birth, were separated by 9 months and were therefore not immediately associated with each other. Indeed, it has been postulated that human understanding of the association between mating and reproduction came through the domestication of animals some 10,000 years ago. In these animals, mating only occurs during a defined period of sexual receptivity termed oestrus, creating an observable link between mating and pregnancy.

On the basis of these observations, the realisation that male semen or 'seed', the only clearly and immediately observable product of copulation, was fundamental to the creation of life became central to the concept of generation. In religious beliefs and in mythology, the male's role in the creation of new life rapidly became dominant. For example, in the Book of Genesis, it is written that Onan 'spilled his seed on the ground' in order to avoid making his sister-in-law pregnant. In Egyptian mythology, the story of creation relates that Atum-Ra created the earth, and the first god and goddess, from his seed through masturbation. This semen/seed analogy dominated all subsequent thinking about generation.

Contributions of the Ancient Greeks

In Europe, up to the second half of the seventeenth century, beliefs on virtually every question relating to life science were dominated by the teaching of Ancient Greek philosophers. In the fifth century BCE, the Greek physician **Hippocrates** (*circa* 460 to 370 BCE), considered to be one of the most outstanding figures in the history of medicine, argued that generation took place through the joint action of two kinds of semen, one from the male ejaculate, the other from the female's menstrual blood. A century later, the Greek philosopher and scientist **Aristotle** (384 to 322 BCE) published *De generatione animalium* (*The Generation of Animals*) about 350 BCE, the first work to provide a comprehensive theory of the mechanisms of reproduction in a variety of animals. He described the concepts of oviparity (birth from eggs), viviparity (live birth) and ovoviviparity (production of an egg that hatches inside the body). He also described the holoblastic and meroblastic patterns of cell division (see Chapter 5). He made the important observation that the organs develop gradually in the embryo (epigenesis) and are not preformed. In contrast to Hippocrates, Aristotle believed that only the male's semen or 'seed' contributed to the 'form' of the foetus and that this form was imprinted onto the 'matter' which was provided by the menstrual blood of the female, much like a seal stamping hot wax. Another analogy, which has persisted to the present day, was that semen was like a seed which was sown on fertile ground. Aristotle argued that lower animals such as insects generated spontaneously from decay. This theory corresponded with the everyday experience of observing maggots appearing suddenly on rotting matter, but this concept was ultimately refuted by **Francesco Redi** (1626 to 1698) in the mid 1600s (see below).

In the second century CE, **Galen** (129 to *circa* 200), a prominent Greek physician, surgeon and philosopher in the Roman Empire, supported the assertion of Hippocrates that the seeds of both the male and female contribute to procreation. This was partly due to his mistaken view that women's genitalia were identical to those of men but turned inward. His anatomical reports, based mainly on dissection of monkeys and pigs, remained uncontested until printed descriptions and illustrations of human dissections were published in 1543 in the classical work on human anatomy *De humani corporis fabrica* (*On the Fabric of the Human Body*) by the Belgian anatomist and physician **Andreas Vesalius** (1514 to 1564).

Although Galen adopted Hippocrates' view that there were two types of 'semen' – one male, the other female – acceptance of this theory was hampered by the fact that it was not possible to identify female semen and therefore Aristotle's view persisted.

The emergence of comparative embryology

For several hundred years, controversy persisted as to the respective roles of the male and female in generation. During this time, the ideas of the Ancient Greeks were maintained by Arab thinkers but were not developed beyond those focusing on the role of the male and, for a period, progress in understanding the origin of life did not occur. From the fourteenth century onwards, there was a resurgence in Europe of the ideas of the ancient thinkers. Around this time, among the famous anatomical drawings of the great Italian artist **Leonardo da Vinci** (1452 to 1519) were those of the pregnant bicornuate bovine uterus and of the foetus and foetal membranes with the uterus removed. Da Vinci also depicted a human uterus opened to reveal the foetus and associated membranes.

One of the first major publications on comparative embryology was *De formato foetu* (*The Formed Foetus*) in 1600 by the pioneering Italian anatomist and surgeon **Hieronymus Fabricius** (Girolamo Fabrizio da Acquapendete, 1537 to 1619) which contained many illustrations of embryos and foetuses at different stages of development. Fabricius was a student of **Gabriele Falloppio** (Fallopius, 1523 to 1562) who described the uterine tubes, formerly referred to as the Fallopian tubes. The site of B lymphocyte formation in birds, the bursa of Fabricius, now known as the cloacal bursa, bore his name. A manuscript entitled *De formatione ovi et pulli (On the Formation of the Egg and Chick)*, found among his lecture notes after his death, was published in 1621 and contained the first description of the bursa. Another Italian, **Bartolomeo Eustachius** (1514 to 1574) published illustrations of canine and ovine embryos in 1552. He also extended the knowledge of the anatomy of the internal ear by describing correctly the auditory tube that connects the middle ear with the nasopharynx, and which bears his name (the Eustachian tube).

Many of the concepts associated with embryology were speculative until the invention of the microscope, which allowed detailed observation of embryological structures. **Marcello Malpighi** (1628 to 1694), an Italian professor of medicine and personal physician to Pope Innocent XII, was one of the first supporters of preformationism. He described development of the embryo as a mere unfolding of an already miniature adult organism. He published the first microscopic examination of chick embryo development in 1672, identifying the neural groove, the somites and blood flow to the yolk sac. Because of the importance of his early work, a number of anatomical structures were named after him, including Malpighian (renal) corpuscles in the kidney and the Malpighian layer in the epidermis. He observed that even the unincubated chick egg was considerably structured, leading him to question the concept of epigenesis and to believe that a preformed version of the chicken resided in the egg. These observations were subsequently questioned, as his 'unincubated' eggs had in fact been left exposed to warm environmental temperatures. Nonetheless, these experiments opened up one of the great debates in embryology: whether the organs of the embryo formed *de novo* at each generation (epigenesis) or were already present in miniature form within the egg or sperm (preformation).

A period of intense discovery in the seventeenth century laid the foundations for the unravelling of sex, life and growth and for our current knowledge on the origins of life. It was during this period that fundamental discoveries relating to biological events associated with procreation were made, although their full meaning remained unclear. **William Harvey** (1578 to 1657), a one-time student of Falloppio and personal physician to King James I and King Charles I, best known for his discovery of blood circulation, undertook one of the first detailed investigations in embryology. In 1651 Harvey published his book *Exercitationes de generatione animalium* (*On the Generation of Animals*) with the now famous frontispiece illustrating the Greek god, Zeus, liberating all creation from an egg bearing the inscription '*ex ovo omnia*' (all things come from the egg) (Fig 1.1). Harvey was convinced that the egg, rather than sperm, was fundamental to generation, apparently challenging Aristotle's belief that sperm were of greatest importance, although what exactly he meant by 'egg' is unclear. He had no understanding of there being equivalent male and female gametes and no idea of what might be contained in semen. In the 1630s Harvey carried out a now famous experiment in which he dissected the deer of King Charles I during rutting and mating. He found no trace of semen in the uterus, nor did he find any changes in the female 'testicles', the generally accepted term at the time for what we now call ovaries. In addition, he failed to recognise the filamentous conceptus characteristic of ruminants. He ultimately concluded that Aristotle was correct and that semen acted in some way by shaping menstrual blood.

At the University of Leiden in the 1660s three medical students, **Nicolas Steno** (Niels Stensen, 1638 to 1686), **Jans Swammerdam** (1637 to 1680) and **Regnier de Graaf** (1641 to 1673) made a significant impact on our knowledge of generation. All three were heavily influenced by their professor, **Johannes van Horne** (1621 to 1670) and, in the case of Swammerdam and Steno, by the French author and scientist **Melchisedec Thévenot** (1620 to 1692). Both scientists encouraged the three students to investigate generation and the origins of life.

In 1667, Steno, who by this time was in Florence, published what turned out to be his most influential scientific work, *Elementorum myologiae specimen* (*A Model of Elements of Myology*), in which he accurately described the function of muscles, using both dissection and mathematical models. He included a comparison between the anatomy of the viviparous dogfish and of egg-laying rays and concluded, based on his observations, that the 'testicles' of women were analogous to the ovaries of the dogfish.

Swammerdam initially focused on the generation of insects and, through careful observation and dissection, he came to the radical conclusion that all animals derive from eggs laid by females of the same species. In his 1669 book, *Historia generalis insectorum*, he put forward a revolutionary classification

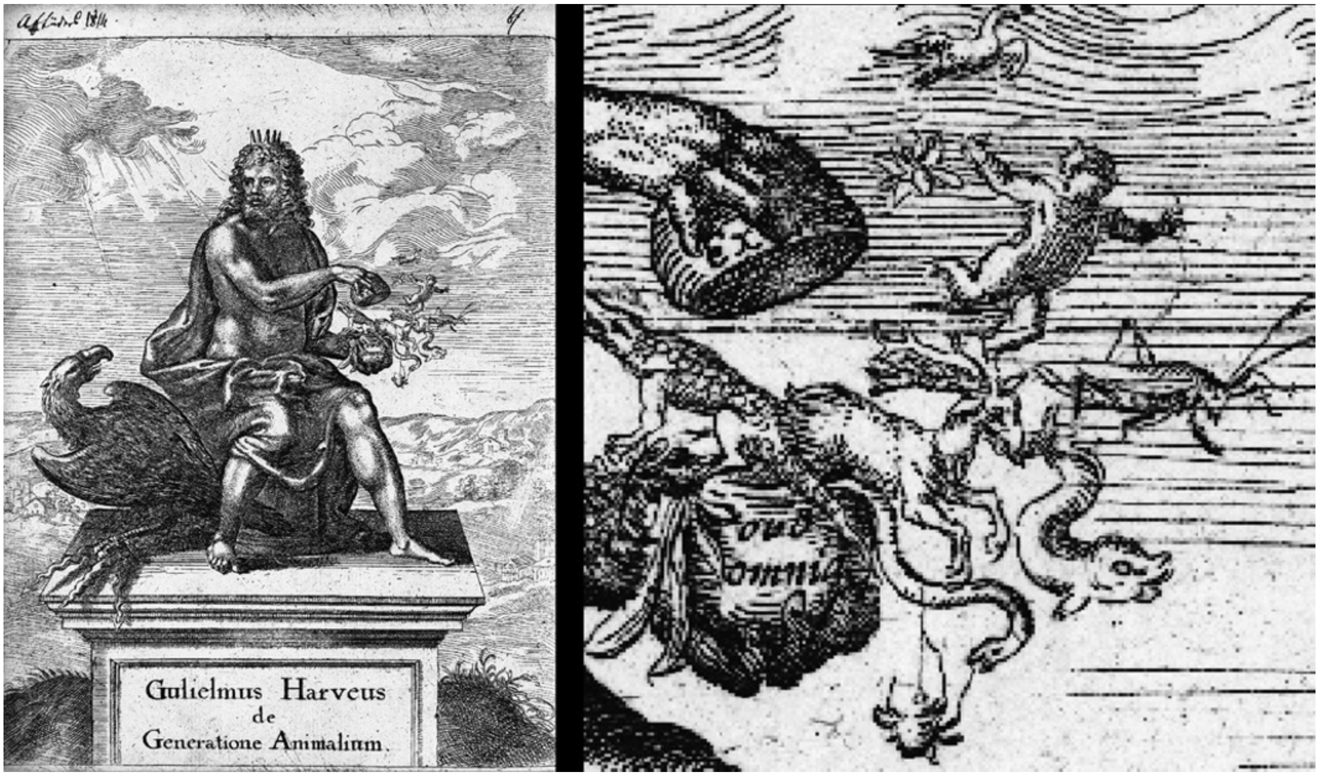

Figure 1.1 The frontispiece of William Harvey's *Exercitationes de generatione animalium*, published in 1651, showing Zeus liberating all living things from an egg bearing the inscription '*ex ovo omnia*' (magnified on right). Courtesy of Wellcome Library, London.

of insects based on their modes of development which is still in use. Together with the work of the Italian biologist Francesco Redi, Swammerdam's study showed that insects did not generate spontaneously, as had previously been thought, but were the product of an egg laid by a female of the same species and that the same organism persists through various stages, namely larva, pupa, juvenile, adult. Redi refuted the notion of spontaneous generation by demonstrating, through simple experimentation, that maggots appearing on decaying matter came from the eggs of flies. His most famous experiments are described in *Esperienze intorno alla generazione degl'insetti* (*Experiments on the Generation of Insects*), published in 1668.

In 1671, de Graaf published a brief outline of his work, in which he summarised his view of how 'eggs' in the female 'testicle' became 'fertile' through the action of the 'seminal vapour' rising up from the uterus via the uterine tubes. In 1672, he published *De mulierum organis generationi inservientibus tractatus novus* (*New Treatise Concerning the Generative Organs of Women*). The book contained dissections of humans, rabbits, hares, dogs, pigs, sheep and cows as well as a section on mating and pregnancy in rabbits where de Graaf referred to the follicles or their contents as eggs. He used careful dissection to show that, in rabbits, the follicles ruptured following mating and that three days after copulation small spherical structures could be found in the uterine tubes. Like Harvey, de Graaf looked and failed to find any signs of semen in the uterus and Fallopian tubes. He concluded that only a 'seminal vapour' reached the eggs and fertilised them. de Graaf's name continues to be associated with ovarian ('Graafian') follicles, which he believed to be eggs. He also described the correct function of the uterine ('Fallopian') tubes.

In 1672, in response to de Graaf's work, Swammerdam published his own account of human generation, *Miraculum naturae, sive uteri muliebris fabrica* (*The Miracle of Nature, or the Structure of the Female Uterus*). The two men became embroiled in a bitter dispute over who was the first to discover that females had eggs. Both wrote to the Royal Society in London presenting their evidence and asked the Society to adjudicate on who was correct. To their surprise and, presumably, their disappointment, the Royal Society decided the honour should go to Steno, who had suggested several years earlier, in 1667, that the structures that had hitherto been referred to as female testicles were in fact ovaries. Interestingly, Steno had drifted away from science, eventually became a Catholic bishop and was ultimately beatified by Pope John Paul II in 1988.

Despite this controversy, de Graaf is ultimately remembered for the discovery that female mammals produce eggs, Steno is largely remembered for his work on geology and Swammerdam has been largely forgotten.

The discovery of sperm

By the mid 1670s, the 'egg' theory of generation was widely accepted by thinkers and the general public. This was a remarkable change of direction from the notion of a 'seed', but it did not persist for long. The discovery of microscopic organisms during the period 1665 to 1683 was made by two Fellows of the Royal Society, **Robert Hooke** (1635 to 1703) and **Antonie van Leeuwenhoek** (1632 to 1723). In *Micrographia* (1665), Hooke presented the first-published depiction of a microganism, the fungus *Mucor*. He is credited with coining the term 'cell' after observing empty spaces contained by walls in a thin section of cork which reminded him of monastic cells. Later, van Leeuwenhoek observed and described microscopic protozoa and bacteria. These important revelations were made possible by the ingenuity of both men in fabricating and using simple microscopes that magnified objects from about 25-fold to 250-fold and afforded an opportunity for the closer examination of other biological samples, including semen.

van Leeuwenhoek, a Dutch draper from Delft, was entirely self-taught. He did not speak or write Latin, the scientific language of the day. He had been introduced to the Royal Society by his friend de Graaf, in 1672, as a maker of exceptional microscopes. The Royal Society subsequently asked him to examine a variety of bodily fluids including semen. He felt that looking at semen would be inappropriate, so he did not accede to the request. A few years later, in 1674, a student, **Nicolaas Hartsoeker** (1656 to 1725) and van Leeuwenhoek were the first to examine semen microscopically, a situation that would later lead to a dispute between them over the discovery of sperm. Hartsoeker postulated the existence of a preformed individual in the sperm, consistent with his spermist theory of preformation, and produced the now famous drawing of a tiny man or 'homunculus' inside the sperm (Fig 1.2). A contemporary, **Dalenpatius** (Francois de Plantade, 1670 to 1740) published drawings in 1699 of homunculi in sperm, a concept later exposed as a hoax.

In 1677, a student from the medical school at Leiden, **Johannes Ham** (1651 to 1723), took a specimen of semen to van Leeuwenhoek, ostensibly collected from a man with gonorrhea, in which Ham had found small living 'animalcules' with tails. van Leeuwenhoek subsequently resumed his own observations and in his own semen, acquired, he stressed, not by sinfully defiling himself, but as a natural consequence of conjugal coitus, he observed a multitude of 'animalcules' less than a millionth the size of a coarse grain of sand and with thin, undulating transparent tails.

In the summer of 1677, he reported his findings to Lord Brouncker, president of the Royal Society, urging him not to publish them if he thought it would give offence. Following further experimentation, his findings were eventually published in January 1679, in Latin, presumably due to their delicate nature. The drawing accompanying the article represented sperm of rabbits and dogs (Fig 1.3). van Leeuwenhoek's letter to Brouncker

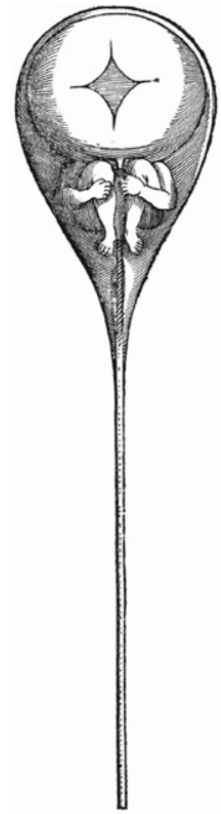

Figure 1.2 Illustration of a homunculus in sperm, drawn by Nicolaas Hartsoeker, published as part of his 1694 French-language paper entitled *Essai de Dioptrique*, a semi-speculative work describing the potential new scientific observations that could be made using magnifying lenses. Courtesy of Wellcome Library, London.

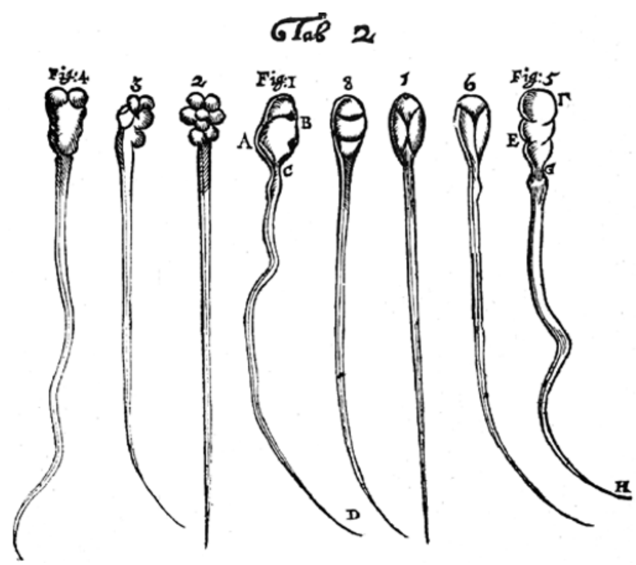

Figure 1.3 Sperm from rabbits and dogs, drawn by Antonie van Leeuwenhoek. Published in *Philosophical Transactions*, the journal of the Royal Society, London, 1678. Courtesy of Wellcome Library, London.

challenged the prevailing ideas about animal generation and represented a return to the ancient Greek view on the origin of life, a sperm-centric view.

Experimental embryology

The division between ovists and spermists persisted for many years. Although he was originally unaware of their involvement in reproduction, in 1685 van Leeuwenhoek wrote that sperm were seeds and that the female merely provided the nutrient soil in which the seeds were planted, thus returning to the notion promulgated by Aristotle some 2000 years earlier. Indeed, although sperm were discovered in the 1670s, the detailed events associated with fertilisation were not elucidated until 1876. Thus, for some 200 years, the role of sperm in generation was unclear.

The uncertainty of the role of sperm in generation was further compounded in 1744, when the Swiss naturalist **Charles Bonnet** (1720 to 1793) published *Traite d'insectologie*, in which he described parthenogenesis in aphids which could apparently breed for numerous generations in the absence of males. This provided further support for the ovist theory of generation. In *Philosophical Palingests, or Ideas on the Past and Future of Living Beings*, he argued that females carry within them all future generations in miniature form. He felt that the theory of preformation was 'one of the greatest triumphs of rational thought over sensual conviction'. As a proponent of the preformist theory, he believed that future generations pre-existed within the germ cells, analogous to the famous Russian Matryoshka dolls of decreasing size, placed one inside another. The fact that eventually such dolls cease to get smaller did not trouble Bonnet, who stated 'Nature works as small as it wishes'. As **Mattias Schleiden** (1804 to 1881) and **Theodor Schwann** (1810 to 1882) did not formulate their 'cell theory' until 1839, Bonnet and his contemporaries lacked scientific evidence to refute their hypothesis.

Bonnet's contemporary and one of ovism's greatest champions, **Albrecht von Haller** (1708 to 1777), examined chick embryos under the microscope and noted that the yolk appeared to be attached to the embryonic chick's small intestine. On this basis, he concluded that the embryo must be created at the same time as the yolk and that, since unfertilised eggs also contain yolks, the embryo existed there prior to fertilisation.

The French mathematician and biologist, **Pierre Maupertuis** (1698 to 1759), refuted the preformationist theories, and from his study of the inheritance of genetic traits proposed various ideas which pre-empted the genetic theory of inheritance. He applied the concept of probability to genetic problems and introduced experimental breeding as a means of studying the inheritance of genetic traits in animals. Maupertuis argued that the embryo could not be preformed, either in the egg or in the sperm, since hereditary characteristics could be passed on equally through the male or the female parent.

One of the last supporters of ovism was the Italian priest and physiologist **Lazzaro Spallanzani** (1729 to 1799). More than 100 years after the discovery of sperm, and building on novel experiments by the French scientist, **René Antoine Ferchault de Réaumur** (1683 to 1757), Spallanzani placed 'trousers' made of taffeta on male frogs to prevent semen from coming into contact with eggs. These experiments provided the first hard evidence of the importance of sperm in reproduction and demonstrated that actual physical contact between the egg and the sperm was necessary for embryo development to occur. In 1784, Spallanzani reported the first successful artificial insemination in a dog, resulting in the birth of three puppies 62 days later, followed soon after by the first successful artificial insemination in humans, in 1790, by the renowned Scottish anatomist and surgeon, **John Hunter** (1728 to 1793). While many of Spallanzani's experiments clearly indicate that sperm are necessary for fertilisation, he did not draw this conclusion at the time. Instead, he became further convinced, as suggested in his *Experiences pour servir a l'histoire des animaux et des plantes*, that the egg contained a fully formed tadpole that only needed to be exposed to seminal fluid to begin development.

French naturalist **Jean-Baptiste Lamarck** (1744 to 1829) is widely remembered for a theory of inheritance of acquired characteristics, called soft inheritance or Lamarckism, which proposed that an organism can pass on characteristics that it acquired during its lifetime to its offspring, which he described in his 1809 *Philosophie zoologique*. This notion was eventually abandoned with the emergence of the laws of Mendelian inheritance following the famous pea plant experiments conducted between 1856 and 1863 by **Gregor Johann Mendel** (1822 to 1884) which established many of the rules of heredity. German anatomist **Johann Friedrich Meckel** (1781 to 1833), a pioneer in the science of teratology, in particular the study of birth defects and abnormalities that occur during embryonic development, adopted Lamarck's evolutionary beliefs. Together with French embryologist **Étienne Serres** (1786 to 1868), he defined a theory of parallelism between the stages of ontogeny and the stages of a unifying pattern in the organic world, which became known as the Meckel-Serres Law, based on a belief that within the entire animal kingdom there was a single unified body type, and that, during development, the organs of higher animals matched the forms of comparable organs in lower animals. This theory applied to both vertebrates and invertebrates, and also stated that higher animals go through embryological stages analogous to the adult stages of lower life forms in the course of their development, a version of the recapitulation theory later captured in the statement 'ontogeny recapitulates phylogeny' of **Ernst Haeckel** (1834 to 1919).

In the late eighteenth century, the German embryologist **Kaspar Friedrich Wolff** (1734 to 1794), in his dissertation *Theoria generationis* published in 1759, revived and supported the theory of epigenesis, previously proposed by Aristotle and Harvey and discredited that of preformation, leading to criticism from von Haller and Bonnet. Through detailed study of the development of chick embryos, Wolff demonstrated that the adult bird developed from tissues having no counterpart in the embryo. In Wolff's *De formatione intestinorum*, published in 1768 and 1769, he established the principles of formation of organs from foliate layers, through proliferation, folding and wrapping, thus laying the foundations of the theory of germ layers in the embryo which subsequently, under Pander and von Baer, became the fundamental concept in structural embryology. His name remains associated with the Wolffian or mesonephric duct.

In spite of Wolff's contribution, the preformation theory persisted until the 1820s, by which time a combination of new staining techniques, improved microscopes and the efforts of a talented group of scientists transformed embryology into a defined specialised branch of science. Three friends, **Christian Heinrich Pander** (1794 to 1865), **Karl Ernst von Baer** (1792 to 1876) and **Heinrich Rathke** (1793 to 1860), all of whom came from the Baltic region, significantly contributed to this advancement of research in embryology.

In his studies of the chick embryo, Pander extended the observations made by Wolff and discovered the germ layers, three distinct regions of the embryo that give rise to the differentiated cell types and specific organ systems (see Chapter 9). He demonstrated that the germ layers did not give rise to their respective organs autonomously, but rather that all three influenced each other, a concept of tissue interaction now known as induction. Thus, he showed that the theory of preformation was erroneous, since organs derive from interactions between simpler structures. His dissertation, *Historia metamorphoseos quam ovum incubatum prioribus quinque diebus subit*, published in 1817, included detailed illustrations by **Eduard Joseph d'Alton** (1772 to 1840). Pander's name was associated with blood islands, sometimes known as Pander's islands, structures around the developing embryo which contribute to many different parts of the circulatory system.

Pander's studies of the chick embryo were continued by von Baer, who expanded Pander's concept of germ layers to include all vertebrates, recognising that there is a common pattern of vertebrate development, and in so doing laid the foundation for comparative embryology. von Baer was the first to observe and describe the mammalian egg (oocyte), first in the dog, in 1826, and then in other species, establishing beyond doubt that mammals originated from eggs and thus ending a search that had begun with Harvey and de Graaf in the seventeenth century and had been avidly pursued by others in the eighteenth and early nineteenth centuries. He published this discovery in *De ovi mammalium et hominis genesi* (*On the Mammalian Egg and the Origin of Man*) in 1827. Together with Pander, and based on the work by Wolff, he described the germ layer theory of development as a principle in a variety of species, laying the foundation for comparative embryology in the book *Über Entwickelungsgeschichte der Thiere* (*On the Development of Animals*, vol. 1, 1828; vol. 2, 1837). He identified the neural folds as precursors of the nervous system, discovered the notochord, described the five primary brain vesicles and studied the functions of the extra-embryonic membranes. This pioneering work established embryology as a distinct subject in its own right.

von Baer's embryological discoveries ultimately led him to a view of development that supported epigenesis and refuted long-held thinking about preformation. He encapsulated his thinking into four statements that are often referred to as 'von Baer's Laws'. These laws state:

1 General characteristics of the group to which an embryo belongs develop before special characteristics.
2 General structural relations are likewise formed before the most specific appear.
3 The form of any given embryo does not converge upon other definite forms, but separates itself from them.
4 The embryo of a higher animal form never resembles the adult of another animal form, such as one less evolved, but only its embryo.

The first two laws were intended to refute preformationism while the second two were intended to refute the laws of parallelism promoted by von Baer's contemporaries, Meckel and Serres.

In 1828, von Baer reported that he had two small embryos preserved in alcohol which he had forgotten to label. He was unable to determine the genus to which they belonged, suggesting that 'they may be lizards, small birds or even mammals'. The observations of von Baer suggested that there was a 'phylotypic' stage at which the embryos of different vertebrate classes all have a similar physical structure, a topic that was to be controversially revisited several decades later.

Along with von Baer and Pander, Rathke is recognised as one of the founders of modern embryology. Rathke followed the intricate development of the vertebrate skull, excretory and respiratory systems, showing that these became increasingly complex and took on different routes of development in different classes of vertebrates. He was the first to describe the brachial clefts and gill arches in the embryos of mammals and birds. In 1839, he was the first to describe the embryonic structure, now known as Rathke's pouch, from which the anterior lobe of the pituitary gland develops. He was the first to describe the pharyngeal arches and showed that these ephemeral formations became gill supports in fish and the jaws and ears, among other structures, in mammals.

Contemporaneously, in 1824, **Jean-Louis Prevost** (1790 to 1850) and **Jean-Baptiste Dumas** (1800 to 1884) claimed that, rather than being parasites, sperm were the active agents of fertilisation and they proposed that the sperm entered the egg and contributed to the next generation. In *Sur les animalcules spermatiques de divers animaux* published in 1821, written with Dumas, Prevost made a histological examination of spermatozoa and demonstrated that these cells originate in certain tissues of the male sex glands. His observations were the culmination of a series of experiments, based on those of Spallanzani, which prepared the way for modern discoveries in fertilisation.

In collaboration with Dumas, Prevost published three memoirs in 1824 on generation in the *Annales des Sciences Naturelles* that are now considered the foundation of experimental embryology. These claims were largely disregarded until the 1840s when the Swiss anatomist and physiologist **Rudolph Albert von Kölliker** (1817 to 1905) described the formation of sperm from cells in the adult testes. Advances in staining and microscopy during the nineteenth century allowed more detailed observations on the initial cleavage stages in the rabbit by German biologist **Theodor Ludwig Wilhelm von Bischoff** (1807 to 1882) and by von Kölliker in humans and domestic species. von Kölliker published *Entwicklungsgeschichte des Menschen und der höheren Tiere* in 1861, the first textbook on embryology in humans and higher animals. However, it was not until 1876 that two zoologists, the German **Oscar Hertwig** (1849 to 1922) and the Swiss investigator **Hermann Fol** (1845 to 1892), independently demonstrated entry of the sperm into the sea urchin egg and the subsequent union of their two nuclei. Thus, after decades of experimentation, fertilisation was finally recognised as the union of the sperm and egg.

Later in the nineteenth century, the Belgian embryologist **Edouard van Beneden** (1846 to 1910) described the early phases of egg development in the rabbit and in bats, including the formation of the three basic layers. **Albert Brachet** (1869 to 1930), a student of van Beneden, was one of the first to confirm the possibility of inducing unfertilised oocytes to develop parthenogenetically by mechanical stimulation, and was a pioneer in experimental attempts to culture mammalian embryos *in vitro*.

In 1890, Hertwig reported the occurrence of parthenogenesis in the animal kingdom, namely in a starfish. In the same year, **Walter Heape** (1855 to 1919) carried out the first successful embryo transfer in mammals by transferring embryos from the biological mother, an Angora rabbit, to a foster rabbit of a Belgian line resulting in the birth of live offspring. Heape concluded that 'a uterine foster-mother has no power of modifying the breed of her foster-children, and that her uterus during gestation, and the nourishment she supplies to the embryo, is analogous to a bed of soil with its various nutrient constituents' (quoted in Biggers 1991, p. 175). It is on this basis that commercial embryo transfer in cattle is carried out today (see Chapter 27).

Evolutionary embryology

As a consequence of the work of Pander, von Baer and Rathke, the theory of preformation all but disappeared in the 1820s. However, the concept survived for another 80 years as some scientists regarded the cells of the early cleavage stage embryo as representing right and left sides of the body as it took form, implying that information for building the body is regionally segregated in the egg. In 1893, the German evolutionary biologist **August Weismann** (1834 to 1914) published *The Germ Plasm: A Theory of Heredity* as an extension of this idea, suggesting that inheritance only takes place by means of the germ cells. He proposed that the sperm and egg provide equal chromosomal contributions. In postulating the germ plasm model, Weismann claimed that the first cleavage division separated the future right and left halves of the embryo.

Ernst Haeckel (1834 to 1919) was a leading German anatomist, a student of von Kölliker and contemporary and supporter of **Charles Darwin** (1809 to 1882). He developed the influential, but no longer widely held, recapitulation theory ('ontogeny recapitulates phylogeny') claiming that an individual organism's biological development, or ontogeny, parallels and summarises its species' evolutionary development, or phylogeny. This became known as the 'biogenetic law'. An accomplished artist, Haeckel published a set of 24 drawings, first in 1866 in his *Generalle Morphologie der Organismen*, and repeated in 1874 in his more popular *Anthropogenie*, which were to become some of the most iconic images in biology (Fig 1.4). These images purport to show embryos of fish, salamander, turtle, chicken, pig, cow, rabbit and human in three stages of development. Haeckel claimed that members of all vertebrate classes pass through an identical conserved phylotypic stage. However, his famous and much reproduced drawings have since been shown to be oversimplified, apparently deliberately so, to the point of obscuring important differences between classes of vertebrates.

The Swiss anatomist and microtome inventor, **Wilhelm His** (1831 to 1904), was among the first to dispute the veracity of Haeckel's drawings of embryos. He studied under von Kölliker, amongst others, and introduced the word endothelium, distinguishing these internal membranes, which formerly had been grouped with epithelia, and described their relationship to the germ layers during development. He is also remembered for his identification of a germinative zone within the developing vertebrate metencephalon that he later termed the rhombic lip.

In 1888, in order to test Weismann's hypothesis, the German embryologist and student of Haeckel, **Wilhelm Roux** (1850 to 1924), published the results of experiments in which individual blastomeres of two- and four-cell frog embryos were destroyed with a hot needle. He reported that they grew into half-embryos and surmised that the separate function of the two cells had already been determined. This led him to propose his 'mosaic' theory of epigenesis, which held that, following a number of cell divisions, the embryo would be like a mosaic, each cell playing its own unique part in the entire design. Later, Roux's theory was refuted by the studies of his colleague, **Hans Driesch** (1867 to 1941), and subsequently, with more precision, the German **Hans Spemann** (1869 to 1941) showed that, while as a rule, Driesch's conclusions were correct, results such as those of Roux could be obtained depending on the plane through which the cells were manipulated. Under the supervision of Haeckel, Driesch used cell separation, instead of Roux's cell destruction, and observed very different results. Using early cleavage stage sea urchin eggs, he demonstrated that each of the cells was able to develop into a small but complete embryo. This important refutation of both preformation and the mosaic theory of Roux

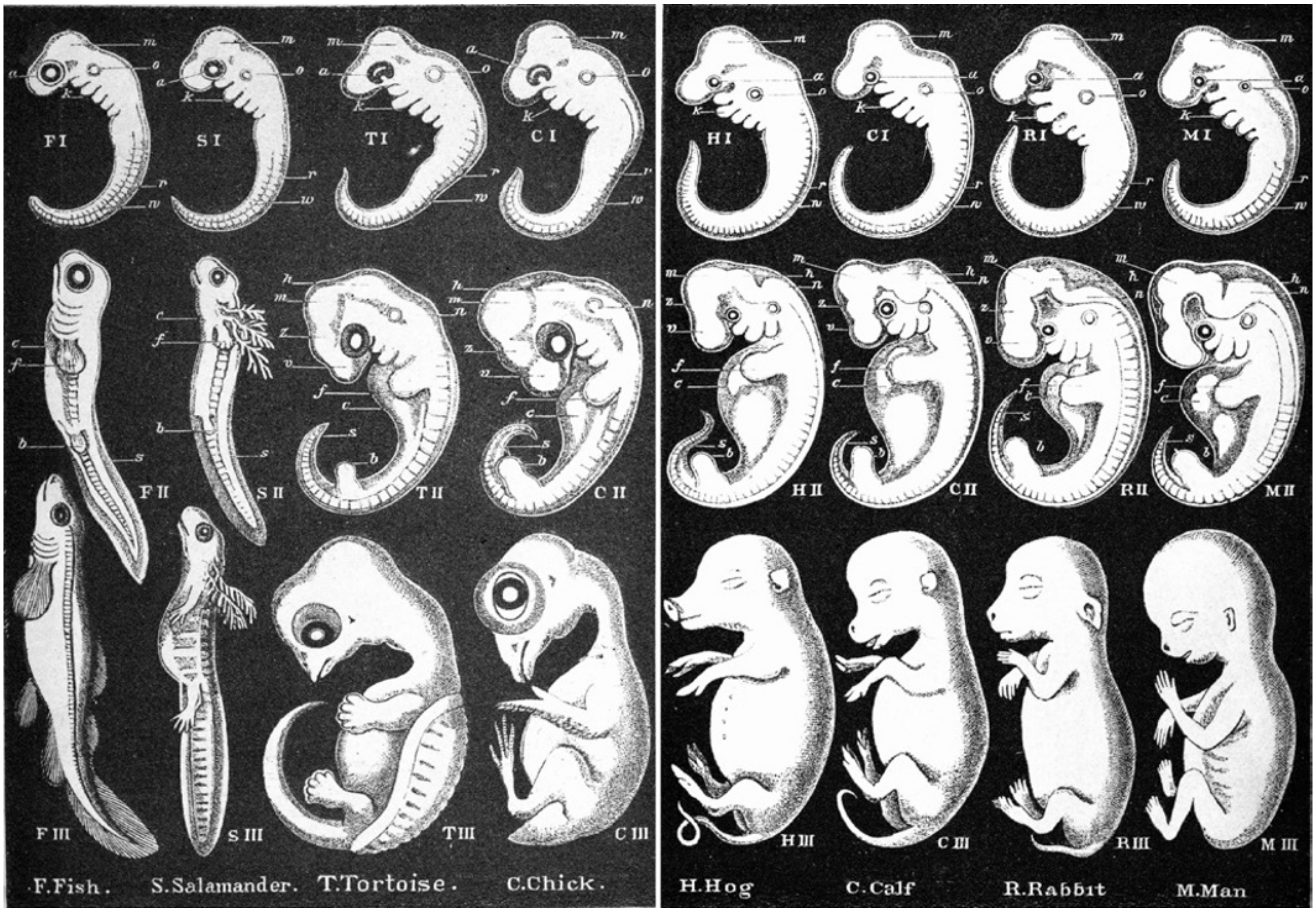

Figure 1.4 Ernst Haeckel's now discredited illustration of eight species compared at three stages of development. Left to right: fish, salamander, turtle, chicken, pig, cow, rabbit and human. From the second edition of *Anthropogenie*, published in 1874. Courtesy of Wellcome Library, London.

was the subject of much discussion in the ensuing years and caused friction between Driesch, Roux and Haeckel. Driesch's findings brought about the adoption of the terms 'totipotent' and 'pluripotent', referring, respectively, to the ability of the cell to generate every cell type, or multiple cell types, in an organism.

Driesch's results were confirmed with greater precision by Spemann, who provided the final evidence against the Roux-Weismann theory. Spemann succeeded in dividing the cells of the early salamander embryo with a noose of his baby son's hair. He found that one half could indeed form a whole embryo, but observed that the plane of division was crucial to the outcome. In conjunction with his graduate student, **Hilde Mangold** (1898 to 1924), he carried out experiments grafting a 'field' of cells (the primitive knot) from one embryo onto another, the results of which were published in 1924. They described an area in the embryo, the portions of which, upon transplantation into a second embryo, organised or 'induced' secondary embryonic primordia regardless of location. Spemann called these areas 'organiser centres'. Later he showed that different parts of the organiser centre produce different parts of the embryo. In 1928, he was the first to perform somatic cell nuclear transfer using amphibian embryos. He was awarded the Nobel Prize in 1935. His theory of embryonic induction by organisers is described in his book *Embryonic Development and Induction* (1938).

Decades before it became technically feasible, Spemann proposed the use of nuclear transfer to clone entire organisms. His experiments paved the way for **Robert Briggs** (1911 to 1983) who in 1952, together with **Thomas Joseph King** (1921 to 2000), cloned a frog, *Rana pipiens*, by transplanting blastula nuclei into enucleated eggs, which then developed into normal embryos. This represented the first successful nuclear transplantation performed in metazoans. However, these successful transplants involved undifferentiated nuclei. **John Gurdon** (1933–), an English developmental biologist then at the University of Oxford, working on *Xenopus laevis* in the late 1950s and early 1960s, extended the work of Briggs and King, culminating in his seminal 1962 paper describing the transplantation of intestinal epithelial cell nuclei from *Xenopus* tadpoles into enucleated frog eggs resulting in the development of normal tadpoles. The implication of Gurdon's success – that the nuclei of differentiated cells retain their totipotency – provided a key conceptual advance in developmental biology. In 2012, Gurdon was awarded, jointly with **Shinya Yamanaka** (1962–), the Nobel Prize for Physiology or Medicine for the discovery that mature cells can be reprogrammed to become pluripotent. Subsequently, in the mid 1980s, **Steen Malte Willadsen** (1943–), a Danish scientist working at the Institute of Animal Physiology, Cambridge, successfully used cells from early embryos to clone sheep by nuclear transfer. This procedure was modified about a decade later by the team led by **Ian Wilmut** (1944–) and including **Keith Campbell** (1954 to 2012), leading to the birth in 1996 of a Finn Dorset lamb named Dolly, the first mammal to be cloned from fully differentiated adult mammary cells. This landmark achievement represented the first demonstration that the nucleus of an adult mammalian somatic cell could be reprogrammed to give rise to the development of an entire organism.

Genes and heredity

The behavior of chromosomes and their importance in heredity was a contentious topic at the turn of the twentieth century. Many scientists, including **Edmund Beecher Wilson** (1856 to 1939) and his colleagues were working on this problem. The chromosome theory of inheritance is credited to **Walter Sutton** (1877 to 1916), a student of Wilson's, as well as to independent work by a friend of Wilson's, **Theodor Boveri** (1862 to 1915) around the same time. Boveri was studying sea urchins, in which he found that a full complement of chromosomes had to be present for normal embryonic development to take place. Sutton's work with grasshoppers showed that chromosomes occur in matched pairs of maternal and paternal chromosomes which separate during meiosis. This groundbreaking work led Wilson to name the chromosome theory of inheritance the Sutton-Boveri Theory. Some time later, the American embryologist **Thomas Hunt Morgan** (1866 to 1945) won the Nobel Prize in Physiology or Medicine in 1933 for discoveries elucidating the role of chromosomes in heredity. Morgan demonstrated that genes are carried on chromosomes and are the mechanical basis of heredity. These discoveries formed the basis of genetics as a modern scientific subject.

Conrad Hal Waddington (1905 to 1975), a British developmental biologist, demonstrated that the principles of embryological development discovered by Spemann in amphibians were also valid in avian species. With **Joseph Needham** (1900 to 1995) and **Jean Brachet** (1909 to 1988), son of Albert Brachet, he initiated a series of experiments in order to determine the chemical nature of the substances produced by the organiser centres previously described by Spemann. At the end of the 1930s, Waddington spent a year in Morgan's laboratory which lead him to reorient his work towards *Drosophila* and the role that genes play in development. Waddington fully agreed with the model proposed by Morgan in his 1934 book *Embryology* that development is the result of an ongoing dialogue between genes and the cytoplasm. He addressed the causal link between embryology and genetics by isolating several genes that caused wing malformations in *Drosophila*. His representation of the epigenetic landscape affecting initial cell differentiation in the embryo is still used today to describe the factors which influence stem cell development.

Creating life *in vitro*

The first attempt at *in vitro* fertilisation of mammalian oocytes is attributed to Austrian embryologist **Samuel Leopold Schenk** (1840 to 1902) in 1878. Working with rabbits and guinea pigs, Schenk noted that cell division occurred in cultures after sperm were added to oocytes. Initial claims by **Gregory Pincus** (1903 to 1967) that he had achieved the first successful pregnancy following *in vitro* fertilisation in rabbits in 1934 were subsequently questioned, as the gametes had been co-incubated *in vitro* for only a short time before transfer to the uterine tube and, in all likelihood, fertilisation actually occurred *in vivo*. This and other studies were later described in Pincus' seminal work, *The Eggs of Mammals*, published in 1936.

A colleague of Pincus, **Min Chueh Chang** (1908 to 1991), and **Colin Russell Austin** (1914 to 2004) independently reported in 1951 that mammalian spermatozoa require a period of time in the female reproductive tract to render them competent to fertilise an oocyte, a process termed capacitation. Chang subsequently reported the birth of live offspring following *in vitro* fertilisation in rabbits in 1959. In the intervening period, **John Rock** (1890 to 1984), a clinical professor of obstetrics and gynaecology at Harvard Medical School and collaborator with Pincus on the development of the human contraceptive pill, together with his technician **Miriam Menkin** (1901 to 1992) reported the first successful *in vitro* fertilisation in humans, published in 1944. Despite the absence of any pregnancies resulting from the embryos created in their experiments, Rock and Menkin still made their mark on the history of embryology, providing proof that an embryo could be created outside a human body. **Landrum Shettles** (1909 to 2003) repeated their experiment years later in preliminary attempts at obtaining a successful pregnancy from *in vitro* fertilisation. In 1960, Shettles published *Ovum humanum*, a book containing a collection of colour photographs showing details of the human egg never before seen, which became the standard visual reference used by scientists researching embryos and early human development at the time. These pioneering studies ultimately lead to the birth of the first baby following *in vitro* fertilisation in 1978 by **Robert G. Edwards** (1925 to 2013) and **Patrick Steptoe** (1913 to 1988), for which Edwards was subsequently awarded the 2010 Nobel Prize in Physiology or Medicine.

Progress in reproductive biology, from a point in the past when the origins of human life were not understood to recent decades where oocyte maturation, fertilisation and early embryo development outside the body is feasible, represents a phenomenal achievement. Figure 1.5 documents the contribution Greek philosophers, scholars and scientists have made over two millennia to the gradual establishment of embryology as a progressive biological subject not only in human but also in animal reproduction.

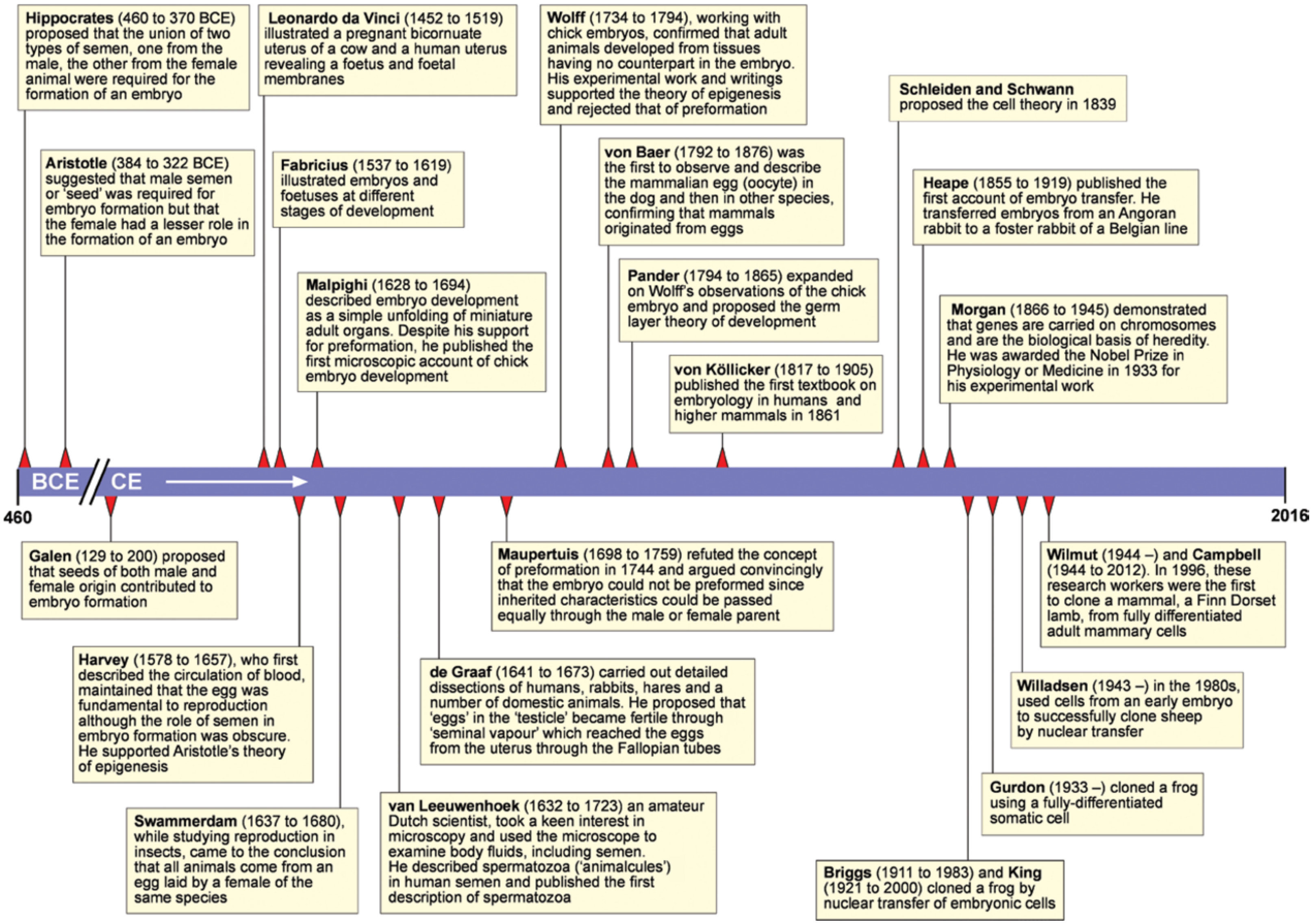

Figure 1.5 Timeline illustrating the contribution of key philosophers, scholars and scientists over two millennia to the gradual establishment of embryology as a progressive biological subject, not only in human but also in animal reproduction.

More than three decades after the birth of the first human baby following IVF, five million babies have been born employing this procedure. Currently, more than one million cattle embryos are transferred worldwide annually, and the possibilities for utilising advances in reproductive technology in the human population and in animal populations are vast. Rapid progress in the understanding of the underlying molecular and regulatory mechanisms that govern embryonic development, as well as the ability to alter the expression of individual genes for specific purposes, permit manipulations of embryos that the early pioneers in reproductive biology could never have imagined.

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Chapter 2

Division, growth and differentiation of cells

Key Points

- Somatic cell division consists of nuclear division, mitosis, which can be divided into four stages, prophase, metaphase, anaphase, telophase, followed by cytoplasmic division, cytokinesis.
- In somatic cells, the highly regulated cell cycle can be divided into four sequential phases, namely G_1, S, G_2 and M, and a quiescent phase, G_0.
- In germ cells, cell division referred to as meiosis takes place, where the daughter cells contain half the number of recombined chromosomes of the progenitor germ cell.
- During the first phase of meiosis a chiasma forms and there is a reciprocal exchange of genetic material between non-sister homologous chromatids.
- The non-disjunction of chromosomes during meiosis results in numerical alteration and structural defects in chromosomes.

The mammalian body is composed of an array of organs, tissues and individual cells which function in a specialised and highly coordinated manner. Although these cells, tissues and organs exhibit considerable diversity in both structure and function, they all derive from a single cell, a fertilised oocyte. The fertilised oocyte is the product of the fusion of two specialised reproductive cells, gametes, of male and female origin. Following fertilisation, the zygote undergoes a series of mitotic divisions which ultimately lead to the formation of totipotent stem cells, from which all cells, tissues and organs of the body arise.

Cells associated with tissue formation and regeneration are described as somatic cells. Specialised reproductive cells, referred to as germ cells, include gametes and their precursors of male and female origin.

Coordinated and regulated cell division is essential for embryonic development. Somatic cell division consists of nuclear division, mitosis, followed by cytoplasmic division, cytokinesis. In mitotic division of somatic cells, the daughter cells produced are genetically identical. A form of cell division distinctly different from mitosis occurs in germ cells. In this form of cell division, referred to as meiosis, the cells produced contain half the number of chromosomes of the progenitor germ cell and are not genetically identical. Somatic cell division combined with other cellular processes such as progressive differentiation, migration, adhesion, hypertrophy and apoptosis are prerequisites for embryonic development.

The cell cycle

As part of the cell cycle, somatic cells undergo a series of molecular and morphological changes. These changes occur in four sequential phases, namely G_1, S, G_2 and M, and also a quiescent phase, termed G_0 (Fig 2.1). The G_1 and G_2 phases are termed resting phases. In these phases, the cell is metabolically active, fulfilling its specialised function preparatory to the next phase of the cycle, but DNA replication does not take place. During the S phase, DNA synthesis takes place prior to chromosomal replication. This is followed by mitosis which occurs during the M phase. Collectively, the G_1, S and G_2 phases constitute the interphase (Fig 2.1). Cells which enter a G_0 state may remain transiently or permanently in that state. Certain fully differentiated cells, such as neurons, do not divide and continue to function permanently in a G_0 state. Other cell types, such as epithelial cells and hepatocytes, can re-enter the cell cycle from G_0 and proceed to mitotic division in response to appropriate stimuli.

A number of stimuli such as growth factors, mitogens and signals from other cells and from the extracellular matrix can induce cells in a G_0 state to re-enter the cell cycle near the end of the G_1 phase. Growth factors which bind to cell surface receptors activate intracellular signalling pathways. In most mammalian cells, the activation of genes encoding cyclins and cyclin-dependent kinases (CDKs) specific to the G_1 phase regulate the cell cycle and commit the cell to enter the S phase. This process is initiated at the restriction point, a stage at which mammalian cells become committed to entering the S phase and are then capable of completing the cell cycle independent of extracellular influences.

Veterinary Embryology, Second Edition. T.A. McGeady, P.J. Quinn, E.S. FitzPatrick, M.T. Ryan, D. Kilroy and P. Lonergan.

Companion website: www.wiley.com/go/mcgeady/veterinary-embryology

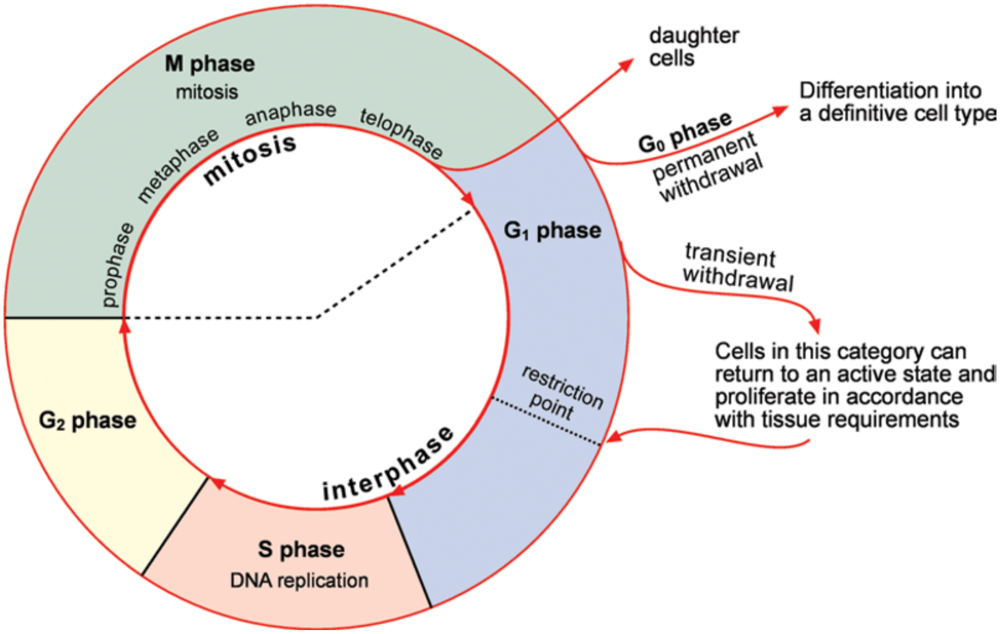

Figure 2.1 Stages in somatic cell division indicating the major phases of the cell cycle.

The rate of cell division varies in different cell types and at different stages of differentiation. Variations in cell cycle length are largely attributed to differences in the length of the G_1 phase, which can range from six hours to several days. Early embryonic development is characterised by rapid cell division but, as cells become more differentiated during organ development, the rate of cell division generally decreases.

Mitosis

The nuclei of somatic cells of each mammalian species have a defined number of chromosomes (Table 2.1). A somatic cell with a full complement of chromosomes is referred to as diploid and given the designation 2n. The term mitosis is used to describe nuclear division of somatic cells, a process which usually results in the production of two cells with the same chromosome complement as the progenitor cell from which they derived. Mitosis is essential for embryonic growth and development and for repair and replacement of tissue throughout life. The stages of mitosis occur as a distinct sequence of cytological events which are part of the cell cycle.

Table 2.1 The number of chromosomes in human and animal diploid cells.

Species	Number of chromosomes (2n)
Humans	46
Cats	38
Cattle	60
Chickens	78
Dogs	78
Donkeys	62
Goats	60
Horses	64
Pigs	38
Rabbits	44
Rats	42
Sheep	54

The stages of mitosis are usually followed by cytoplasmic division or cytokinesis (Fig 2.2 F).

Stages of mitosis

In preparation for mitosis, the chromosomes are replicated in the S phase of the cell cycle, forming sister chromatids. Within the nuclear envelope, sister chromatids remain attached at a constricted region of the chromosome called a centromere. Following the G_2 phase (Fig 2.2A), mitosis, which can be divided into four stages, prophase (Fig 2.2B), metaphase (Fig 2.2C), anaphase (Fig 2.2D) and, finally, telophase (Fig 2.2E), begins.

Prophase

The first stage of mitosis is prophase (Fig 2.2B). During this period, the chromosomes, consisting of closely associated sister chromatids, condense. Outside the nucleus, the centrosomes, composed of paired centrioles previously replicated during interphase, begin to form microtubule spindles or asters. The microtubule spindles facilitate the movement of the centrosomes to opposite poles of the dividing cell.

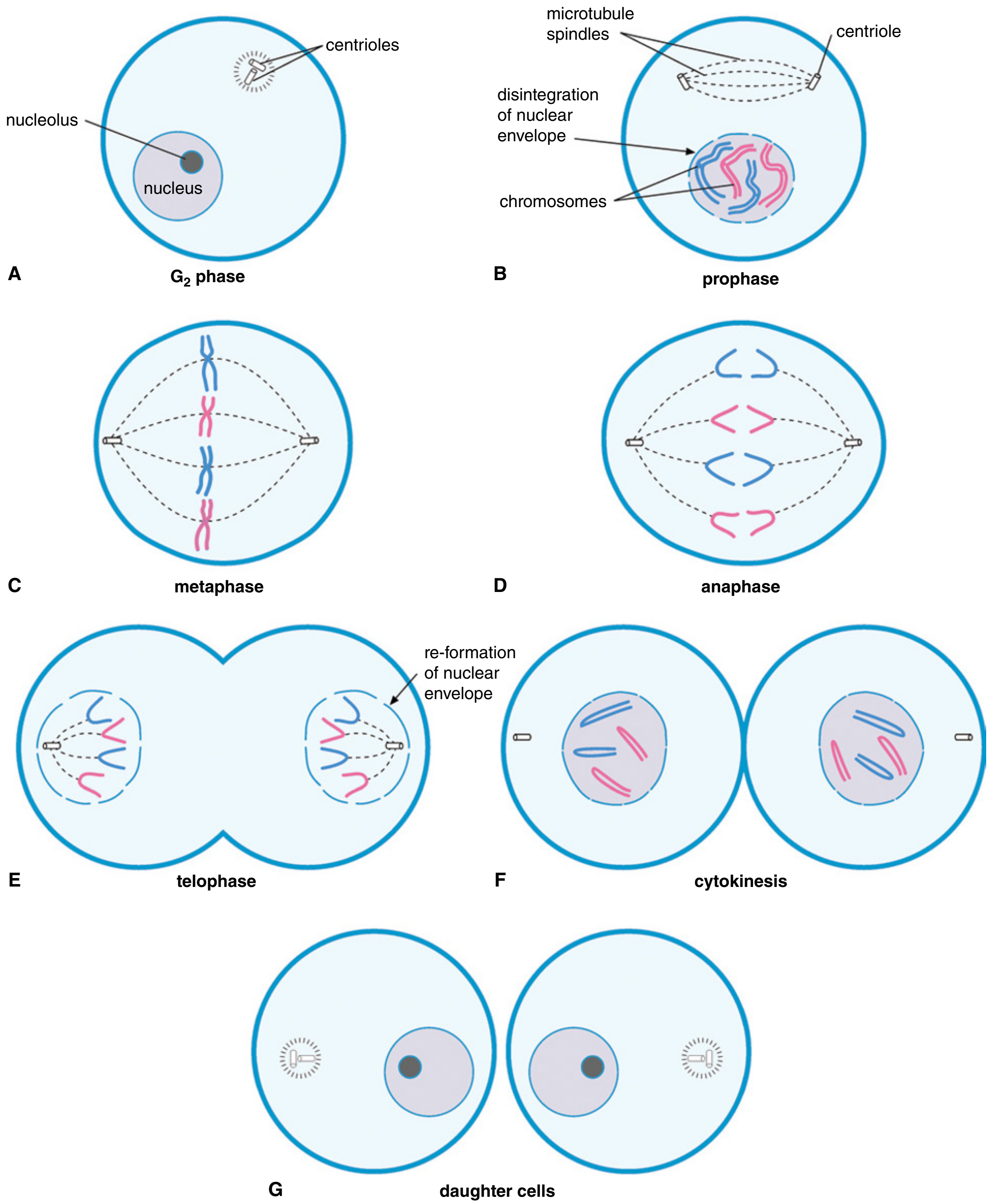

Figure 2.2 An outline of the sequential stages in mitosis (A to G). After the G_2 phase, prophase commences, followed by metaphase, anaphase, telophase and cytokinesis, leading to the formation of two daughter cells.

Microtubules, an essential component of the mitotic apparatus, are visible microscopically only during the M phase. Individual microtubules are cylindrical structures, composed of 13 parallel protofilaments consisting of alternating α-tubulin and β-tubulin subunits. An individual microtubule may grow or shrink by a process of polymerisation of α-tubulin and β-tubulin. A growing microtubule has a structure referred to as a guanidine triphosphate (GTP) cap. The β-subunit of a microtubule contains GTP which is hydrolysed to guanidine diphosphate (GDP). This, in turn, alters the conformation of the subunits, resulting in shrinking of the microtubules. If GTP hydrolysis occurs more rapidly than subunit addition, the cap is lost and the microtubule shrinks. Shrinking and growing are dynamic processes and these changes enable the microtubules to actively orientate and move chromosomes during mitosis and meiosis.

Metaphase

Events during the metaphase stage of mitosis can be divided into two phases, pro-metaphase and metaphase. Disintegration of the nuclear envelope marks the beginning of pro-metaphase. The kinetochore, a protein complex which forms on the centromeres during late prophase, acts as a platform for attachment to microtubules. Chromosomes attach to the microtubules via their kinetochores and the combination of these two latter structures is termed a kinetochore microtubule. Formation of the kinetochore microtubule enables chromosome movement to take place. During metaphase, the chromosomes are positioned midway between the poles of the cell at a region termed the metaphase plate. Each sister chromatid is attached to the centrosome by its kinetochore microtubule (Fig 2.2C). The initial capture of microtubules by the kinetochores is both asynchronous and stocastic and errors in attachment frequently occur. These transient and erroneous associations are corrected as mitosis progresses, where sister kinetochores become attached to microtubules from opposite spindle poles, supporting the faithful segregation of chromosomes. A core control network regulates the stability of the kinetochore–microtubule attachments and promotes error correction. Proteins comprising the core control network include SAC, PLK1, Aurora A and B kinases and cyclin-CDK.

Anaphase

During the anaphase stage, the pairs of conjoined sister chromatids synchronously separate as the centromeres split and the attached kinetochore microtubules shorten. The newly separated chromatid sets are drawn towards opposite poles of the cell (Fig 2.2D).

Telophase

The two groups of identical chromosomes (former chromatids), clustered at their respective poles, de-condense and a nuclear envelope forms around each set. The formation of nuclear envelopes marks the end of mitosis, a process which results in equal and symmetrical division of the nucleus (Fig 2.2E).

Cytokinesis

Following the formation of the nuclear envelope, a contractile ring of actin and myosin pinches the cell wall and divides the cytoplasm, resulting in the formation of two daughter cells (Figs 2.2F and 2.2G). This latter process, termed cytokinesis, typically results in the formation of two equally-sized daughter cells. Occasionally, unequal amounts of cytoplasm or organelles may be distributed to the daughter cells during cytokinesis. In some instances mitosis may occur without subsequent cytokinesis, resulting in the formation of binucleate or, occasionally, multinucleate cells.

In lower organisms, such as amphibians, the cytokinesis which occurs early in development can generate daughter cells in which the factors which direct the fate of the cells may not be uniformly distributed, resulting in differing developmental potential in individual daughter cells. In mammals, experimental evidence indicates that cell divisions which give rise to totipotent cells occur early in development. This suggests that, in mammals, cytoplasmic determinants are shared uniformly between daughter cells and that the initial stages of differentiation may arise as a result of cell communication and microenvironmental factors.

Regulation of mitosis

Close cooperation between cyclin-dependent kinases (CDK), cyclins and CDK inhibitors all ensure the orderly and regulated progression through the cell cycle. While there are many types of these enzymes (there are in excess of 20 members of the CDK family), concentrations of each change throughout the cell cycle, reflecting their different roles at each stage. The enzyme M-cyclin-dependent kinase (M-CDK) has a central role in the initiation of mitosis following the G_2 phase of the cell cycle. This heterodimeric protein, which is a complex of CDK1 and M-cyclin, is activated by the removal of inhibitory phosphate groups in the late G_2 phase. M cyclin concentrations rise as the cell begins to enter mitosis and peaks at metaphase. The M-CDK protein induces events essential for mitosis, including phosphorylation of the proteins which control microtubule dynamics, chromatin condensation, rearrangement of both the cytoskeleton and organelles and, finally, dissolution of the nuclear envelope. Although the mitotic cell cycle is normally highly regulated, undesirable alterations in the functioning of the genes known as proto-oncogenes or tumour suppressor genes, responsible for the control of cell proliferation or differentiation, may lead to malignant transformation of normal tissue. Typically, changes in two or more of these regulatory genes appear to be required for cells to undergo malignant transformation.

Mitotic division in successive generations of cells derived from a neoplastic cell continues to produce abnormal cells which are not subject to normal regulatory processes. Neoplastic conditions such as leukaemia, lymphoma and myeloma can arise from gene alteration within a single cell in the bone marrow or in peripheral lymphoid tissue. With the production and accumulation of large populations of abnormal cells, clinical effects of neoplasia become evident.