

Cambridge University Press
978-0-521-17851-8 - Infections, Infertility, and Assisted Reproduction
Kay Elder, Doris J. Baker and Julie A. Ribes
Excerpt
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Part I

Overview of microbiology

Introduction

History of microbiology

The history of microbiology, the scientific study of microorganisms, had its origins in the second half of the seventeenth century, when Anton van Leeuwenhoek (1632–1723), a tradesman in Delft, Holland, learned to grind lenses in order to make microscopes that would allow him to magnify and observe a wide range of materials and objects. Although he had no formal education and no knowledge of the scientific dogma of the day, his skill, diligence and open mind led him to make some of the most important discoveries in the history of biology. He made simple powerful magnifying glasses, and with careful attention to lighting and detail, built microscopes that magnified over 200 times. These instruments allowed him to view clear and bright images that he studied and described in meticulous detail. In 1673 he began writing letters to the newly formed Royal Society of London, describing what he had seen with his microscopes. He corresponded with the Royal Society for the next 50 years; his letters, written in Dutch, were translated into English or Latin and printed in the *Philosophical Transactions of the Royal Society*. These letters include the first descriptions of living bacteria ever recorded, taken from the plaque between his teeth, and from two ladies and two old men who had never cleaned their teeth:

I then most always saw, with great wonder, that in the said matter there were many very little living animalcules, very prettily a-moving. The biggest sort . . . had a very strong and swift motion, and shot through the water (or spittle) like a

pike does through the water. The second sort . . . oft-times spun round like a top . . . and these were far more in number . . . there were an unbelievably great company of living animalcules, a-swimming more nimbly than any I had ever seen up to this time. The biggest sort . . . bent their body into curves in going forwards . . . Moreover, the other animalcules were in such enormous numbers, that all the water . . . seemed to be alive.

His *Letters to the Royal Society* also included descriptions of free-living and parasitic protozoa, sperm cells, blood cells, microscopic nematodes, and a great deal more. In a Letter of June 12, 1716, he wrote:

. . . my work, which I’ve done for a long time, was not pursued in order to gain the praise I now enjoy, but chiefly from a craving after knowledge, which I notice resides in me more than in most other men. And therewithal, whenever I found out anything remarkable, I have thought it my duty to put down my discovery on paper, so that all ingenious people might be informed thereof.

At this time, around the turn of the eighteenth century, diseases were thought to arise by ‘spontaneous generation’, although it was recognised that certain clinically definable illnesses apparently did not have second or further recurrences. The ancient Chinese practice of preventing severe natural smallpox by ‘variolation’, inoculating pus from smallpox patients into a scratch on the forearm, was introduced into Europe in the early 1800s. The English farmer, Benjamin Justy, observed that milkmaids who were exposed to cowpox did not develop smallpox, and he inoculated his family with cowpox pus to prevent smallpox. Long before viruses had been recognized as an entity, and with no knowledge of their

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properties, the physician Edward Jenner (1749–1823) was intrigued by this observation, and started the first scientific investigations of smallpox prevention by human experimentation in 1796. Jenner used fluid from cowpox pustules on the hand of a milk-maid to inoculate the 8-year-old son of his gardener, and later challenged the boy by deliberately inoculating him with material from a real case of smallpox. The boy did not become infected, having apparently developed an immunity to smallpox from the cowpox vaccination. Jenner's early experiments led to the development of vaccination as protection against infectious disease and laid the foundations for the science of immunology, which was further developed during the nineteenth century by Pasteur, Koch, von Behring and Erlich.

The nineteenth century was a 'Golden Age' in the history of microbiology. The Hungarian doctor, Ignaz Semmelweiss (1818–1865), observed that puerperal fever often occurred when doctors went directly from the post-mortem room to the delivery room, and seldom occurred when midwives carried out deliveries. He thus introduced the notion that infectious agents might be transmitted, and suggested hand washing with chlorinated lime water. Significant discoveries about bacteria and the nature of disease were then made by Louis Pasteur, Joseph Lister, Paul Ehrlich, Christian Gram, R. J. Petri, Robert Koch (1843–1910) and others. Louis Pasteur and Robert Koch together developed the 'germ theory of disease', disproving the 'spontaneous generation' theory held at the time. Louis Pasteur (1822–1895) developed the scientific basis for Jenner's experimental approach to vaccination, and produced useful animal and human vaccines against rabies, anthrax and cholera. In 1876 Robert Koch provided the first proof for the 'germ theory' with his discovery of *Bacillus anthracis* as the cause of anthrax. Using blood from infected animals, he obtained pure cultures of the bacilli by growing them on the aqueous humour of an ox's eye. He observed that under unfavourable conditions the bacilli could form rounded spores that resisted adverse conditions, and these began to grow again as bacilli when suitable conditions were restored. Koch went on to invent new methods of cultivating

bacteria on solid media such as sterile slices of potato and on agar kept in the recently invented Petri dish. In 1892 he described the conditions, known as Koch's Postulates, which must be satisfied in order to define particular bacteria as a cause of a specific disease:

- (i) The agent must be present in every case of the disease.
- (ii) The agent must be isolated from the host and grown in vitro.
- (iii) The disease must be reproduced when a pure culture of the agent is inoculated into a healthy susceptible host.
- (iv) The same agent must be recovered once again from the experimentally infected host.

Koch's further significant contributions to microbiology included work on the tubercle bacillus and identifying *Vibrio* as a cause of cholera, as well as work in India and Africa on malaria, plague, typhus, trypanosomiasis and tickborne spirochaete infections. He was awarded the Nobel Prize for Physiology or Medicine in 1905, and continued his work on bacteriology and serology until his death in 1910.

During the 1800s, agents that caused diseases were being classified as filterable – small enough to pass through a ceramic filter (named 'virus' by Pasteur, from the Latin for 'poison') – or non-filterable, retained on the surface of the filter (bacteria). Towards the end of the century, a Russian botanist, Dmitri Iwanowski, recognized an agent (tobacco mosaic virus) that could transmit disease to other plants after passage through ceramic filters fine enough to retain the smallest known bacteria. In 1898 Martinus Beijerinck confirmed and developed Iwanowski's observations, and was the first to describe a virus as *contagium vivum fluidum* (soluble living germ). In 1908 Karl Landsteiner and Erwin Popper proved that poliomyelitis was caused by a virus, and shortly thereafter (1915–1917), Frederick Twort and Felix d'Herelle independently described viruses that infect bacteria, naming them 'bacteriophages'. These early discoveries laid the foundation for further studies about the properties of bacteria and viruses, and, more significantly, about cell genetics and the transfer of genetic information between cells. In the 1930s, poliovirus was grown in cultured

cells, opening up the field of diagnostic virology. By the 1950s, plasmids were recognized as extranuclear genetic elements that replicate autonomously, and Joshua Lederberg and Norton Zinder reported on transfer of genetic information by viruses (Zinder & Lederberg, 1952).

Following the announcement of the DNA double helix structure by Watson and Crick in 1953, the properties of bacteria, bacteriophages and animal viruses were fully exploited and formed the basis of a new scientific discipline: molecular biology, the study of cell metabolic regulation and its genetic machinery. Over the next 20 years, *Escherichia coli* and other bacterial cell-free systems were used to elucidate the molecular steps and mechanisms involved in DNA replication, transcription and translation, and protein synthesis, assembly and transport. The development of vaccines and antimicrobial drugs began during the 1950s, and antibiotic resistance that could be transferred between strains of bacteria was identified by 1959 (Ochiai *et al.*, 1959). In 1967, Thomas Brock identified a thermophilic bacterium *Thermus aquaticus*; 20 years later, a heat-stable DNA polymerase was isolated from this bacterium and used in the polymerase chain reaction (PCR) as a means of amplifying nucleic acids (Brock, 1967; Saiki *et al.*, 1988). Another significant advance in molecular biology came with the recognition that bacteria produce restriction endonuclease enzymes that cut DNA at specific sites, and in 1972 Paul Berg constructed a recombinant DNA molecule from viral and bacterial DNA using such enzymes (Jackson *et al.*, 1972). The concept of gene splicing was reported by 1977, and in that same year Frederick Sanger and his colleagues elucidated the complete nucleotide sequence of the bacteriophage ϕ X174, the first microorganism to have its genome sequenced (Sanger *et al.*, 1977). Berg, Gilbert and Sanger were awarded the Nobel Prize for Chemistry in 1980.

These discoveries involving microorganisms established the foundation for genetic engineering. Gene cloning and modification, recombinant DNA technology and DNA sequencing established biotechnology as a new commercial enterprise: by 2002 the biotechnology industry had worldwide

drug sales in excess of 10 billion US dollars. The first genetically engineered human protein, insulin, was available by 1982, and the first complete genome sequence of a bacterium, *Haemophilus influenzae*, was published in 1995. Hormones and other proteins manufactured by recombinant DNA technology are now used routinely to treat a variety of diseases, and recombinant follicle stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotrophin (hCG) are available for routine use in assisted reproductive technology.

A parallel line of investigation that was also a key feature in molecular biology and medicine during the latter part of the twentieth century came from the study of retroviruses, novel viruses that require reverse transcription of RNA into DNA for their replication. During the 1960s, Howard Temin and David Baltimore independently discovered viral reverse transcription, and in 1969 Huebner and Todaro proposed the viral oncogene hypothesis, subsequently expanded and confirmed by Bishop and Varmus in 1976. They identified oncogenes from Rous sarcoma virus that are also present in cells of normal animals, including humans. Proto-oncogenes are apparently essential for normal development, but can become cancer-causing oncogenes when cellular regulators are damaged or modified. Bishop and Varmus were awarded the Nobel Prize for Medicine or Physiology in 1989. In 1983, Luc Montagnier discovered a retrovirus believed to cause the acquired immune deficiency syndrome (AIDS) – the human immunodeficiency virus (HIV). By the end of the twentieth century, the total number of people affected by this novel virus exceeded 36 million.

Around this same time, another novel pathogen of a type not previously described also came to light: in 1982 Stanley Prusiner discovered that scrapie, a transmissible spongiform encephalopathy (TSE) in sheep, could be transmitted by a particle that was apparently composed of protein alone, with no associated nucleic acid – the prion protein (Prusiner, 1982). This was the first time that an agent with neither DNA or RNA had been recognized as pathogenic, challenging previous dogmas about disease pathogenesis and transmission.

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The field of microbiology continues to grow and elicit public concern, both in terms of disease pathology and in harnessing the properties of microbes for the study of science, especially molecular genetics. During the past 25–30 years, approximately 30 new pathogens have been identified, including HIV, hemorrhagic viruses such as Ebola, transfusion-related hepatitis C-like viruses, and, most recently, the coronavirus causing sudden acute respiratory syndrome (SARS). The first SARS outbreak occurred in the Guangdong province of China in November 2002 and had spread as a major life-threatening pneumonia in several countries by March 2003. The infectious agent was identified during that month, and a massive international collaborative effort resulted in elucidating its complete genome sequence only 3 weeks later, in mid-April 2003. The genome sequence reveals that the SARS virus is a novel class of coronavirus, rather than a recent mutant of the known varieties that cause mild upper respiratory illness in humans and a variety of diseases in other animals. Information deduced from the genome sequence can form the basis for developing targeted antiviral drugs and vaccines, and can help develop diagnostic tests to speed efforts in preventing the global epidemic of SARS. At the beginning of June 2003, 6 months after the first recorded case, the World Health Organization reported 8464 cases from more than two dozen countries, resulting in 799 deaths.

These new diseases are now being defined within a context of ‘emergent viruses’, and it is clear that new infectious diseases may arise from a combination of different factors that prevail in modern society:

- (i) New infectious diseases can emerge from genetic changes in existing organisms (e.g. SARS ‘jumped’ from animal hosts to humans, with a change in its genetic make-up), and this ‘jump’ is facilitated by intensive farming and close and crowded living conditions.
- (ii) Known diseases may spread to new geographic areas and populations (e.g. malaria in Texas, USA).
- (iii) Previously unknown infections may appear in humans living or working in changing ecologic conditions that increase their exposure to insect

vectors, animal reservoirs, or environmental sources of novel pathogens, e.g. prions.

- (iv) Modern air transportation allows large numbers of people, and hence infectious disease, to travel worldwide with hitherto unprecedented speed.

Other areas that can contribute to pathogen emergence include events in society such as war, civil conflict, population growth and migration, as well as globalization of food supplies, with changes in food processing and packaging. Environmental changes with deforestation/reforestation, changes in water ecosystems, flood, drought, famine, and global warming can significantly alter habitats and exert evolutionary pressures for microbial adaptation and change. Human behaviour, including sexual behaviour, drug use, travel, diet, and even use of child-care facilities have contributed to the transmission of infectious diseases. The use of new medical devices and invasive procedures, organ or tissue transplantation, widespread use of antibiotics and drugs causing immunosuppression have also been instrumental in the emergence of illness due to opportunistic pathogens: normal microbial flora such as *Staphylococcus epidermidis* cause infections on artificial heart valves, and saprophytic fungi cause serious infection in immunocompromised patients.

Microorganisms can restructure their genomes in response to environmental pressures, and during replication there is an opportunity for recombination or re-assortment of genes, as well as recombination with host cell genetic elements. Some viruses (e.g. HIV) evolve continuously, with a high frequency of mutation during replication. Retroviruses are changing extraordinarily rapidly, evolving sporadically with unpredictable patterns, at different rates in different situations. Their genetic and metabolic entanglement with cells gives retroviruses a unique opportunity to mediate subtle, cumulative evolutionary changes in host cells. Viruses that are transmitted over a long time period (HIV) have a selective advantage even when their effective transmission rates are relatively low.

Assisted reproduction techniques are now being used to help people who carry infectious diseases

(including those that are potentially fatal and may have deleterious effects on offspring) to have children. This potential breach of evolutionary barriers raises new ethical, policy and even legal issues that must be dealt with cautiously and judiciously (Minkoff & Santoro, 2000).

History of assisted reproduction

Assisted reproduction may also be said to have its origin in the seventeenth century, when Anton van Leeuwenhoek first observed sperm under the microscope and described them as 'animalcules'. In 1779, Lazzaro Spallanzani (1729–1799), an Italian priest and scientist was the first to propose that contact between an egg and sperm was necessary for an embryo to develop and grow. He carried out artificial insemination experiments in dogs, succeeding with live births, and went on to inseminate frogs and fish. Spallanzani is also credited with some of the early experiments in cryobiology, keeping frog, stallion and human sperm viable after cooling in snow and re-warming. The Scottish surgeon, John Hunter (1728–1793), was the first to report artificial insemination in humans, when he collected sperm from a patient who suffered from hypospadias and injected it into his wife's vagina with a warm syringe. This procedure resulted in the birth of a child in 1785. The next documented case of artificial insemination in humans took place in 1884 at Jefferson Medical College in Philadelphia, USA:

A wealthy merchant complained to a noted physician of his inability to procreate and the doctor took this as a golden opportunity to try out a new procedure. Some time later, his patient's wife was anaesthetised. Before an audience of medical students, the doctor inseminated the woman, using semen obtained from 'the best-looking member of the class'. Nine months later, a child was born. The mother is reputed to have gone to her grave none the wiser as to the manner of her son's provenance. The husband was informed and was delighted. The son discovered his unusual history at the age of 25, when enlightened by a former medical student who had been present at the conception. (Hard, AD, Artificial Impregnation, *Medical World*, 27, p. 163, 1909)

By the turn of the nineteenth century, the use of artificial insemination in rabbits, dogs and horses had been reported in several countries. In 1866 an Italian physician, Paolo Mantegazza, suggested that sperm could be frozen for posthumous use in humans and for breeding of domestic animals, and in 1899 the Russian biologist Ivanoff reported artificial insemination (AI) in domestic farm animals, dogs, foxes, rabbits and poultry. He developed semen extenders, began to freeze sperm and to select superior stallions for breeding. His work laid the foundation for the establishment of artificial insemination as a veterinary breeding technique.

Around this same time in Cambridge UK, the reproductive biologist, Walter Heape, studied the relationship between seasonality and reproduction. In 1891 he reported the recovery of a pre-implantation embryo after flushing a rabbit oviduct and transferring this to a foster mother with continued normal development (Heape, 1891). His work encouraged others to experiment with embryo culture; in 1912 Alain Brachet, founder of the Belgian School of Embryology, succeeded in keeping a rabbit blastocyst alive in blood plasma for 48 hours. Pregnancies were then successfully obtained after flushing embryos from a number of species, from mice and rabbits to sheep and cows. Embryo flushing and transfer to recipients became a routine in domestic animal breeding during the 1970s.

Artificial insemination

By 1949, Chris Polge in Cambridge had developed the use of glycerol as a semen cryoprotectant, and the process of semen cryopreservation was refined for use in cattle breeding and veterinary practice. The advantages of artificial insemination were recognized: genetic improvement of livestock, decrease in the expense of breeding, the potential to increase fertility, and a possible disease control mechanism. Almquist and his colleagues proposed that bacterial contaminants in semen could be controlled by adding antibiotics to bovine semen (Almquist *et al.*, 1949). The practice of artificial insemination was

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soon established as a reproductive treatment in humans. Methods for cryopreserving human semen and performing artificial insemination were refined in the early 1950s (Sherman & Bunge, 1953), and a comprehensive account of Donor Insemination was published in 1954 (Bunge *et al.*, 1954). By the mid-1980s, however, it became apparent that donor insemination had disadvantages as well as advantages, including the potential to transmit infectious diseases. Before rigorous screening was introduced, HIV, *Chlamydia*, Hepatitis B and genital herpes were spread via donor semen (Nagel *et al.*, 1986; Berry *et al.*, 1987; Moore *et al.*, 1989; McLaughlin, 2002).

In vitro fertilization

Advances in reproductive endocrinology, including identification of steroid hormones and their role in reproduction, contributed significantly to research in reproductive biology during the first half of the twentieth century. During the 1930s–40s, the pituitary hormones responsible for follicle growth and luteinization were identified, and a combination of FSH and LH treatments were shown to promote maturation of ovarian follicles and to trigger ovulation. Urine from postmenopausal women was found to contain high concentrations of gonadotrophins, and these urinary preparations were used to induce ovulation in anovulatory patients during the early 1950s.

Parallel relevant studies in gamete physiology and mammalian embryology were underway by this time, with important observations reported by Austin, Chang and Yanagimachi. In 1951, Robert Edwards began working towards his Ph.D. project in Edinburgh University's Department of Animal Genetics headed by Professor Conrad Waddington and under the direction of Alan Beatty. Here he began to pursue his interest in reproductive biology, studying sperm and eggs, and the process of ovulation in the mouse. After 1 year spent in Pasadena at the California Institute of Technology working on problems in immunology and embryology, in 1958 he joined the MRC in Mill Hill, where he worked with

Alan Parkes and Bunny Austin, continuing to explore his interest in genetics, mammalian oocytes and the process of fertilization. During this period he started expanding his interests into human oocyte maturation and fertilization, and with the help of the gynaecologist Molly Rose began to observe human oocytes retrieved from surgical biopsy specimens. In 1962 he observed spontaneous resumption of meiosis in a human oocyte in vitro for the first time. After a brief period in Glasgow with John Paul, he was appointed as a Ford Foundation Fellow in the Physiological Laboratory in Cambridge in 1963. By this time Chang (1959) had successfully carried out in vitro fertilization with rabbit oocytes and sperm, and Yanagimachi (1964) subsequently reported successful IVF in the golden hamster. Whittingham was working towards fertilization of mouse eggs in vitro, and reported success in 1968. In Cambridge, Bob Edwards began on the slow and arduous road that eventually led to successful human in vitro fertilization. He continued his studies using the limited and scarce material available from human ovarian biopsy and pathology specimens, and published his observations about maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes (Edwards, 1965). Working with several Ph.D. students, Edwards fertilized mouse and cow eggs in vitro, and obtained mouse offspring. With his students, he studied the growth of chimaeric embryos constructed by injecting a single or several inner cell mass cells from donor blastocysts into recipient genetically marked blastocysts. The birth of live chimaeras confirmed the capacity of single stem cells to colonize virtually all organs in the recipient, including germline, but not trophoctoderm. They also removed small pieces of trophoctoderm from live rabbit blastocysts and determined their sex by identifying whether they expressed the sex chromatin body. Embryos with this body were classified as females and the others as males. The sex of fetuses and offspring at birth had been correctly diagnosed, signifying the onset of preimplantation genetic diagnosis for inherited characteristics. At this time, anomalies observed in some rabbit offspring caused them some concern, but a large study by Chang on in vitro fertilization in mice proved that the anomalies were due

to the segregation of a recessive gene, and not due to IVF.

In 1968 Edwards began his historic collaboration with Patrick Steptoe, the gynaecologist who pioneered and introduced the technique of pelvic laparoscopy in the UK. Using this new technique in his clinical practice in Oldham General Hospital near Manchester, Steptoe was able to rescue fresh pre-ovulatory oocytes from the pelvis of patients who suffered from infertility due to tubal damage. Bob Edwards and his colleague, Jean Purdy, traveled from Cambridge to Oldham in order to culture, observe and fertilize these oocytes in vitro. The team began to experiment with culture conditions to optimize the in vitro fertilization system, and tried ovarian stimulation with drugs in order to increase the number of oocytes available for fertilization. After observing apparently normal human embryo development to the blastocyst stage in 1970, they began to consider re-implanting embryos created in vitro into the uteri of patients in order to achieve pregnancies: the first human embryo transfers were carried out in 1972. Despite the fact that their trials and experiments were conducted in the face of fierce opposition and criticism from their peers at the time, they continued to persevere in their efforts, with repeated failure and disappointment for the next 6 years. Finally, their 10 years of collaboration, persistence and perseverance were rewarded with the successful birth of the first IVF baby in 1978.

The modern field of assisted reproductive technology (ART) arrived with the birth of Louise Brown on July 25, 1978. After a 2-year lag, when no funds or facilities were available to continue their pioneering work, Steptoe and Edwards opened a private clinic near Cambridge: Bourn Hall Clinic, dedicated solely to treating infertility patients using in vitro fertilization and embryo transfer. The first babies were conceived within days, and many more within 3 months. Their rapid clinical success in achieving pregnancies and live births led to the introduction of IVF treatment worldwide throughout the 1980s and 1990s. The first babies born after transfer of embryos that had been frozen and thawed were born in 1984–85, and cryopreservation of embryos as well as semen became routine. Experiments with cell cultures and

co-culture allowed the development of stage-specific media optimized for embryo culture to the blastocyst stage. Advances in technique and micromanipulation technology led to the establishment of assisted fertilization (intracytoplasmic sperm injection, ICSI) by a Belgian team led by André Van Steirteghem and including Gianpiero Palermo (Palermo *et al.*, 1992) by the mid 1990s. Other microsurgical interventions were then introduced, such as assisted hatching and embryo biopsy for genetic diagnosis. Gonadal tissue cryopreservation, in vitro oocyte maturation and embryonic stem cell culture are now under development as therapeutic instruments and remedies for the future.

The first live calves resulting from bovine IVF were born in the USA in 1981. This further milestone in reproductive biotechnology inspired the development of IVF as the next potential commercial application of assisted reproduction in domestic species, following on from AI and conventional transfer of embryos produced in vivo from superovulated donors. The assisted reproductive techniques continued to be refined so that by the 1990s IVF was integrated into routine domestic species breeding programmes. Equine IVF has also been introduced into the world of horse breeding (although reproductive technology procedures cannot be used for thoroughbreds). China used artificial insemination to produce the first giant panda cub in captivity in 1963, and assisted reproduction is now used in the rescue and propagation of endangered species, from pandas and large cats to dolphins. Artificial insemination, and, in some cases, in vitro fertilization are used routinely in specialist zoos throughout the world.

In the field of scientific research, application of assisted reproductive techniques in animal systems has helped to unravel the fundamental steps involved in fertilization, gene programming and expression, regulation of the cell cycle and patterns of differentiation. Somatic cell nuclear transfer into enucleated oocytes has created 'cloned' animals in several species, the most famous being Dolly the sheep, who was born in Edinburgh in 1997; Dolly was euthanized at an early age in February 2003, after developing arthritis and progressive lung disease.

Advances in molecular biology and biotechnology continue to be applied in ART. Preimplantation genetic diagnosis (PGD), introduced in 1988, is used to screen embryos for sex-linked diseases or autosomal mutations in order to exclude chromosomally abnormal embryos from transfer. Molecular biology techniques can identify chromosomes with the use of fluorescently labelled probes for hybridization, or to amplify DNA from a single blastomere using PCR. This technique is also used for gender selection, now used routinely in animal breeding programmes.

Assisted reproductive technology (ART) and microbiology

Assisted reproduction is a multidisciplinary field that relies on close teamwork and collaboration between different medical and scientific disciplines that must

cover a wide range of expertise, including microbiology. Culture media and systems used to process and culture gametes and embryos are designed to encourage cell growth, but this system can also encourage the growth of a wide variety of microbes. Whereas cell division in preimplantation embryos is relatively slow, with each cell cycle lasting approximately 24 hours, microbes can multiply very rapidly under the right conditions. Under constant conditions the generation time for a specific bacterium is reproducible, but varies greatly among species. The generation time for some bacteria is only 15–20 minutes; others have generation times of hours or even days. Spore-forming organisms have the ability to go into ‘suspended animation’, allowing them to withstand extreme conditions (freezing, high temperatures, lack of nutrients). This important property allows the organisms to survive in central heating and air conditioning systems or cooling towers

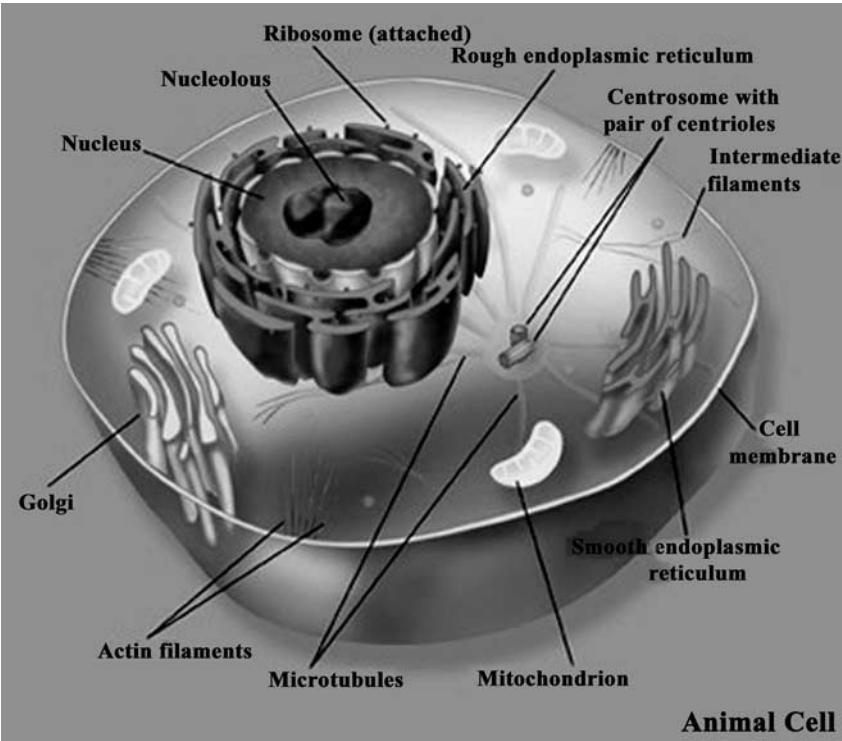


Fig. 1.1. Schematic diagram of a typical eukaryotic cell, illustrating characteristic intracellular organelles.

for indefinite periods: the 'Ice Man' fungus survived at least 5300 years, and live bacterial spores were found in ancient pressed plants at Kew Gardens dating back to the seventeenth century. Viruses do not form spores, but some can survive dry on handkerchiefs, or cleaning or drying cloths if protein is present, e.g. in droplets from a sneeze or cough. These properties presents special problems in the ART laboratory since many agents used to inhibit or destroy microorganisms are toxic to sperm, oocytes and embryos. An ART laboratory must incorporate strict guidelines for maintaining necessary sterile conditions without compromising the gametes and embryos.

Patients presenting for infertility treatment often have a background of infectious disease as a factor in their infertility, and it is now acknowledged that some chronic infectious diseases may be 'silent' or inapparent, but transmissible in some patients (e.g. Herpes, *Ureaplasma*, *Chlamydia*). Some patients may be taking antimicrobial drugs that will have a negative effect on gamete function: for example, bacterial protein synthesis inhibitors affect sperm mitochondrial function, adversely affecting sperm motility. The trend for worldwide travel also introduces new contacts and potentially infectious agents across previous geographic barriers.

There are many factors to be taken into account when assessing the risk from microorganisms, and some that are unique to ART are complex and multifaceted. Formal assessment for quantifying risk requires experimental study data, epidemiological information, population biology and mathematical modelling – this is not possible in assisted reproductive practice. Instead, ART laboratory practitioners need to collate, review and evaluate relevant information, bearing in mind that ART practises breach biological barriers, increasing the risk:

Wisdom lies in knowing what one is doing and why one is doing it – to take liberties in ignorance is to court disaster – each fragment of knowledge teaches us how much more we have yet to learn. (John Postgate *Microbes and Man*, 2000)

Chance favours the prepared mind (Louis Pasteur, 1822–1895)

Overview of microbiology

Naturam primum cognoscere rerum

First . . . to learn the nature of things (Aristotle, 384–322 BC)

Living things have been traditionally classified into five biological Kingdoms: Animals (Animalia), Plants (Plantae), Fungi (Fungi), Protozoa (Protista) and Bacteria (Monera). Animals, plants, fungi and protozoa are eukaryotic, with nuclei, cytoskeletons, and internal membranes (see Fig. 1.1). Their chromosomes undergo typical reorganization during cell division. Bacteria are prokaryotes, i.e. they have no well-defined nucleus or nuclear membrane and divide by amitotic division (binary fission) (Fig. 1.2). The world of microbes covers a wide variety of different organisms within the Kingdoms of Fungi, Protista and Monera, with a diverse range

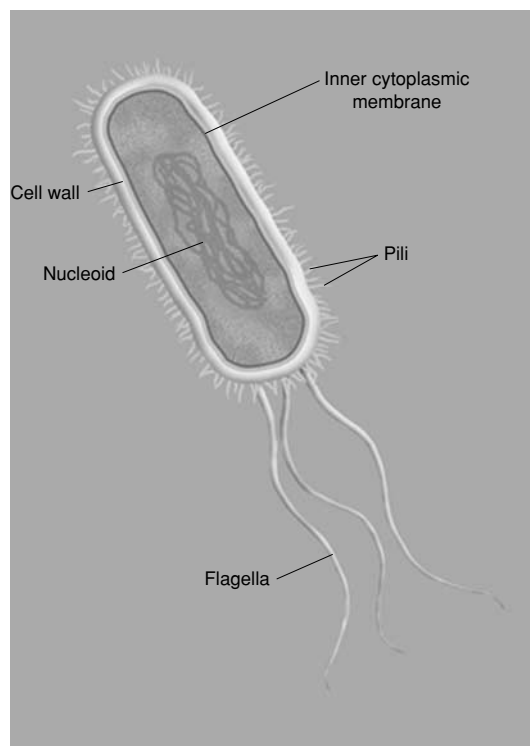


Fig. 1.2. Schematic diagram of a typical prokaryotic (bacterial) cell, illustrating the nucleoid, inner cytoplasmic membrane, cell wall, pili and flagella.