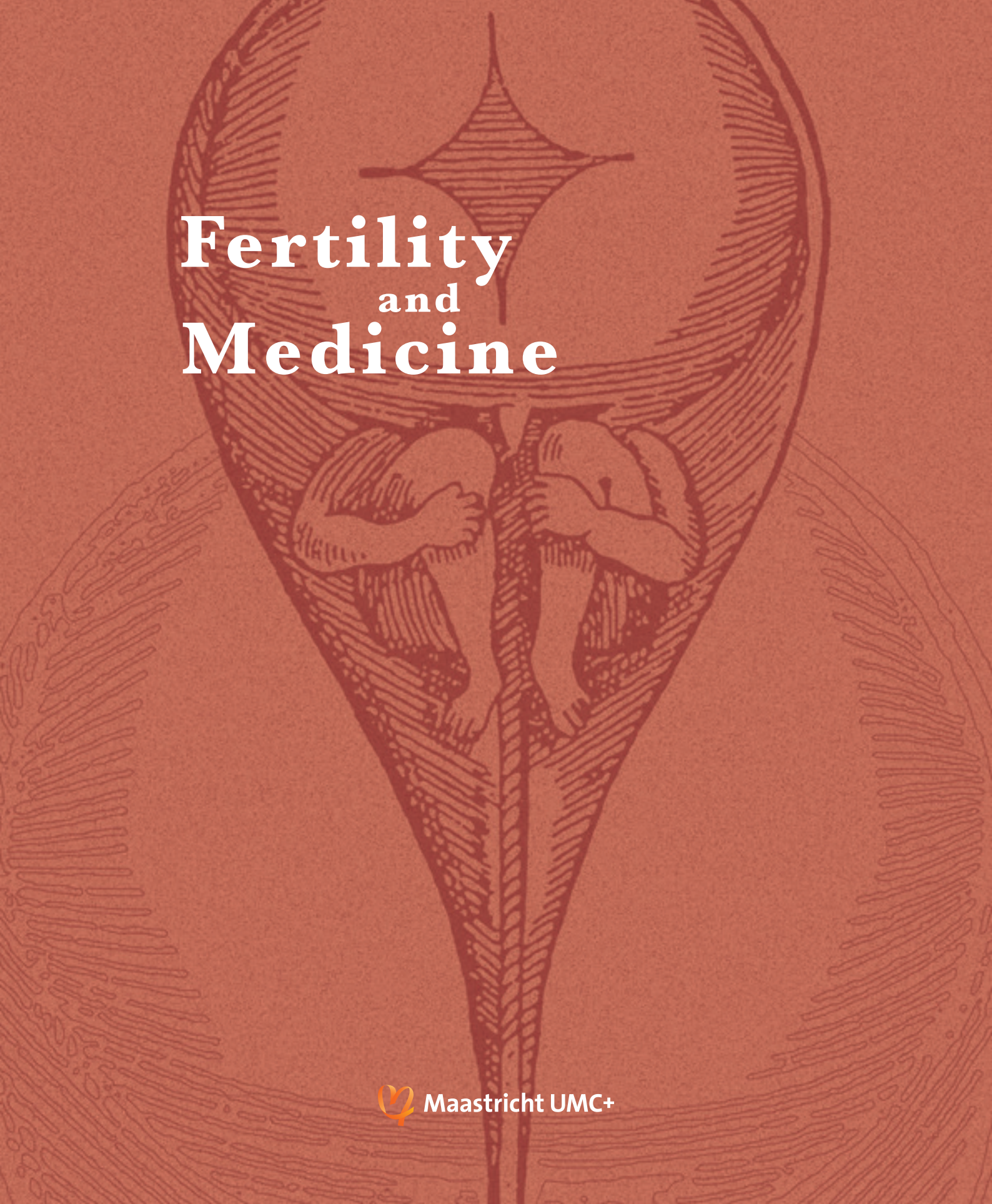
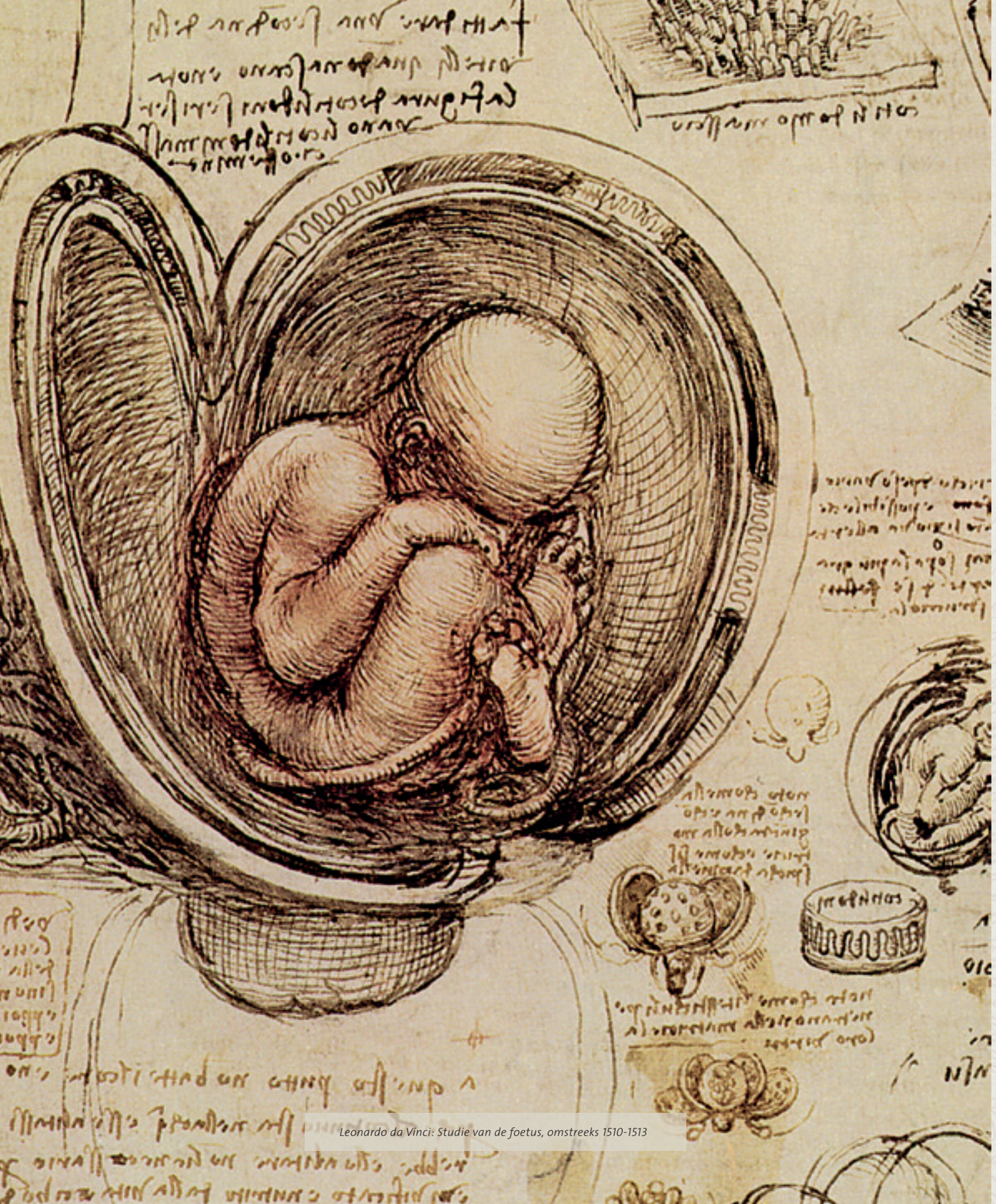


Fertility and Medicine

Maastricht UMC+



Leonardo da Vinci: Studie van de foetus, omstreeks 1510-1513

Fertility and Medicine

* Scientific thinking about fertility and reproduction
* Heredity, genes and genetic disorders
* Pregnancy tests through the ages
* Involuntary childlessness
* Medical treatments for childlessness and genetic diseases
* Medically assisted reproduction and ethics

Published to accompany the exhibition ‘Fertility and Medicine’
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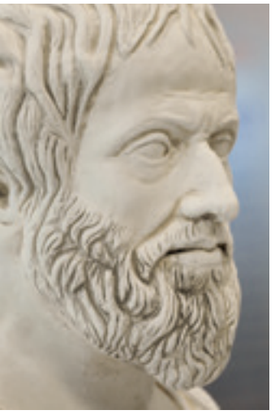
Compiled by Eddy Houwaart, Frans Ramaekers, Hans Evers and Guido de Wert on behalf of the Maastricht UMC+ Medical History Working Group.
In conjunction with Museum Boerhaave, Joep Geraedts and John Dumoulin.
Design and realisation: Maastricht UMC+ Graphic Service

Foreword

As the cornerstones of humanity’s continued existence, fertility and pregnancy are understandably both a source of awe and an important driver of research. Over the centuries, Dutch scientists have made vital contributions to knowledge in this field. Reinier de Graaf and Jan Swammerdam, for instance, became famous for documenting the female reproductive organs and the follicles that hold egg cells, while Antoni van Leeuwenhoek was the first scientist to describe the sperm cell.
Yet pregnancy is not something we can take for granted. Involuntary childlessness is an age-old problem, but these days in vitro fertilisation (IVF) has made it possible to give nature a helping hand. Genetic embryo selection after IVF, meanwhile, can guard against hereditary anomalies in our children, though there are ethical dilemmas. This exhibition seeks to place advances in knowledge about fertility in a historical perspective and to illustrate how medical and genetic developments at Maastricht UMC+ have helped to pave the way for medically assisted reproduction and DNA analysis for embryo selection. It also highlights the ethical and political issues raised by these developments.

On behalf of the MUMC+ Medical History Working Group:
Eddy Houwaart, professor of Medical History in the Metamedica Department, and Frans Ramaekers, professor of Molecular Cell Biology and scientific director of the GROW School for Oncology & Developmental Biology.

Scientific thinking about fertility and reproduction



Aristotle (384-222 BC.)
The ideas of the Greek natural scientist and philosopher Aristotle shaped European approaches to biology up through the seventeenth century.

The desire to understand the miracle of new life in humans as well as animals goes all the way back to the ancient Greeks. Western medicine's founding father, Hippocrates (460 BC), postulated the existence of two semens, male and female, which he thought the mother and father produced by way of their spinal cords, kidneys and reproductive organs. When two partners' seminal fluids mixed during sexual intercourse, an embryo could result. The proportion of male to female semen determined the sex of the child. More influential were the insights of the natural scientist and philosopher Aristotle (384-222 BC). Rejecting the idea of female semen, Aristotle said women's menstrual blood was the basic substance from which embryos were formed, while male sperm was merely the spark that triggered the transformation, or metamorphosis, of blood into an embryo. Subsequent events inside the uterus progressed in a kind of clockwork leading inexorably to a complete foetus (a process called epigenesis).

1 2 3

Conception according to Aristotle
Sixteenth-century illustrations of the Aristotelian idea that women's menstrual blood was the basic substance from which embryos were formed.
Fig. 1 Six days after fertilisation, blood vessels and small white patches that will become the liver, heart and brains begin to form on the surface of the yolky mass of blood and semen.
Fig. 2 After twelve days, the blood vessels reveal the outline of a human being.
Fig. 3 After eighteen days the foetus is fully formed and needs only to grow.

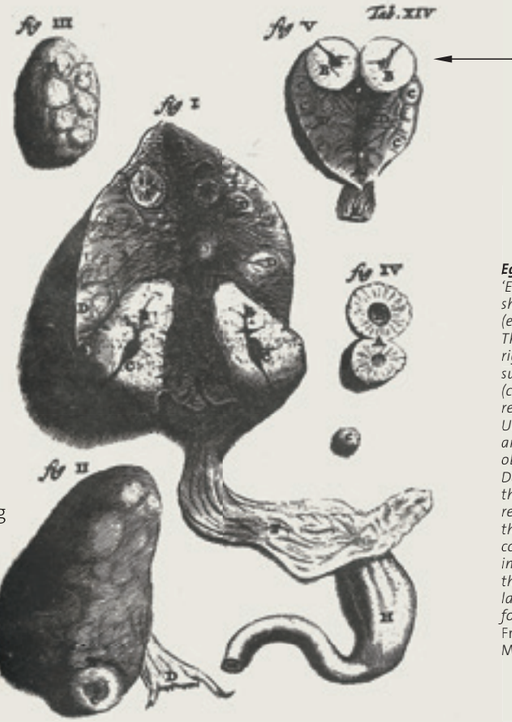
From: Jacob Rueff, De Conceptu et Generatione Hominis. Zurich, 1554.

The egg cell and the sperm cell

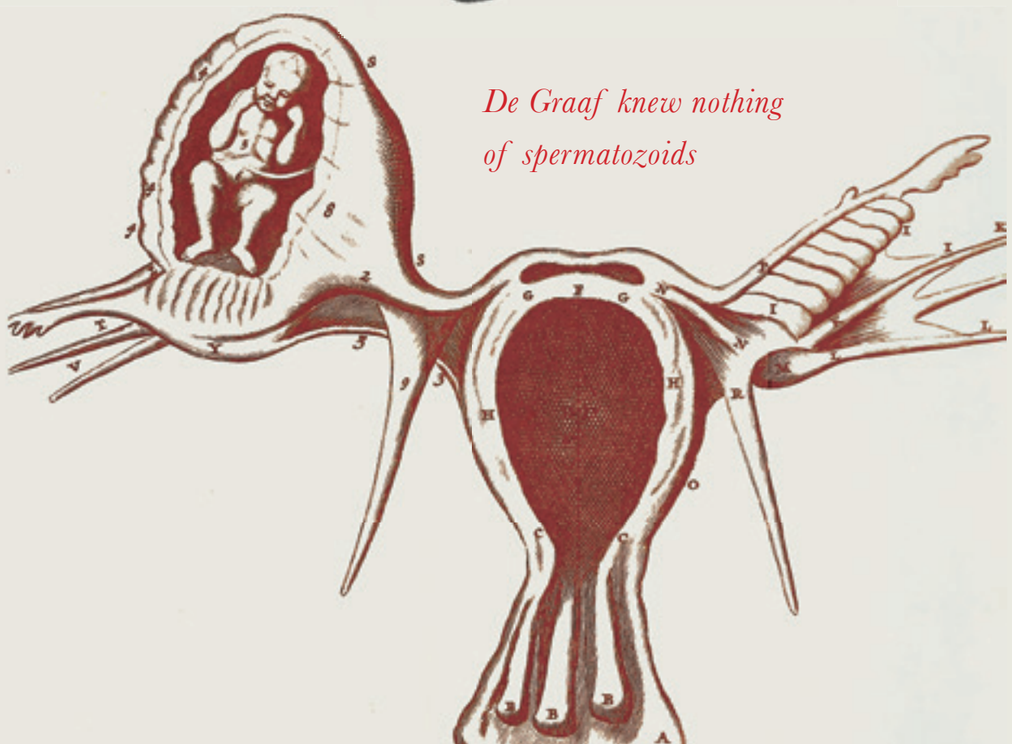
Aristotelian views shaped the world of natural scientific research up through the seventeenth century, when the first modern anatomical studies of female reproductive organs were published. In the years after 1600 there was mounting scientific criticism of the Aristotelian concepts of metamorphosis and epigenesis. There were serious doubts, for example, about the role of the so-called female testes. Ancient physicians such as Galen (AD 130-200) assumed that women had two testicles in their,abdomen, comparable to men's external testicles. But anatomical research in the sixteenth century suggested that the female testes were in fact 'egg nests' – ovaries – and in the seventeenth century this was finally confirmed by the English physician and anatomist William Harvey (1578-1659) and the Dutch researchers Reinier de Graaf (1641-1673) and Jan Swammerdam (1637-1680). In his De Generatione Animalium, published in 1651, Harvey stated that no matter how hard he tried, he was unable to find a shred of evidence to support Aristotle's claim that embryos arose from a mixture of blood and sperm. He went on to hypothesise that mammalian offspring actually came out of eggs (omne vivum ex ovo). Twenty years later, the Delft physician Reinier de Graaf published a meticulous study documenting the internal and external reproductive organs.

Reinier de Graaf (1641-1673)
Portrait of the Delft physician and anatomist Reinier de Graaf: 'All people and other animals have their origins in an egg.' Reinier de Graaf spent years studying male and female reproductive organs in cows, sheep and rabbits. In 1672 he published a book in Leiden, titled 'A New Treatise Concerning the Generative Organs of Women, Which Serve Reproduction', in which he detailed the development of the ovaries following fertilisation. The book quickly won national and international acclaim, not least on account of its clear illustrations.
R. de Graaf, De mulierum organis generatione inservientibus tractatus novus. Leiden, 1672.

Based on his dissections of rabbits, De Graaf was able to report that, after mating, ‘follicles filled with fluid, nerve and seed-forming vessels could be observed in the female testes’. According to him, these ‘small globes’ contained the germ out of which, once fertilised, an individual could develop. Though De Graaf almost certainly never observed them directly, he realised that these follicles, later named Graafian follicles in his honour, held the female eggs. He was convinced that ‘both humans and all other animals that are called viviparous, have their origins in an egg just as the egg-laying animals do’. Having no knowledge of spermatozoids, De Graaf believed the eggs were fertilised in the ovaries and made their way from there into the uterus.



Egg nests of a cow and an ewe
‘Egg nests’ of a cow and an ewe, showing the changes in the ‘ova’ (eggs) following fertilisation. The letters BB (see arrow, top right) mark the glandulous substance found in the ovary (cut lengthwise) following release of the fertilised egg. Unlike his contemporaries, and though he never actually observed a fertilised egg himself, De Graaf was convinced that these follicles were crucial to reproduction. They contained the germ that, once fertilised, could develop into a new individual. Effectively, therefore, they were ‘eggs’. Many years later, they were named ‘Graafian follicles’.
From: Reinier de Graaf, Mulierum organis 1672), plate 15

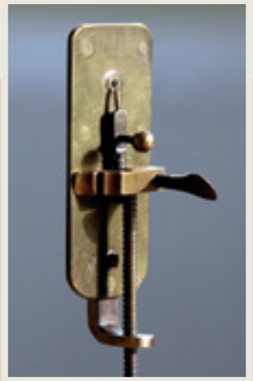


De Graaf knew nothing of spermatozoids

De Graaf knew nothing of spermatozoids. He assumed that, once in the vagina, male semen released a ‘seminal vapour’ that permeated the ovaries and triggered a kind of fermentation in the egg. The egg then began to develop and was expelled into the uterus.

In the same year that De Graaf overturned the entire science of embryology, new revelations were also being made about the nature of the male sperm. In 1677, the Delft tradesman and scholar Antoni van Leeuwenhoek (1637-1723) announced that he had seen ‘live organisms’ in semen samples observed under one of his handmade microscopes. Soon others also began to confirm the existence of spermatozoa. This led, quite naturally, to debates about the role that sperm played in fertilising the female egg. Van Leeuwenhoek, for his part, asserted that these animalcula were the basic germ of the new animal or human being. His theory was supported by Nicolaas Hartsoeker (1656-1725), who in 1695 published his own hypothesis that sperm cells already contained a tiny, complete human (homunculus).

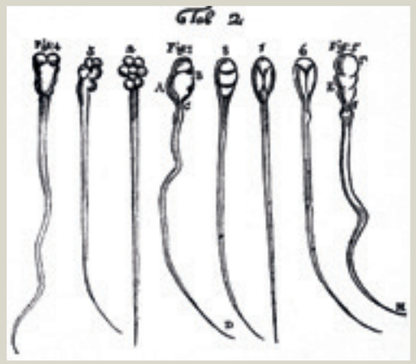
Antoni van Leeuwenhoek (1632-1723)
The second half of the seventeenth century saw a surge in microscopic research. At the forefront of this research, alongside the Englishman Robert Hooke, the Italian Marcello Malpighi and the Amsterdam physician Jan Swammerdam, was the Delft cloth merchant, wine-gauger, surveyor, glass-blower and autodidact Antoni van Leeuwenhoek.



Replica of a Leeuwenhoek microscope
Van Leeuwenhoek enjoyed great success with his design for a microscope made from a single lens fitted between two plates. The object of study was affixed to a rod whose position relative to the lens could be adjusted by means of screws. Van Leeuwenhoek blew and polished the glass for the lenses them himself, achieving a magnification of 100x to 250x. He made more than five hundred microscopes of this type. Only ten have survived.

Like Reinier de Graaf, Van Leeuwenhoek studied the male and female reproductive organs of rabbits and dogs. But his conclusions were diametrically different. In 1677, Van Leeuwenhoek reported to the Royal Society in London, where he had been introduced four years earlier by De Graaf, that he had discovered ‘live organisms’ – sperm cells – in human semen. Between 1678 and 1685 he released a string of new data intended to prove De Graaf and his fellow ‘ovists’ wrong: ‘The seed of the man alone creates the fruit,’ he asserted.

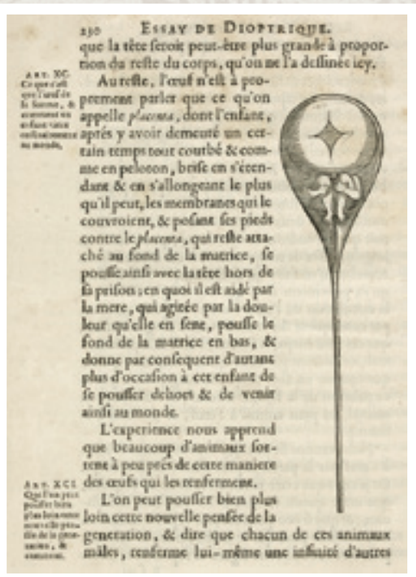
Observationes D. Anthonii Lewenhoeck, de natis e semine genitali animalculis. Philosophical Transactions Royal Society Vol. 12 (1677-1678), p. 1040-1046.



Van Leeuwenhoek's drawings of spermatozoa
Illustrations of sperm cells drawn by Van Leeuwenhoek himself (1678). Figures 1-4 show human sperm cells (three dead and one live); figures 5-8 show canine sperm cells. Van Leeuwenhoek thought

that animals existed fully-formed in miniature within sperm.

Therefore, he not only supported the theory of preformation but also maintained that embryos were generated exclusively from sperm, and not from an egg. Disagreement between the ovists (who held that new individuals arise from an egg) and the animalculists (who argued that new individuals arise from sperm) continued far into the eighteenth century.



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que la tête seroit peut-être plus grande à proportion du reste du corps, qu'on ne l'a destinée icy.

Au reste, l'œuf n'est à proprement parler que ce qu'on appelle *placenta*, dont l'enfant, après y avoir demeuré un certain temps tout courbé & comme en peloton, brise en s'étendant & en s'allongeant le plus qu'il peut, les membranes qui le couvroient, & poussant ses pieds contre le *placenta*, qui reste attaché au fond de la matrice, se pousse ainsi avec la tête hors de sa prison; en quoi il est aidé par la mere, qui agitée par la douleur qu'elle en sent, pousse le fond de la matrice en bas, & donne par conséquent d'autant plus d'occasion à cet enfant de se pousser dehors & de venir ainsi au monde.

L'experience nous apprend que beaucoup d'animaux sortent à peu près de cette maniere des œufs qui les renferment.

L'on peut pousser bien plus loin cette nouvelle pensée de la generation, & dire que chacun de ces animaux mâles, renferme lui-même une infinité d'autres

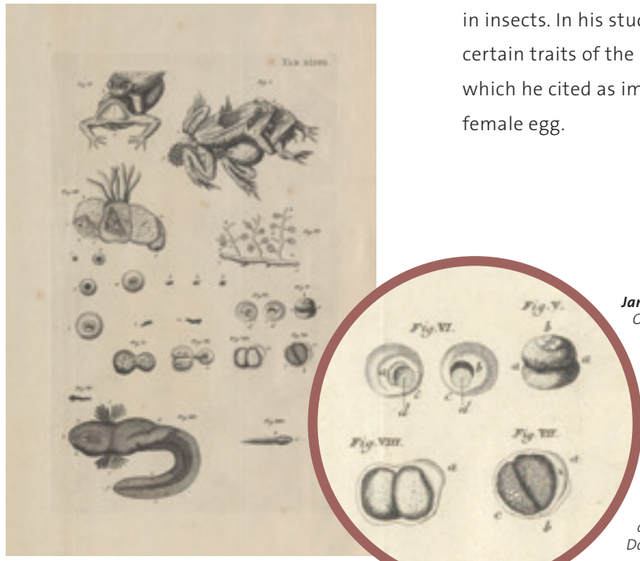
Sperm according to Nicholas Hartsoeker (1656-1725)
In Amsterdam, the researcher Nicholas Hartsoeker believed, like Van Leeuwenhoek, that sperm cells contained the adult animal in miniature form. In an illustration published in 1794, he depicted

the head of a sperm cell containing a tiny person (homunculus).

What he drew was not the result of his actual observations, but how a sperm cell would look had he had a better microscope!
N. Hartsoeker, Essay de Dioptrique Paris 1694, p. 230.

Preformation theory

By the seventeenth century, anatomical research was far enough advanced to disprove the medical and biological assumptions of Antiquity. Prevailing anti-Aristotelian sentiment led to the rejection of the entire concept of metamorphosis and epigenesis. Whereas Aristotle's ideas relied on the notion of vital forces to explain the genesis of the embryo, seventeenth- and eighteenth-century scientists preferred to seek mechanical explanations such as those formulated in the field of physics by the Englishman Isaac Newton. Most scientists believed that the fertilisation of the egg and development of the embryo was a relatively straightforward mechanical process. With the discovery of eggs and sperm, it made sense to assume that the new individual and all its specific traits were stored in miniature inside them. Once a sperm and an egg had come together, this ready-made organism needed only nine months to grow into a viable infant. This early modern alternative to Aristotelian doctrine has gone down in history as the theory of preformation. Yet there was one pivotal question on which scientists still disagreed. According to Van Leeuwenhoek and contemporaries like Hartsoeker and Leibniz, it was from the spermatozoon that individuals germinated. Their opponents, by contrast, believed that the miniature human resided in the female egg. It was this latter group of scientists that worked out and fervently defended the preformation theory. Among them was Jan Swammerdam, who conducted extensive research into reproduction in insects. In his study of butterflies, Swammerdam discovered that certain traits of the adult animal are already present in the caterpillar, which he cited as important evidence in favour of preformation in the female egg.



Jan Swammerdam (1637-1680): Oocyte in two-cell stage (fig. VII and VIII)
One of the first-ever illustrations of the division and two-cell stage of an egg cell in a frog, drawn by the Amsterdam physician Jan Swammerdam sometime between 1665 and 1675. Swammerdam went on to become a pioneering microscopist and specialist in insects and butterflies and is considered one of the founders of the preformation theory: the hugely influential theory that the adult animal already exists fully formed in the ovum, or egg cell. Swammerdam and De Graaf had been friends in their student days, but in 1672 the two men clashed over the question of which of them had been first to describe the ovum inside the ovary. They took their dispute all the way to the Royal Society in London, which decided that, in fact, neither of the Dutchmen had been first to document the mammal ovum. That honour belonged to the Dane Niels Stensen (1638-1686).

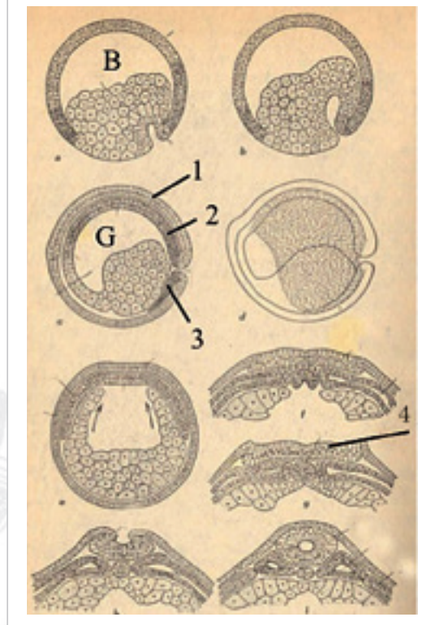
Germ layers and cell division

Thanks in large part to Swammerdam, but also other scientists such as the Italian Marcello Malpighi (1628-1694), the preformation theory dominated the eighteenth-century understanding of biology, even if some had their reservations. A new phase in scientific thinking about fertility and embryogenesis dawned in the mid-nineteenth century with the more meticulous study of embryonic development and subsequent discovery of the cell as the basic physiological building block of all living nature.

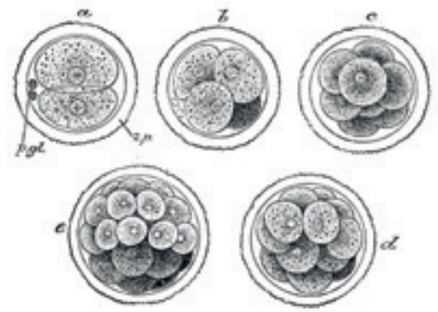
In 1827, 155 years after De Graaf, Karl Ernst von Baer (1792-1876) published a study furnishing the first evidence of the existence of the mammalian ovum. Von Baer's findings were definitive proof that the embryogenesis of all vertebrates begins with an egg. The following year, he documented in impressive detail how the fertilised egg began as a homogeneous substance and gradually differentiated into complex structures. Male sperm, Von Baer said, functioned only to set this process in motion. He showed that the development of the fertilised oocyte always begins with the formation of two clear tissue layers, called germ layers. These germ layers then divide and eventually give rise to a specific set of organs, such as the digestive system and nervous system.

Karl Ernst von Baer (1792-1876)
A native of Estonia, Karl Ernst von Baer was a physician and is considered the founder of modern embryology. In 1827 he furnished the first microscopic evidence of De Graaf's hypothesised mammalian oocyte. K.E. von Baer, Über die Bildung des Eies der Säugethiere und des Menschen. Leipzig, 1827.

Above is a letter that Von Baer sent to the Academy of Science in St Petersburg in 1828 describing his discovery of the mammalian oocyte.



From bicellular to blastomeres and germ layers
In 1828, Von Baer convincingly documented how the mammalian oocyte, once fertilised, initially consisted of a homogeneous substance that subsequently differentiated into leaf-like layers, or 'germ layers', and from there into complex structures. His publication overturned the preformation theory once and for all.
K.E. von Baer, Über die Entwicklungsgeschichte der Thiere. Königsberg, 1828.



Drawing of the first stage of development of a fertilised oocyte by the Scottish physician and renowned anatomist and embryologist Allen Thomson (1809-1884).
After fertilisation, the egg cell begins to divide. After three days it has become a clump of eight non-differentiated cells, called the morula, which continue to divide. Upon reaching the 32-cell stage, the cells move apart, leaving a hollow sphere called the blastula (illustrated on the left). The outer cell layer around this cavity is called the trophoblast; the structure as a whole is known as the blastocyst cavity. The trophoblast primarily goes on to form the placenta. In a later stage (not shown here), the germ layers become distinguishable in the cell mass, after which organs begin to form.

Twenty years later, the Polish-German scientist Robert Remak (1815-1865) would label these germ layers the ectoderm, endoderm and mesoderm, a classification scientists still use today.

A second important breakthrough was the discovery of the cell. Cells (cellulae) had already been observed in the seventeenth century by the Englishman Robert Hooke (1635-1703) as well as by Van Leeuwenhoek, but they were unsure as to what they had seen. With microscopes becoming more powerful from 1820 onwards, two German scientists, the biologist Theodor Schwann (1810-1882) and the botanist Matthias Schleiden (1804-1881), began to zoom in on the cell's contents. They documented cellular fluid, or protoplasm, and the cell nucleus, and they formulated a theory whose central tenet was that all plants and animals are composed of the same basic unit: the cell. Furthermore, they postulated cell division as the standard reproductive mechanism.

'Ovology' according to Auzoux (1797-1880)
Embryology came into its own as an important discipline in the nineteenth century. Around 1860 the French physician and anatomist Louis Auzoux created these papier-maché models of the female reproductive organs and embryonic stages to use as teaching aids. His representation of the development of human embryos was in fact based on that of tadpoles and chickens, as knowledge of embryogenesis in humans was still incomplete at the time.

In 1855 the German physician Rudolf Virchow (1821-1902) summed up the new cell theory in the now-famous statement 'omnis cellula a cellula'. He would go on to work out his ideas about the division of cell nuclei not long thereafter, establishing himself as the founder of cellular pathology.

Rudolph Virchow (1821-1902)
The period after 1830 saw the introduction of new types of microscope lenses that offered increasingly clearer images of the cell contents and nucleus. Scientists concluded that all plants and animals are made up of cells and that the standard method of replication was cell division. This insight was what led the German physician and founder of cell pathology Rudolf Virchow to utter his now famous statement in 1855: 'Omnis cellula a cellula' – all cells arise from cells.
Arch. für pathologische anatomie und Physiologie 8 (1855) 23.

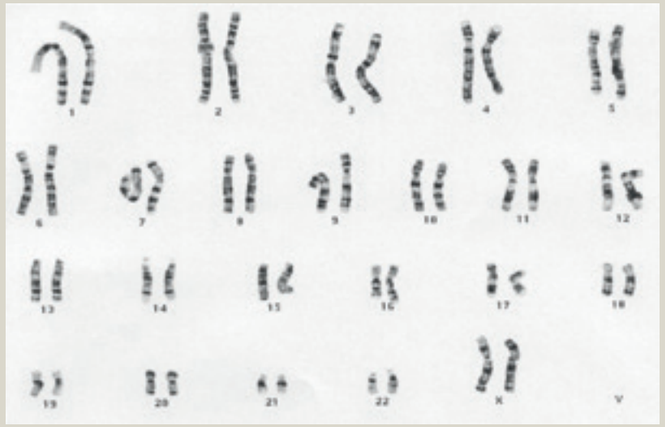
"Ich formuliere die Lehre von der pathologischen Generation, von der Neoplasie im Sinne der Cellularpathologie einfach: Omnis cellula a cellula."

Heredity, genes and genetic disorders

Towards the end of the nineteenth century extensive microscopic research and biological experiments led to a series of discoveries that definitively established the modern disciplines of embryology and reproductive science. Among the first of these was Hermann Fol's (1845-1892) important discovery in 1877 that spermatozoa could penetrate the oocyte, and his subsequent observation of two nuclei inside it that could be seen to 'merge'. This finally overturned the age-old idea that the sperm cell was merely the catalyst that caused the oocyte to germinate into an embryo.

Shortly thereafter, around 1880, the biologists Eduard Strasburger (1844-1912) and Walther Flemming (1843-1905) discovered that chromosomes, which arise from a process of condensation in the nucleus, contain the cell's hereditary material and pass this material on to daughter cells when the parent cell divides. Flemming also discovered that during cell division each chromosome consists of two chromatids.

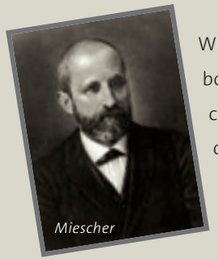
Walther Flemming (1843-1905): Cell division and nuclear division
Illustrations of cells with chromosomes. During nuclear division, chromosomes first replicate and then separate into pairs through a series of steps called mitosis. In 1882 the biologist Walther Flemming published these illustrations in what became the standard work on cell division and nuclear division. A number of penetrating studies by several German biologists subsequently led to the conviction around 1885 that all hereditary material is concentrated in the cell nucleus. Inside the nucleus, the most important components came to be identified in 1888 as chromosomes.
W. Flemming, Zellsubstanz, Kern- und Zelltheilung. Leipzig, 1882.



Humans have 46 chromosomes
By the turn of the twentieth century scientists had finally concluded that the nuclei of human body cells contain chromosomes and that these are the carriers of hereditary traits. But it was not until 1956 that Joe-Hin Tjio (1919-2001) and Albert Levan (1905-1998) made the finding that humans normally have 46 chromosomes – 23 pairs, each made up of one chromosome originating from the mother and one from the father. One pair of the 23 pairs consists of either two X chromosomes (in women, as shown above) or one X and one Y chromosome (in men).

A few decades earlier, in 1865, the Czech monk Gregor Mendel (1822-1884) had completed and published a series of inheritance experiments conducted on pea plants, but at the time he was the only one to realise the significance of his findings. Indeed, it was Mendel who first discovered genes and their inheritance patterns. Not until the plant physiologist Hugo de Vries (1848-1935) and botanists Carl Correns (1864-1933) and Erich von Tschermak (1871-1962) replicated his results in the early twentieth century would the time prove ripe to combine insights from cell theory with these studies on heredity and finally reveal the link between genes and chromosomes.

From cell nucleus to DNA



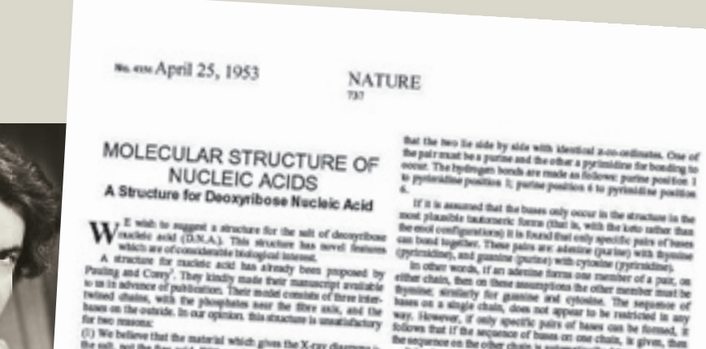
Where conceptualisations of the cell had largely been the domain of botanists and plant physiologists through the end of the nineteenth century, in the twentieth century biochemists took over, delving deeper into the molecular composition of the cell and its nucleus. The first chemical analyses of cell nuclei had actually been performed much earlier however, in the 1860s and 1870s, by Swiss physician and chemist Johan Friedrich Miescher (1844-1895). Miescher managed to isolate the nucleus and identify a substance inside it that he called nuclein, which we now know as DNA. Further chemical characterisation revealed the constituents of this substance and that it was the basic building block of chromosomes, thus making it the molecule of inheritance. Nonetheless, it was not until 1953 that this knowledge could be harnessed to unlock the structure of the DNA molecule and consequently to identify the molecular mechanisms responsible for regulating human heredity. It was James Watson (born 1928) and Francis Crick (1916-2004) who eventually consolidated the work of scientists such as Rosalind Franklin (1920- 1958) and many others in their double helix model of the DNA molecule, a breakthrough that represents perhaps the greatest advance in our understanding of life in recent centuries.

Discovery of the DNA structure
In 1953 two Cambridge scientists – the British physicist Francis Crick (1916-2004) and the American geneticist James Watson (born 1928) – demonstrated their double helix model of DNA. As they conceived it, the double helix model solved two problems:

1. *It established a clear molecular structure for DNA, just two years after it was discovered to be the main constituent of the genes in chromosomes.*
2. *It answered a question that had dominated genetics since 1900, namely: How is hereditary information coded and how is this code passed on from cell to cell – that is, from one generation to the next?*

The key to the mystery of DNA structure was provided by Rosalind Franklin (1920-1958), a researcher who worked in London and Paris and studied the composition of molecules using X-rays (crystallography). Photos: Sciencesource Images

Model of the DNA molecule's double helix structure
Deoxyribonucleic acid, abbreviated DNA, is the principle carrier of hereditary information in all known organisms. DNA consists of two long strands of what are called nucleotides, each of which contain one nucleobase. A complete DNA molecule contains four different nucleobases which always occur in pairs: Guanine and Cytosine (G-C) make up one pair and Adenine and Thymine (A-T) the other. The sequence of these pairs in the DNA (for instance, GCTACCT) provides unique hereditary information. Just as letters are used to write words, sentences and books, the letters of DNA serve to code genetic information. Chromosomes can contain anywhere from several dozen to hundreds of millions of these letter pairs. Human chromosome 1, for example, contains almost 250 million base pairs.



No. 4356 April 25, 1953 NATURE 737

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons:

(1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions.

Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J.D.W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J.D. WATSON
F.H.C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 2.

Watson and Crick's famous 1953 publication on the DNA structure
J.D.Watson and F.H.C.Crick, Molecular structure of nucleic acids, Nature 171 (1953) 737-738.

Gene codes and hereditary disorders

Watson and Crick discovered that the blueprint for inheritance is embedded in the structure of DNA molecules, which is found in the cell nucleus. These extremely long molecular strands can be conceptualised as a nearly endless code consisting entirely of four letters arranged in a virtually infinite number of combinations. In essence, they are like gigantic barcodes, which modern geneticists can easily read out. We now know that our heredity is laid down in the 46 chromosomes inside each cell nucleus – 23 from our mother and 23 from our father. Each individual inherited trait is determined by a distinct gene that makes up part of these long chains of DNA, with a single gene spanning around 300,000 bars of code, on average. An entire human DNA chain contains several tens of thousands of genes, which in turn makes up just 10% of our overall DNA. The rest of our DNA – the lion's share, in fact – regulates the error-free transcription of gene codes, among other things. Indeed, before the information encoded in nuclear DNA can do its work in the cell, it first must go through a process of conversion into proteins, the actual agents of heredity.

Given the vast number of genes involved in these processes, it is not surprising that errors sometimes slip in. Cells are efficient at repairing errors in DNA in most cases, but not all. This has resulted in many thousands of known hereditary illnesses in humans that are the result of permanent changes in a gene, known as gene mutations. These hereditary disorders, of which sickle cell anaemia, muscular dystrophy, hypercholesterolemia and cystic fibrosis are just a few examples, occur in approximately 1% of all newborns. Furthermore, everything that damages genes can also give rise to cancer. These days, thousands of researchers all over the world are working to map human DNA so that the thousands of hereditary and spontaneous DNA abnormalities can be not only documented but also traced back to specific characteristics of individual, possibly diseased cells. Once it is established how a faulty DNA code causes a particular illness, it should also be possible to develop more targeted treatments in the form of dietary adjustments, medicine or gene therapy.

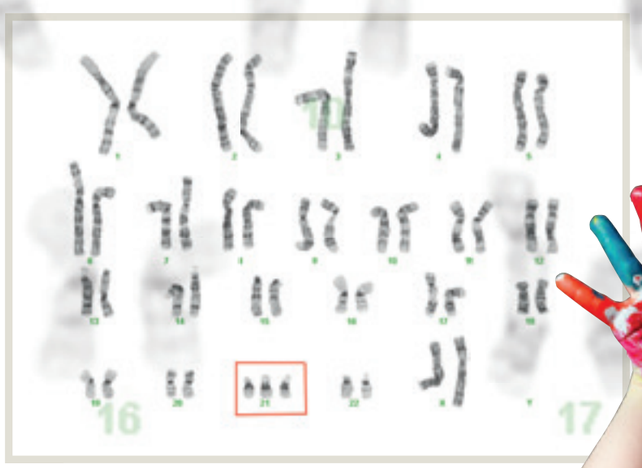
Genetically determined disorders

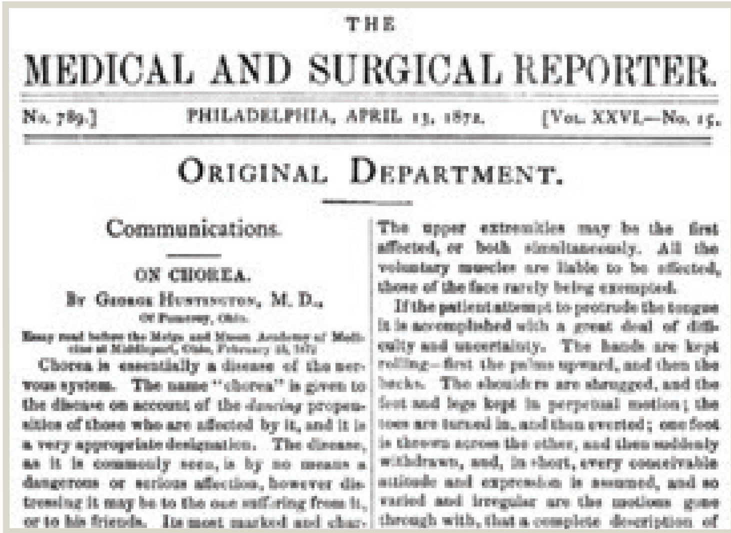
Important genetically determined disorders for which diagnostic methods have existed for quite some time include Down syndrome and Huntington's disease.

Down syndrome is the most widely known chromosomal disorder. John Langdon Haydon Down, for whom it is named, published the first detailed clinical description of the condition in 1866.

Children with Down syndrome usually exhibit characteristics of the disorder from birth. There may occasionally be some uncertainty, but even if the diagnosis seems clear, chromosome screening is always carried out. Down syndrome occurs when a third copy of chromosome 21 is produced during cell division, and for this reason the condition is also known as trisomy 21. While approximately 95% of children born with Down syndrome fit this profile, a smaller percentage of cases are due to what is known as translocation, where part of another chromosome has attached to chromosome 21. Children with this form of Down syndrome are not outwardly distinguishable from those with trisomy 21, and account for roughly 4% of those born with the disorder. However, unlike trisomy 21, which is not inherited, this latter form is hereditary. Another form of the condition is mosaic Down syndrome, where approximately 90% of the cells are normal but the remaining 10% have three copies of chromosome 21 in the cell nucleus. Pregnant women aged 36 and older are offered prenatal screening for Down syndrome as standard, which consists of a nuchal scan plus chorionic villus sampling or amniocentesis. The foetal cells obtained through these procedures are then tested for chromosomal abnormalities. More recently, it has also become possible to perform a DNA test on foetal blood cells or foetal DNA in the mother's blood. The degree of accuracy achieved by this test is so high that it will likely become the standard form of screening in the future. Though there is no treatment for Down syndrome, if detected early, the concomitant disorders can be treated to ameliorate the physical and mental development and social functioning of children who have the condition.

Down syndrome: abnormality in the number of chromosomes
In 1956 it was established that the human cell contains a total of 46 chromosomes (23 pairs). In 1958, the scientists Marthe Gautier (born 1925), Jérôme Lejeune (1895-1998) and Raymond Turpin (1926-1994), working in Paris, discovered that patients with Down syndrome have three instead of two copies of the smallest human chromosome. With the standardised scientific classification of all 23 chromosome pairs in 1960, this smallest chromosome was assigned the number 21, and the chromosomal abnormality was designated 'trisomy 21'. This discovery made physicians, and paediatricians in particular, aware of the value of chromosome screening (karyotyping) in children with developmental disorders.
J. Lejeune, M. Gautier, R. Turpin, Étude des chromosomes somatiques de neuf enfants mongoliens, Comptes Rendus Academie des Sciences 248. (1959) 1721-1722.





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MEDICAL AND SURGICAL REPORTER.

No. 789.] PHILADELPHIA, APRIL 13, 1872. [Vol. XXVI.—No. 15.

ORIGINAL DEPARTMENT.

Communications.

ON CHOREA.

By George Huntington, M. D.,

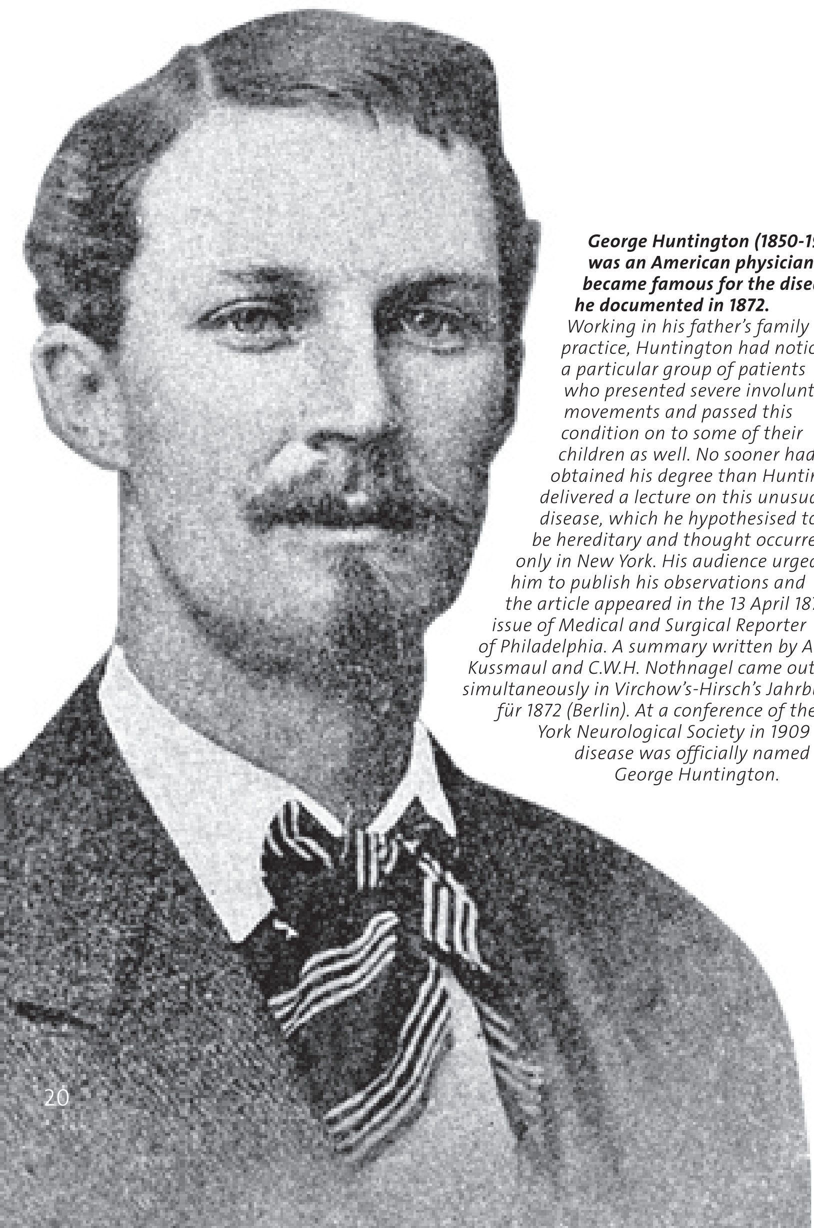
Of Pomeroy, Ohio.

Essay read before the Meigs and Mason Academy of Medicine at Middleport, Ohio, February 15, 1872.

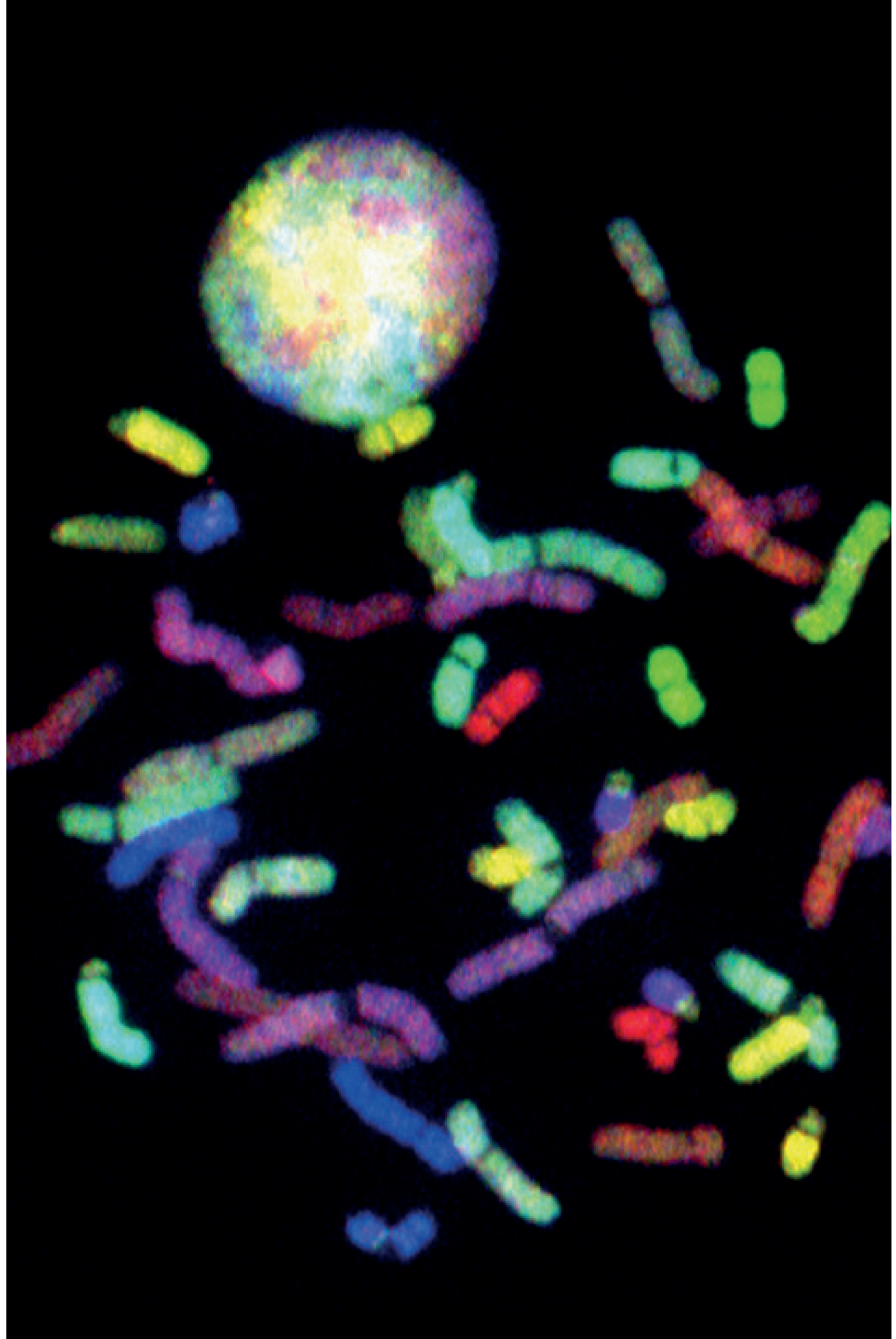
Chorea is essentially a disease of the nervous system. The name "chorea" is given to the disease on account of the dancing propensities of those who are affected by it, and it is a very appropriate designation. The disease, as it is commonly seen, is by no means a dangerous or serious affection, however distressing it may be to the one suffering from it, or to his friends. Its most marked and char-

The upper extremities may be the first affected, or both simultaneously. All the voluntary muscles are liable to be affected, those of the face rarely being exempted.

If the patient attempt to protrude the tongue it is accomplished with a great deal of difficulty and uncertainty. The hands are kept rolling—first the palms upward, and then the backs. The shoulders are shrugged, and the feet and legs kept in perpetual motion; the toes are turned in, and then everted; one foot is thrown across the other, and then suddenly withdrawn, and, in short, every conceivable attitude and expression is assumed, and so varied and irregular are the motions gone through with, that a complete description of



George Huntington (1850-1916) was an American physician who became famous for the disease he documented in 1872.
Working in his father's family practice, Huntington had noticed a particular group of patients who presented severe involuntary movements and passed this condition on to some of their children as well. No sooner had he obtained his degree than Huntington delivered a lecture on this unusual disease, which he hypothesised to be hereditary and thought occurred only in New York. His audience urged him to publish his observations and the article appeared in the 13 April 1872 issue of Medical and Surgical Reporter of Philadelphia. A summary written by A. Kussmaul and C.W.H. Nothnagel came out simultaneously in Virchow's-Hirsch's Jahrbuch für 1872 (Berlin). At a conference of the New York Neurological Society in 1909 the disease was officially named after George Huntington.

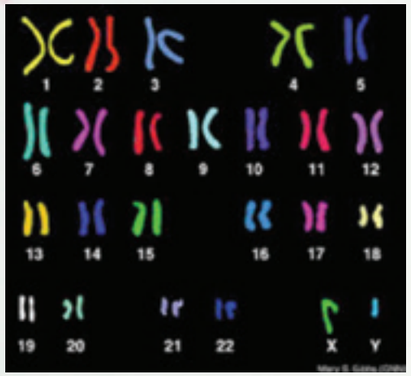


Huntington's disease is a hereditary condition that damages certain parts of the brain and is expressed in involuntary, convulsive movements (chorea) that grow progressively worse, alongside a wide spectrum of psychiatric symptoms including personality changes and mental deterioration. There is no cure for the disease at present, nor any treatments to halt its progress. Though not all members of a family will necessarily have Huntington's disease, they will all feel its effects both emotionally and socially. In the Netherlands there are some 1,700 Huntington's patients. It is equally prevalent among men and women. The offspring of a parent who has the disease has a 50% chance of having inherited the genetic abnormality, and therefore of being a carrier of the Huntington's gene. Disease characteristics normally present themselves between the ages of 30 and 50 years. A rarer form, affecting approximately 6% of patients, is the juvenile or Westphal variant that starts during the teenage years and of which the main symptom is muscle rigidity. Huntington's disease is caused by a genetic abnormality on chromosome 4. The gene for Huntington's disease was discovered in March 1993, making it possible to perform DNA testing to confirm diagnosis of an abnormally long trinucleotide repeat gene (CAG) on chromosome 4 (4p16.3). This test can also be used predictively. Because the CAG repeat is not the same length in all patients with the disease, the symptoms can vary in severity.

Image source: National Human Genome Research Institute

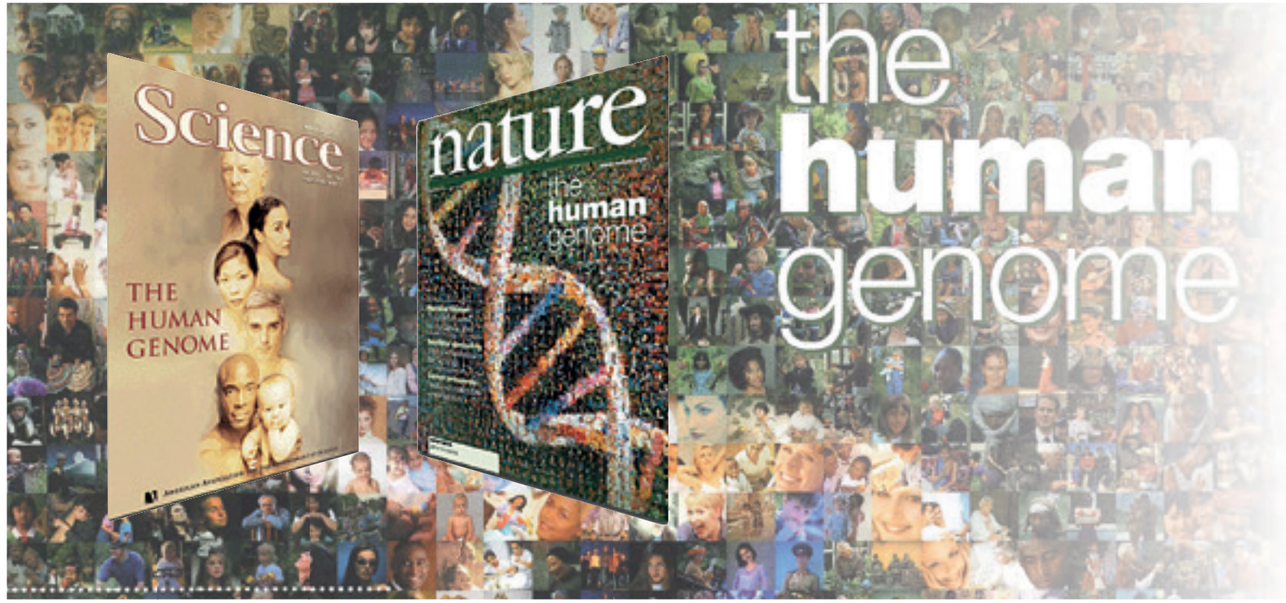
Modern DNA diagnostics

The optical microscope has played a major role in biological research ever since the seventeenth century. In genetic research it has been used specifically to study the numbers of chromosomes in certain diseases. Such research led to the discovery in 1958 of the trisomy of chromosome 21 in patients with Down syndrome, for instance, as described above. Decades later, and even with modern-day advanced gene analyses, the use of microscopes remains every bit as crucial for genetic screening. These days, divergent chromosome numbers or rearrangements of DNA sequences (translocations) are detected using a technique called **fluorescence in situ hybridisation** (FISH), also known as chromosome painting, in which specific DNA sequences are coloured using fluorescent dyes and then analysed under a special microscope.



FISH chromosomes
Using fluorescence technology (called FISH; also known as chromosome painting), individual chromosomes or sections of the chromosomes can be made visible. Currently FISH is mostly used to detect divergent numbers of chromosomes and abnormalities in which chromosome sections have become rearranged (translocation).

As DNA diagnostics entered the twenty-first century, huge advances were made through the **Human Genome Project**. This project (abbreviated HGP) was set up with the aim of sequencing the complete DNA structure, down to the individual base pairs, and identifying and mapping the entire human genome. When it was launched in 1988, this ambition sounded more like wishful thinking, but technologies developed at such a rapid pace that the goal had been largely achieved well before the envisioned deadline. In 1990 the US government provided a grant to get the project up and running for an anticipated period of 15 years. Thanks to extensive international collaboration, technical advances (particularly in sequence analysis) and huge leaps in computer technology, a rough map of the human genome was finished in the year 2000, as jointly announced by former US president Bill Clinton and then British prime minister Tony Blair. The first major publications by the two consortia conducting the project – the International Human Genome Sequencing Consortium and Craig Venter's commercial firm Celera Genomics – appeared in quick succession in February 2001. The HGP was subsequently concluded on 14 April 2003, when it was reported that the project had successfully sequenced 99% of the human genome with an accuracy of 99.99%. Other genome projects are now working on unravelling the genetic structure of other animals and plants. Where the diagnosis of hereditary diseases is concerned, the human genome project has created an immensely valuable point of reference for the detection of mutations and other chromosomal abnormalities.



Publication of the human genome
Science, Vol. 291, no. 5507, 16 February 2001
Nature, Vol. 409, no. 6822, p.745-964, 15 February 2001

Not so long ago, tracking down a genetic error required examining every single gene, one by one. This could take years to pin down – if you ever managed to find the error at all. A new technique called **exome sequencing** now makes it possible to screen all of a person's 20,000 genes simultaneously, boosting the likelihood that the cause of a hereditary condition will be found. The method first looks for errors in sets of genes suspected of being involved in the condition. If no errors can be detected in those genes, the remaining genes are examined.

One of the most recent developments in DNA diagnostics is **whole genome sequencing (WGS)**, consisting of the analysis of an individual's complete DNA, both chromosomal and mitochondrial. Hardware and software advances have refined the sensitivity and efficiency of these analyses, to the extent that we can now lay bare the DNA of a single cell.

Intermezzo:
Pregnancy tests through the ages

Piss prophecy and uroscopy in the Middle Ages
People have sought methods to detect pregnancy since Antiquity, whether to confirm the mother-to-be's suspicions (based on signs such as missed periods, nausea and swollen breasts) or to banish anxious uncertainty. A method that gained prominence during the Middle Ages was piss prophecy, or uroscopy, a technique already applied by the ancient Egyptians, Greeks and Romans and introduced into Western Europe via Byzantium in the eleventh century. Uroscopy became the diagnostic method of choice for medieval medics, and for centuries images of physicians showed them holding up a uroscopy flask to inspect its contents.
Rijksstudio collection.

Pregnancy tests in the sixteenth century
This painting by Samuel van Hoogstraten shows a physician attending to a young woman. He is performing a uroscopy, the verdict of which may well be that the woman in question is pregnant, though perhaps unintentionally. By the end of the sixteenth century, however, uroscopy had lost its credibility, not least as a means of establishing pregnancy. In 1623 Pieter van Foreest, a physician from Delft, published a searing attack entitled 'The arraignment of vrines: wherein are set downe the manifold errors and abuses of ignorant vrine-mongring empirickes, cozening quacksalvers, women-physitians, and the like stuffe', in which he called the 'piss-prophets' who claimed to be able tell if a woman was pregnant by examining her urine outright liars.
Rijksstudio collection.

The urine wheel
To perform a uroscopy, the physician used a glass flask, called a matula, with a thin neck that opened out towards the bottom. The patient's urine was carefully inspected on metrics of clarity, cloudiness, smell, taste, deposits, discharge, blood and especially colour. A special diagram called a urine wheel distinguished twenty different hues, each indicating a particular infirmity. According to some physicians, these hues could also indicate pregnancy.

Laboratory assistant injecting a frog
Laboratories often had aquariums in which they kept a large supply of frogs. The frog test was used principally for special diagnostics between 1950 and 1970.

Frog test in the general practice
In the 1950s and 1960s, GPs would send a small bottle of urine to a special private frog lab in the morning and receive the test results by telephone the same afternoon. The test could be performed from six weeks after conception and cost ten guilders.

Prognosticon: a new pregnancy test in 1960
In 1960 the Swedish physicians Carl Axel Gemzell (1910-2007) and Leif Edvin Wide (born 1934) discovered that immunological techniques could be applied to detect the human chorionic gonadotropin (hCG) hormone used in the frog test in women's urine. They took their findings to a Dutch firm, Organon, and developed the world's first test for use in general practice. For GPs performing the test, meticulous application of the pipette, microscope slide and spatula was of the essence. The results came two hours later. Initially, not everyone in the medical world was enthusiastic. For one, the test too often gave a false positive – unlike the frog test, when done properly. In 1958 Gemzell further demonstrated that another pituitary hormone (FSH) could be used to stimulate ovulation, and therefore to treat infertility. This finding would later prove instrumental in the treatment of ovulatory disorders and development of in vitro fertilisation (artificial insemination).
L. Wide and C.A. Gemzell, *An Immunological pregnancy test. Acta Endocrinologica 35 (1960) 261-267.*
G.J.P.C.M. Kok and H. Beeuwkes, *Een nieuwe serologische zwangerschapsreactie. Nederlands Tijdschrift voor Geneeskunde 106 (1962) 1620.*

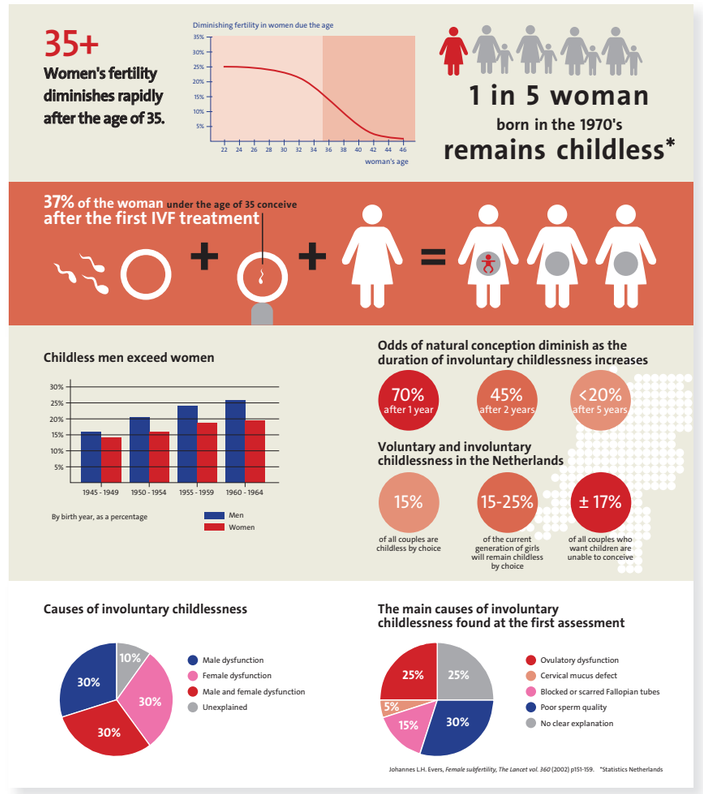
The modern Predictor test
The first pregnancy test for home use was Predictor, in 1971. At the time, it seemed a small miracle of technology. A mere nine days after missing her period, a woman could find out if she was pregnant in less than two hours. According to the manufacturer, actually asking for the pregnancy test at their pharmacy was highly taboo. Predictor therefore included a tear-out coupon in their advertising brochures reading 'I would like a Predictor, please'; this could be discretely passed to the shop clerk, who would then take one of the Predictor pregnancy tests from underneath the counter and hand the unlabelled box to the customer. The very first Predictor was something of a miniature laboratory, comparable to Organon's Prognosticon test. After 1988, however, all that was needed was to dip the test strip in a cup of morning urine. If the hormone was present, the window of the hCG indicator would change colour after a few minutes. If the colour had not changed after 15 minutes, the woman was most likely not pregnant.

Involuntary childlessness

How many childless couples do you know? Are they childless by choice? Or are they unable to conceive? These are sensitive issues, even today. Childlessness is something of a taboo subject – one people do not feel comfortable discussing. Nor, until recently, was there much research on it. One study conducted at Maastricht revealed that of all the couples who had attempted to become pregnant, one in six were either unable to have children or were not able to have as many as they wanted. And these were only the people who actually went to a GP or specialist to seek help; the real number is probably higher. But there are also couples who make a conscious choice not to have children. They prefer to prioritise their careers or put off thinking about having a child until after the age of 35, when female fertility, in particular, diminishes rapidly. The number of couples who are childless 'by choice' was around 5% in the 1970s, but has gone up in recent years. It is estimated that 15-25% of the current generation of girls will choose not to have children. The precise scope of the problem of involuntary childlessness is difficult to ascertain, not only because it is a taboo, but also given the vast grey area between voluntary and involuntary childlessness. Couples who are unable to conceive often present it as a conscious choice to other family members. Another obstacle to determining the actual extent of the problem is how the 'symptom' or 'complaint' is presented. When you go to the GP because your leg is broken or you have migraines, there is a clear symptom on which to base the diagnosis. However in the case of an unfulfilled desire to have children, there is usually no symptom; rather, a couple visits the GP because something is missing – namely, a child – but the physician has no immediate clues to the cause. Within the class of mammals, humans are actually not a particularly fertile species. Whereas 75% of simians generally conceive within one month, and rabbits even after 95% of matings, humans can barely manage 20% within a month. Consequently, it can take a long time even for normal, healthy couples to achieve pregnancy. A chance of 20% in a month amounts to a pregnancy rate of 74% after six months and 92% after one year, and after two years almost all healthy couples who try to become pregnant succeed. Conversely, this means that around 25% of fertile couples do not conceive within six months, and 10% do not succeed within one year, owing purely to chance. So how long should a couple wait before seeking medical advice? It is known that the longer the period of childlessness, the smaller the likelihood that the couple will become pregnant spontaneously. After one year of trying, the odds of ever becoming pregnant without medical intervention are around 70% – not small by any means, but after two years this figure drops to 45%, and after five it decreases to less than 20%. In the Netherlands, couples can request a medical assessment after one year. For the most part, such assessments can easily be carried out by a GP.

In roughly 30% of couples the problem lies with the man (for example, due to a low sperm count), in 30% with the woman (such as due to obstruction of the Fallopian tubes) and in another 30% with the specific combination of partners (the man may have slow sperm, for instance, whilst his partner's ovulation is irregular). Finally, in 10% of cases the couple remain unable to conceive even though all the tests come back normal and no abnormality can be found: the man's sperm is healthy, it reaches the woman's uterus, her Fallopian tubes are unobstructed and she ovulates like clockwork. Not surprisingly, a failure to conceive can lead to a vicious circle of spiralling frustration and stress, and thus even lower chances of success.

The main causes of involuntary childlessness (subfertility) found at the first assessment are:

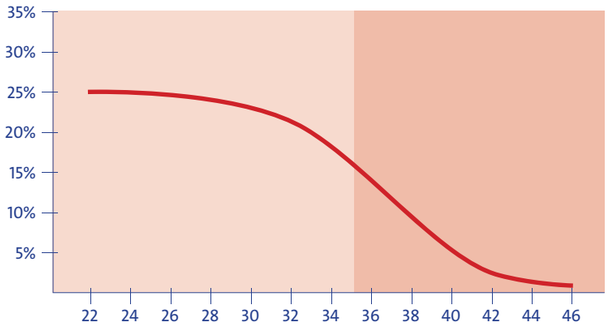


25% *Ovulatory dysfunction*
5% *Cervical mucus defect*
15% *Blocked or scarred Fallopian tubes*
30% *Poor sperm quality*
25% *No clear explanation*

Often, the explanation lies in a combination of factors. While none of these will cause infertility on its own, together they can make it (much) more difficult to conceive unassisted. The group for which there is no evident explanation shows the greatest diminishing odds over time. Many couples do end up conceiving spontaneously after all, however. In such cases, prematurely bringing out the heavy artillery – such as in vitro fertilisation, or IVF – can actually be counterproductive. Indeed, these days, healthcare is increasingly focusing on the question of when medical intervention is not necessary. As such, modern fertility assessments are directed primarily at detecting serious abnormalities; if those can be ruled out, the couple can be given some more time to conceive at home, naturally. Where physicians once looked for a quick diagnosis in order to initiate swift treatment, today the emphasis is much more on weighing the odds of conception. Depending on the prognosis and the couple themselves, the physician will recommend either the most appropriate therapy, or suggest delaying therapy a while longer (if the odds are still good without it), or advise no therapy at all (if the odds without therapy are equally poor). When making this judgement, the physician also considers the urgency of the couple's desire to conceive, their desire to take steps, and the age of the woman, which is one of the weightiest prognostic factors. Another consideration is how far the physician thinks the couple are willing to go. While some are ready to try every medical option up to and including IVF, for others a treatment like IVF is a bridge too far.

There are also other causes of involuntary childlessness, albeit more rare and beyond the scope of this survey. In many cases, IVF or one of its variations can offer a solution. This makes it worthwhile to zoom in a little more closely here on IVF and ICSI, and on preimplantation genetic diagnosis (PGD, also known as embryo screening), a technique allied to IVF which Maastricht University Medical Center (MUMC+) has sole authorisation to carry out in the Netherlands.

Diminishing fertility in women due to age



Medical treatments for childlessness and hereditary diseases

In Vitro Fertilisation (IVF)

In vitro fertilisation is a method of natural insemination in an artificial environment. The first baby to be conceived through IVF was Louise Brown in England in 1978. This milestone was achieved after twenty years of research by the embryologist Robert Edwards (1925-2013) and the gynaecologist Patrick Steptoe (1913-1988). Edwards received the Nobel Prize for his work in 2010; unfortunately Steptoe had already passed away by then.

Robert Edwards (1925-2013) and Patrick Steptoe (1913-1988): In vitro fertilisation
In 1977 the British physiologist Robert Edwards (on the right) and the gynaecologist Patrick Steptoe became the first to perform a successful in vitro fertilisation (IVF), resulting in the birth of Louise Brown. Edwards had been investigating the maturation and fertilisation of eggs outside the body at Cambridge since the 1960s, and in 1965 discovered that an egg needed 37 hours to mature before it could be fertilised successfully. 'In vitro' is a Latin phrase meaning 'in glass', and describes biological procedures performed outside the body of the organism.
R.G. Edwards, Bavister B.D, Steptoe P.C., Early stages of fertilisation in vitro of human oocytes matured in vitro, Nature (London) 1969, 221, 632.
P.C. Steptoe and R.G. Edwards, *Birth after reimplantation of a human embryo, Lancet (1978) 2, 366.*
Photo: Getty Images

The first test-tube baby: Lovely Louise
Louise Brown was the very first 'test-tube baby', delivered at a hospital outside Manchester on 25 July 1978. Her birth made headlines around the world. In the subsequent decade, proponents and opponents of the new technique known as in vitro fertilisation engaged in fierce debate, leading many countries to pass special laws governing the procedure.

The Netherlands' first successful IVF. Gerard Zeilmaker (1936-2002) and Bert Alberda (born 1950), Dijkzigt hospital, Rotterdam.
Photo: NPO

The first Dutch test-tube baby was born in Rotterdam's Dijkzigt hospital on 15 May 1983, the fruit of many years' work by the physiologist Gerard Zeilmaker and the gynaecologist Bert Alberda. They had to develop their own IVF technique, because their British colleagues Edwards and Steptoe refused to divulge their methods.

To perform IVF, a woman's natural menstrual cycle is suspended while she receives hormone injections to stimulate the simultaneous maturation of not one but multiple eggs. As soon as the eggs are mature, a long, thin needle is used to collect them through the vagina, after which they can be inseminated with the man's semen in the lab. In vitro fertilisation thus takes place 'spontaneously', in a plastic dish containing a ratio of 50,000 or more sperm cells to each egg. After 24-48 hours, one or more embryos will have resulted. The best embryo is selected and transferred to the mother's uterus. If there is more than one viable embryo, the others may be frozen to be thawed in turn and implanted in a subsequent month (or much later on, for instance following the first successful pregnancy). With IVF, the odds of carrying a pregnancy to term are around 30%, also when the embryos used have been frozen and thawed. Where male infertility is concerned, apart from artificial insemination using donor sperm (AID) or fertilisation with a combination of ejaculates, no real solution existed until the development of intracytoplasmic sperm injection (ICSI) by André van Steirteghem's group at the Vrije Universiteit in Brussels. Instead of waiting to see if a sperm cell will fertilise an egg, in ICSI the clinical embryologist performs the insemination artificially. A hollow glass needle is used to collect a single sperm and inject it directly into the egg. This procedure is used when a man does not produce enough healthy sperm for ordinary IVF to be likely to succeed.

Tool kit for medically assisted fertilisation (IVF, ICSI)
The process starts with a hormonal treatment to mature several of the woman's eggs simultaneously. A long, thin needle is then used to collect the eggs through the vagina. In IVF, the sperm and the eggs are placed together in a culture medium, where insemination takes place 'naturally'. In ICSI, by contrast, slow-moving sperm cells are injected directly into an egg. After two to five days, one or two of the fertilised eggs (then called embryos) are implanted in the woman's uterus.

2002: Celebration of the birth of the first 1,000 babies conceived through IVF at Maastricht academic hospital. By 2015, this figure had exceeded 3,000.

Cryopreserved embryos and the world's first 'frozen baby'

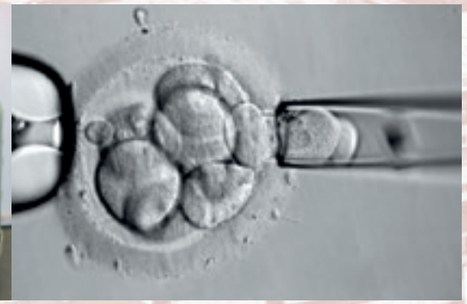
On 3 July 1984, a Rotterdam team made up of the physiologist Gerard Zeilmaker (1936-2002) and the gynaecologist Bert Alberda (born 1950) became the first to successfully cryopreserve and thaw an embryo, resulting in the birth of the first 'frozen baby' at Dijkzigt hospital in Rotterdam. Since the 1990s, it has been customary in Dutch medical practice to freeze the eggs of cancer patients. In 2011 the Dutch Minister of Health approved legislation allowing women who wish to put off having children to cryopreserve their eggs, and in 2012 the country's first oocyte bank opened its doors in Utrecht; as the women's counterpart to sperm banks, couples wishing to have a child can come here to obtain oocytes.

The first 'frozen baby' born in 1984
G.H. Zeilmaker, A.T. Alberda, I. van Gent, C.M. Rijkmans, A.C. Drogendijk, *Two pregnancies following transfer of intact frozen-thawed embryos. Fertility and Sterility vol. 42 (1984) 293-296.*
Photo: ANP

Embryo selection (preimplantation genetic diagnosis PGD)

Not long after the birth of the first IVF baby, it became clear that preimplantation genetic diagnosis (PGD, also known as embryo screening) could be utilised for IVF as well. The screening is aimed at selecting an IVF embryo for implantation that does not have certain specific genetic abnormalities known to occur in the families concerned. The technique was developed in response particularly to objections that parents of children with serious hereditary diseases had regarding abortions connected with prenatal testing. First applied in 1990, when in vitro sex determination and embryo selection were used to exclude serious X chromosome-linked abnormalities, since then many thousands of tests for hereditary diseases and chromosomal abnormalities have been performed in PGD embryos worldwide. Apart from ensuring that the couple's offspring will not inherit the feared condition, this procedure has the further benefit of eliminating that condition from the family entirely.

Frozen embryos
A cryotank containing liquid nitrogen, in which not only sperm and eggs but also fertilised eggs (embryos) can be stored for many years at a temperature of -196 °C. The process does not increase the risk of abnormalities in the embryo and has almost no effect on the success rate of conception.



Isolation of a single cell from an early embryo for PGD
A three- to four-day-old embryo (centre) is affixed to a pipette (left), after which part of the embryo wall is opened and a single cell is collected from it using a second, thin glass pipette (right). This cell is then subjected to genetic (DNA) analysis.

PGD in Maastricht

In 1995, Maastricht academic hospital became the first hospital in the Netherlands to be licensed to perform PGD, enabling it to screen for cystic fibrosis, Huntington's disease, certain cancers and other conditions. Two years later, in April 1997, this resulted in the country's first 'PGD baby'. PGD tests embryos exclusively for abnormalities for which there is a known increased risk, and not for any others. Maastricht UMC+ also performs PGD procedures for the university medical centres in Utrecht, Groningen and Amsterdam. To save patients from having to travel all the way to Maastricht, these partner hospitals also have facilities to collect cells from IVF embryos. The genetic material is then sent to Maastricht to be tested for errors in specific genes. The results are forwarded to the treating physician at the hospital concerned, which can then proceed to implant a healthy embryo.


NRC HA

Embryo op afwijking onderzocht

Gezonde baby na test in reageerbuis

VANDAAG

CULTUREEL

NRC, 25 april 1997.

Five leaders of the Maastricht UMC+ IVF and PGD team
FLTR: Prof. Hans Evers, Prof. Joep Geraedts, Prof. Guido de Wert, Dr John Dumoulin and Prof. Christine de Die

Medically assisted reproduction and ethics

Human reproduction has always been a source of awe. However, as is clear from the preceding chapters, recent decades especially have begun to strip away its mysteries. The genesis of reproductive cells, the process of fertilisation and of embryo development, and the interaction of genetic and environmental factors all seem to hold fewer and fewer secrets. Modern reproductive biology and medicine are both symptoms and catalysts of this demystification. And yet the awe remains: if anything, insight into the complex reality only amplifies our sense of wonder. Reproductive medicine is an exceptionally dynamic field that encompasses two (partially overlapping) branches: 1) infertility treatment and 2) selective reproduction, which chiefly concerns the prevention of the conception or birth of a child with a serious disease or disability. Developments in the field also give rise to continuing ethical and social dilemmas, one central question being: Should we be allowed to exercise all the possibilities that science gives us?

Ethical debate

In 1995 Maastricht academic hospital became the only medical centre in the Netherlands to be licensed to perform preimplantation genetic diagnosis (PGD). Even then, this led to heated debates. Opponents said PGD was unacceptable because selection conflicts with what they consider to be the inviolable status of the preimplantation embryo. Others warned of the burden and risks of IVF for women, as well as the danger of the 'slippery slope' to a future where we design children on demand, as it were, selecting for intelligence, heterosexuality or other desired traits. Proponents, by contrast, cited the value of PGD to prospective parents with regard to prenatal testing and the possible decision to terminate in the event of a foetal abnormality.

In 2006 the Health Council of the Netherlands issued a recommendation at the request of the then State Secretary of Health, Welfare and Sport in which it set out clear and substantiated criteria for the performance of PGD in the Netherlands. PGD for gene defects associated with hereditary breast and ovarian cancer were deemed allowable under certain conditions. Unexpectedly, however, in May 2006 the State Secretary threatened to restrict PGD and even prohibit some of the constituent techniques completely.

Political debate

The public and political debate surrounding this decision peaked in 2007 and 2008 following open criticism by, amongst others, the Maastricht University Professor of Biomedical Ethics Guido de Wert, both in newspapers and on TV. According to de Wert, the decision was ill-founded, inconsistent and based on factual inaccuracies.

After a new government took office in February 2007, the new Labour Party State Secretary decided in May 2008 to give the green light for PGD (embryo screening) to test for risk of hereditary breast and ovarian cancer, albeit without conferring with the other cabinet ministers. The Christian Union party had particularly strong objections to the decision, and there was even talk of a government collapse over the issue. After lengthy cabinet negotiations, however, in July 2008 the ministers and a parliamentary majority were able to agree on legislation that permitted PGD on the basis of clearly defined criteria. In fact, the 'new' criteria on which this approval was based in essence confirmed the methods long applied at Maastricht academic hospital.

Embryo screened for abnormalities. Healthy baby after test tube analysis. NRC, 25 April 1997.

G. de Wert. PGD ban is back-door moralism. Opinions section, NRC, 21 September 2007.

Leonardo da Vinci: Drawing of a foetus, around 1510-1513