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# The road to 1978: a brief history of fertility research

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This issue of *The Biochemist* acknowledges the 40th anniversary of the birth of the first IVF baby, Louise Brown, and with it the emergence of Assisted Reproductive Technology (ART). These developments did not arise in a vacuum; the history of baby making dates back well before 1978. The influence of fecundity (the ability to reproduce) has been deliberated since the Palaeolithic period.

An explanation for the mechanism of reproduction was first proposed by the ancient Greeks. The philosopher and 'Father of Medicine', Hippocrates (460–370 BC) proposed the 'two seed theory' where both males and females produce seeds that combine to give rise to a new human. However, Aristotle (384–322 BC) held the view that the unborn child was preformed, consisting of fluids and menstrual blood (catamenia) in the womb. This catamenia awaited the male's semen to trigger its development.



**Figure 1.** Replica of a Leeuwenhoek microscope (author's own)

He considered that the preformed foetus grew like 'the seeds of plants' eventually developing into the baby. This theory of preformation or 'epigenesis' dominated the understanding of reproduction for over 1000 years until the latter part of the European Renaissance. Scholars such as the anatomist, Hieronymus Fabricius (1537–1619) turned his attention to the mystery of reproduction. Studying the domestic hen, Fabricius noticed that fertile eggs were laid some time after mating with a cockerel. This led him to believe that semen from the cockerel was stored in a little sac near the cloaca in the hen, where it rendered the whole uterus and eggs fertile. This sac was later named the Bursa of Fabricius (although it is not actually used for semen storage but B-cell development in birds). A student of Fabricius, William Harvey (1578–1657) – better known as the discoverer of blood circulation – was also interested in reproduction. He expanded the work of his mentor and investigated the role of semen through dissection of female deer, dogs and rabbits. His dissection of female deer soon after mating, found no 'evidence' of a physical role for semen in reproduction but ascribed its role as more ethereal, a conclusion closer to Aristotle's preformationist idea. Harvey concluded 'that all things come from the egg' (ex ovo omnia) which he published in *Exercitationes de Generatione Animalium* (Exercises on the Generation of Animals), in 1651.

## The first microscopes

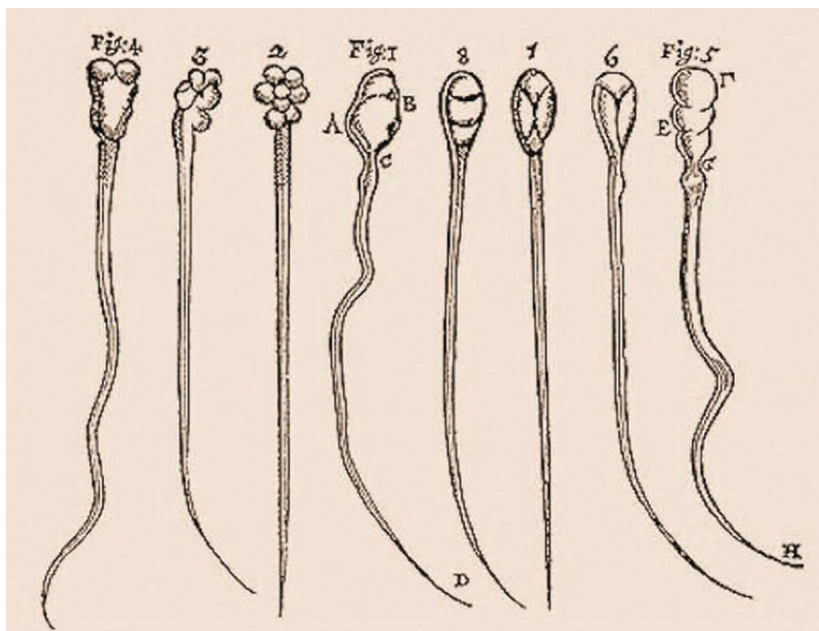
Discoveries in science frequently follow innovations in technology, and the emergence of the microscope is a fine example. Improvements of Gutenberg's printing press made printed books and manuscripts more accessible,

and this in turn generated a market for ground lenses to aid reading. The Dutch spectacle makers, father and son Hans and Zacharias Janssen, and Hans Lipperskey are credited with inventing the first microscope, during the 1600s. They placed two convex lenses at each end of an adjustable tube, with one end functioning as an eyepiece, and the other the objective. This was rather rudimentary, but offered some magnification of objects. Later the English polymath, Robert Hooke (1635–1703) improved on the design, exploring the micro-world in great detail. He published his findings in the magnificent *Micrographica* (published 1665). This masterpiece contained 38 plates, including a large pullout illustration of a flea, and included the first biological reference to cells.

Antonie van Leeuwenhoek (1632–1723) worked as a draper and acted as a minor city official in the Dutch town of Delft. He was a meticulous observer and keen craftsman, designing and making hundreds of simple microscopes, much different to the compound double-lens microscopes of Janssen and Hooke. Leeuwenhoek's were mostly small handheld, single-lens microscopes with impressive magnifying and resolving powers (Figure 1). Through his observations, he made some remarkable discoveries, including the first observations and descriptions of small bacteria and protist in samples of water, which he called 'animalcules'. He sent many letters describing his observations to the Royal Society in London, whose members (including Hooke) were impressed with his microscopic skills and meticulous descriptions. For this, he was elected as a foreign member in 1680.

Leeuwenhoek is most famous for the discovery of sperm. He believed that the generation of animals was from these 'animalcules in the male sperm' and noted their presence in abundance in his own semen and that of the dog, rabbit, and cockerel (Figure 2). Leeuwenhoek had the preformationist view of generation, stating his discovery 'the parts and membranes of the fetus', including the head and the shoulders. He postulated that these animalcules travel to the uterus where they grow and develop – 'the female served only to afford nourishment to the animalcules of the male sperm', akin to a seed planted in nutrient soil (for his letters to the Royal Society see vanleeuwenhoek.com). Nicolas Hartsoeker (1656–1725) a fellow Dutchman, described what he perceived these preformed little men would look like, as depicted in the drawing of the 'homunculi' in these animalcules (Figure 3).

During the seventeenth and eighteenth century, there were two preformationist concepts of reproduction – the ovists, who argued that generation is derived from the preformed foetus residing in the ovum or egg; and the spermists who proposed the idea of the homunculi and the 'planting of the male seed'.



**Figure 2.** Sperm from rabbits and dogs, as described by Antonie van Leeuwenhoek in 1678. Taken from [https://commons.wikimedia.org/wiki/File:Sperm\\_Anton\\_van\\_Leeuwenhoek\\_Rabbit\\_dog.jpg](https://commons.wikimedia.org/wiki/File:Sperm_Anton_van_Leeuwenhoek_Rabbit_dog.jpg), available under Creative Commons Public Domain Mark 1.0.

## The role of the egg

Through studies of insects, amphibians, fish and mammals – the ovists view of the importance of the egg in the generation of animals was further cemented by the work of Dutch biologists, Nicolaus Steno (1638–1683), Jan Swammerdam (1637–1680) and Reinier de Graaf (1641–1673). Steno dissected dogfish and noted that the ovaries were similar to the female 'testis' he observed in women and sheep. This led him to conclude "...I have no doubt that the testicles of women are analogous to the ovary..." , which he believed contained the eggs. Swammerdam and de Graaf were gifted anatomists, and were both keen to confirm the existence of eggs in the woman. They battled to be the first with to be accredited with this discovery. However, de Graaf first published his detailed work, *De Mulierum Organis Generationi Inservientibus Tractatus Novus* (New treatise concerning the generative organs of women), in 1672. In it he describes the follicle and its contents – exclaiming that this organ contains the egg (from where the Graaffian follicle is derived). He also postulated that a 'seminal vapour' reached the eggs through the uterus and fertilized them. However, the true role of both egg and sperm in the generation of animals was unraveled by the careful and conscientious work of the Italian Catholic priest, Lazzaro Spallanzani (1729–1799). Spallanzani was a great experimentalist and thinker, with a broad interest, including physics, chemistry, geology, and biology. He carefully designed controlled experiments,



**Figure 3.** Homunculus as described by Hartsoeker. Taken from the Wellcome Collection (<https://wellcomecollection.org/works/wsxjcdqe?query=Spermatozoon.+Homunculus>); available under a CC-BY licence.

describing the methodology in enough detail for others to repeat his work, and rebutted any critics with more controlled experimental data.

Spallanzani turned his attention to fertilization and reproduction. In one experiment, he examined the nature of the sperm's *aura spermatica*. This was one prevailing preformationists theory, proposing that a 'vapour' emanating from semen triggered embryonic development. Spallanzani set out to investigate this by placing semen from a toad in a watch glass while eggs from the female were placed in the bottom of another watch glass turned upside down. The eggs were separated from the sperm by a few millimetres. After several hours, he noted the eggs were covered 'as if by a dew', from the condensation of the evaporated seminal fluid. However, none of the eggs developed. This disproved the property of the *aura spermatica* of sperm. In another, ingenious experiment, he prized male frogs from their mating amplexus and fitted them with tight taffeta britches, after which he replaced them back in their mating position. With the taffeta barrier, none of the eggs developed. However, when he scraped the semen from the britches and added it to the eggs, they all developed into tadpoles. Hence, Spallanzani was able to demonstrate unequivocally, the role of the egg and semen in the generation of animals. This was one of the earliest demonstrations of IVF and the proof that fertilization took place by the physical contact between semen and egg. However, he, erroneously, concluded that semen had the fertilizing property, not the sperm. It would be another 100 years before direct interaction of sperm and eggs could be characterized.

### Fertilization described

By the mid-1800s, improvements in optics and microscopic techniques, together with the elucidation of the cell theory by Virchow, Schwann, and Schleiden paved the pathway for a more accurate explanation and description of the process of fertilization. In 1879, Oscar Hertwig (1849–1922) and Hermann Fol (1845–1892), who both trained under Ernst Haeckel, independently described sperm entry into the egg and the subsequent union of male and female nuclei in the starfish.

It took nearly 200 years after Leeuwenhoek's discovery of sperm that it was finally confirmed that both egg and sperm had to fuse together, during the process of fertilization, to trigger embryo development.

The nineteenth century saw advances in the study of mammalian reproductive biology.

The Swiss physician Jean-Louis Prevost (1838–1927) and the French scientist, Jean-Baptiste Dumas (1800–1884) concluded that the testis produced sperm and in other experiments, they demonstrated that sperm

was essential for fertilization in the frog. It was the embryologist, Carl Ernst von Baer (1792–1876), in 1826 identified the mammalian egg during his studies on the ovary of a dog and described early embryo development.

The practice of artificial insemination can be dated back to Spallanzani, who successfully artificially inseminated a bitch. However, Dr John Hunter is cited as having performed the first documented artificial insemination in humans (circa 1790). He carried out this with sperm derived from a man with hypospadias using a syringe.

The first reported attempted mammalian IVF was carried out in the 1930s. Gregory Pincus demonstrated this in the rabbit in 1934 stating "that first certain demonstration that mammalian eggs can be fertilized *in vitro*". However, his work was criticized and the 'fertilized' eggs were suggested to be parthenotes, as spontaneous egg activation was common in cultured rabbit eggs. The main impediment to mammalian *in vitro* fertilization was the physiology of sperm. Sperm must undergo biochemical and physiological changes in the female reproductive before they have the capacity to fertilize an egg. This process is called capacitation and was first identified by both Colin Austin (1914–2004) and M.C. Chang (1908–1991), who both published their findings separately in 1951. Austin further described another essential sperm process, the acrosome reaction, which enables the sperm to penetrate the outer layer of the egg (the zona pellucida). In 1959, Chang collected mature unfertilized eggs from albino female rabbits. Sperm used for insemination were collected from uteri of albino females mated with albino males 12 hours previously. The fertilized eggs were cultured until the 4-cell stage, where they were then transferred to black surrogate females, that subsequently delivered live albino young. Chang's work represented a significant advancement. However, the necessity to pre-incubate sperm in the uterus of a pregnant female prior to attempting to fertilize the eggs complicated this process. It was Chang and Austin's work on capacitation that led to the belief that sperm must reside in the female reproductive tract in order to contribute to fertilization *in vitro*. However, Ryuzo Yanagimachi and Chang in 1963 showed that this was not essential, when they developed experimental *in vitro* conditions through which sperm (from hamster) without prior *in vivo* activation could fertilize eggs *in vitro*, with subsequent embryo development to the 2-cell stage. Following this, many other mammalian species (mouse, guinea pig, cat, sheep and pig) were fertilised *in vitro*. These important contributions laid the groundwork that would eventually lead to successful human IVF.

## Human IVF

In 1963 Robert Edwards (1925–2013) set up a laboratory at Cambridge University to investigate human fertilization. His energy, enthusiasm and ambition to obtain successful human IVF were admirable. However, in trying to achieve this he encountered a number of scientific obstacles. He needed a regular supply of human eggs and a means to capacitate sperm *in vitro*.

His meeting, and eventual long collaboration with Patrick Steptoe (1913–1988), solved the problem with obtaining human eggs. Steptoe was a Consultant Obstetrician at Oldham General Hospital, Greater Manchester, where he had been pioneering the development and use of the laparoscope in gynaecological surgery. Edwards' interest in collaborating with Steptoe was to initially to remove capacitated sperm from the oviduct using laparoscopy. However, that problem was solved by his appointment of Barry Bavister (Austin's PhD student) on to the project. Bavister developed a culture media that produced higher rates of fertilization in hamster eggs (Bavister's media). Steptoe would become the valuable source for human ovarian tissue and eggs.

This collaborative work between Edwards, Steptoe and Bavister led to their landmark 1969 *Nature* paper, which described convincingly for the first time the fertilization of human eggs *in vitro*. This paper ended with the humble concluding remarks, "Human oocytes have been matured and fertilized by spermatozoa *in vitro*. There may be certain clinical and scientific use for human eggs by this procedure".

The continuing work of Edwards, Steptoe and an additional team member, Jean Prudy (1946–1985) eventually led to the birth of the first IVF baby, Louise Brown, on the 25 July 1978. Since then approximately 6 million babies have been born through ART.

## Summary

Since the birth of Louise Brown, the field of ART has advanced both in our understanding of human reproduction, the causes of infertility and developments in ART.

Infertility is defined as the inability to conceive naturally after 12 months of actively trying and affects 1 in 7 people in the UK (15% of the global population according to the World Health Organization).

The advent of a variant of IVF, Intracytoplasmic Sperm Injection (ICSI), in which a single spermatozoon is selected and injected into the egg cytoplasm with a fine pipette, has revolutionized the treatment of male infertility, in particular in cases where sperm numbers are very low or their swimming ability (motility) is impaired.

Combining advances in molecular genetics and embryology has given rise to techniques such as Preimplantation Genetic Diagnosis (PGD) and Preimplantation Genetic Screening (PGS), which are used to screen and test for a specific genetic disease that may be embryo lethal or result in severe abnormalities and miscarriages.

Developments in sperm, egg and embryo cryostorage (freezing) has enabled couples and single would-be parents to preserve their fertility, especially after cancer treatment.

The history of baby making is rich and diverse – with many important contributors playing essential parts. This article is a synopsis of some of the key events that took place over the course of history. ■

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