

A CONCISE HISTORY OF ART

To mark the year that the first test tube baby, Louise Brown, turned 40, independent researcher **Zara Josephs**, looks back over the history of artificial reproductive technology.

Theories of conception have always abounded, from pre-historic ideas about fertility goddesses to the nature-based explanations of the early Greek philosophers. Such theories included pangenesis, in which the germinal cells were postulated to originate from the whole body; spermism, which held that the structure and form of offspring, were contributed solely by the male parent (the female providing only nourishment); and the particularly popular preformationism, in which a miniature human housed in the sperm developed in the female's womb following implantation, emerging in due course as the neonate.

Aristotle, the first to formulate a coherent theory of conception, saw the sperm as containing a pre-existing

“principle” for specifying the generation of new offspring following a specific blueprint. The important point was that this principle (to be identified millennia later as the genetic code) for the building up of the organism was something other than the material constituting the final form of the organism.

In 1677, Antonie van Leeuwenhoek (hailed as the father of microbiology) became the first person to observe and describe sperm cells. Karl Ernst von Baer discovered the mammalian ovum in 1827, while Edgar Allen was first to observe the human ovum in 1928. The first reported fertilisation event, recorded in 1876 by Oskar Hertwig, was the fusion of spermatozoa and ova in starfish.

Successful engineering of the fertilisation process was first observed with Lazzaro Spallanzani's artificial insemination of dogs in 1780, a feat that

was followed a decade later by Dr John Hunter, who performed the first recorded artificial insemination in humans. In 1963, Chang and Yanogumachi demonstrated, through experiments in hamsters, that *in vitro* fertilisation (IVF) lay within the realms of possibility.

Human IVF was made possible by collaboration between Robert Edwards, Patrick Steptoe and Barry Bavister, which led to a 1969 *Nature* paper describing the *in vitro* fertilisation of human eggs.

The continuing collaboration of Edwards, Steptoe and Jean Purdy eventually led to the birth of Louise Brown on 25th July 1978.

Developments in human reproductive technology have been galvanised by a number of revolutionary tools and concepts, including the polymerase chain reaction (PCR); recombinant DNA techniques; transgenic technology; somatic cell nuclear transplantation; and microRNAs. These tools have enabled the further refinement of disease prevention and treatment strategies. Techniques dealing specifically with infertility all come under the heading of artificial reproductive technology (ART): the *in vitro* manipulation of sperm,

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eggs or embryos in order to achieve successful fertilisation, implantation and clinical pregnancy.

Oocytes and ageing

Much of ART has focused on women, for reasons that appear obvious: while men continue to produce sperm cells throughout life, women are born with all the egg cells they are ever going to produce, and these decline with age, in both quality and quantity. Furthermore, older age at conception is a risk factor for chromosomal errors in the embryo, which can cause miscarriages or the birth of a child affected with a genetic disorder. The most common of these disorders is trisomy 21 (Down Syndrome), which is linked with mental and physical problems and a shortened lifespan.

Most chromosomal errors arise during meiosis, the cell division that produces haploid eggs or sperm cells from a diploid stem cell. Eggs are formed in the ovaries of the fetus before birth, and their number declines continually from birth until menopause. Pioneering work by Hartshorne and others has shed light on mechanisms underlying chromosomal aberrations in older mothers. Chromosome pairs are held

together by binding molecules which are laid down prenatally. In meiosis 1, two chromosomes must arrive at the same spindle pole; in meiosis 2, they have to separate. Thus the binding proteins must be flexibly adhesive. With time, these binding molecules disintegrate, and in older eggs come unstuck. By the time the chromosomes segregate just before ovulation, they have been in an arrested state for many years. The kinetochores (protein clusters) which attach chromosomes to the spindle tubules control the orientation of the chromosome attachments and ensure that the chromosomes are pulled in the right directions at meiosis 1.

In older women, the paired kinetochores are further apart at metaphase of meiosis than in younger women. Thus, in humans, kinetochores move apart with time, a fact linked with the higher incidence of chromosomal aberrations in fetuses of older women. Significantly, in species such as mice, in which the kinetochores are fused together (and thus held firmly together during the pre-birth meiotic arrest), chromosomal anomalies occur far less frequently than in humans. Women planning to delay conception are currently advised to avoid risk by



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banking their eggs while still young, for later use; or by accepting eggs from younger donors.

Male fertility testing

The relative lack of attention to male fertility notwithstanding, the evidence shows a link between increased paternal age and declining fertility, as well as serious birth defects. In fact, 50% of infertility cases arise from poor sperm function, yet diagnostic and management tools for male infertility are few in number.

The main diagnostic tool is semen analysis using reference figures published by the World Health Organization (WHO), which allows classification into different categories: normal (normozoospermia); reduced motility (asthenozoospermia); containing high numbers of dead sperm cells (necrozoospermia); displaying morphology abnormalities (teratozoospermia); low in count (oligozoospermia); and zero count (azoospermia). However, the WHO criteria leave many cases of male infertility unsolved. Thus, the new assay developed by Dimakopoulou and Jayasera at

Hammersmith Hospital, London, is a welcome development. High levels of reactive oxygen species (ROS) are known to be a leading cause of male factor infertility. Based on the association of ROS with cell pathology, the new chemiluminescence-based test directly assays ROS through measuring light emitted following the oxidation of luminol. While the endogenously generated ROS in sperm cells promotes sperm motility and egg fertilisation, excessive ROS levels can peroxidise the sperm plasma membrane and damage DNA in both nucleus and mitochondria. The result is dysfunctional sperm that are incapable of fertilising eggs. The ROS assay is especially relevant for men leading harmful lifestyles or harbouring infections. The medical consensus is that management by antibiotics and by reducing dietary fat intake may be valuable for couples with unexplained recurrent miscarriage.

Steps to fertilisation

The expression “miracle of birth” is aptly worded: many steps can and do go wrong

between gametogenesis and birth. Any break in the chain of events leading to fertilisation will derail the process. First, the sperm cells (approximately 180m in each ejaculate) navigate towards the egg cell through the hostile environment of the female reproductive tract, suffering immune system attacks en route. The few sperm cells that arrive at the utero-tubal junction unscathed undergo a multi-step selection and activation process to ensure error-free fertilisation:

- **Capacitation:** The first of these steps, takes place in the fallopian tube reservoir. Here, spermatozoa undergo molecular changes that trigger hyperactivity and greatly increased motility that propels them towards the ovum in the *ampulla*. Capacitation also involves destabilisation of the sperm’s cell membrane in readiness for the next step.

- **Acrosomal reaction:** The egg is ringed by the *zona pellucida*, a protective glycoprotein coat which contains receptors for sperm cells. Contact of the sperm with the *zona pellucida* elicits the fusion of the sperm membrane with the acrosome, a cap-like organelle at the tip of the sperm cell. The fusion releases enzymes that break down the *zona pellucida*, allowing the sperm to bind to the egg cell membrane and initiate fertilisation.

- **Cortical reaction:** As soon as the first sperm cell fuses with the egg, the egg releases cortical granules that deactivate all sperm receptors, preventing penetration by any further sperm cells (fertilisation by more than one sperm - polyspermy - almost inevitably leading to early embryonic death).

- **Meiosis reactivation:** The oocyte, hitherto suspended in metaphase of meiosis 2, is released from meiotic arrest though signals carried in calcium oscillations triggered by the sperm-egg fusion.

- **Nuclear fusion:** The sperm and egg nuclei migrate towards each other along a pathway of microtubules derived from

Right. Cell-division of two fertilised human embryos during the third day of embryonic development following IVF treatment at a private clinic in London.

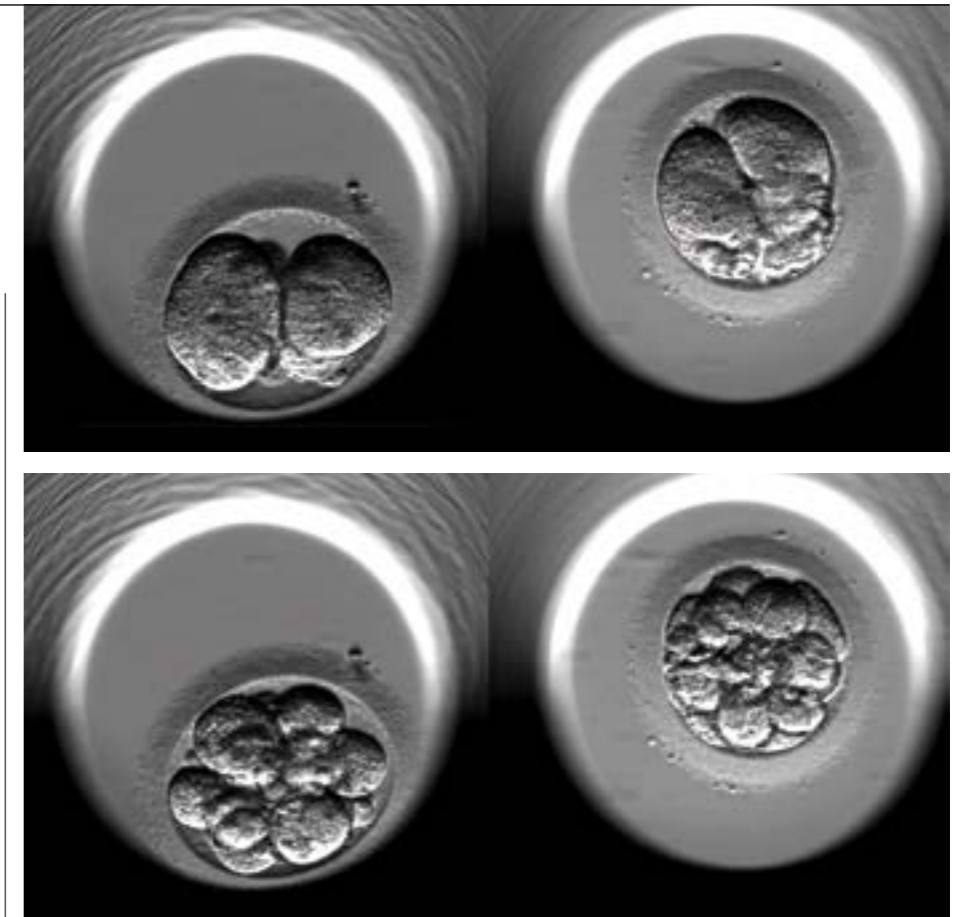
both sperm and egg, fusing to form the diploid zygote nucleus.

The sperm factor

The theory holds that sperm contain a specific trigger that initiates conversation between the two gametes when they meet. This “sperm factor” was identified as a unique phospholipase C (PLC) form called phospholipase zeta (PLC ζ), found in the sperm’s cytoplasm. PLC ζ , activated by fertilisation, triggers calcium release from the egg’s endoplasmic reticulum. Thus, it mediates communication between sperm and egg. The calcium is released in a characteristic oscillatory pattern. Research shows a link between infertility and PLC ζ absence, deficiency or abnormal localisation. Thus, PLC ζ assays enabling detection of egg activation ability have been developed, which clinicians can use to develop appropriate clinical management strategies.

AOA as male infertility treatment

New diagnostic tools to treat oocyte activation deficiency (OAD) have been developed to diagnose and manage cases of infertility that fall outside the WHO criteria. Many couples with a history of fertilisation failure have benefited from artificial oocyte activation (AOA). In this process, an artificial agent is applied to the oocyte during conventional intracytoplasmic sperm injection (ICSI), to induce calcium release and initiate activation events. This method may improve fertilisation outcomes in azoospermic males, or couples for whom conventional ICSI has failed. However, AOA fails to replicate the natural pattern of calcium oscillations (instead, releasing the Ca²⁺ all at once), or the control of gene expression in the embryo characteristic of PLC ζ , and could therefore exert a negative impact on the developing embryo.



Research is ongoing into recombinant PLC ζ , which may better mimic the effects of the naturally generated kind.


From A to zeta

Aristotle postulated a conception theory that in principle agrees with the discoveries today, almost two thousand years later. Over the intervening centuries, major breakthroughs in knowledge appear to have been characterised by the same

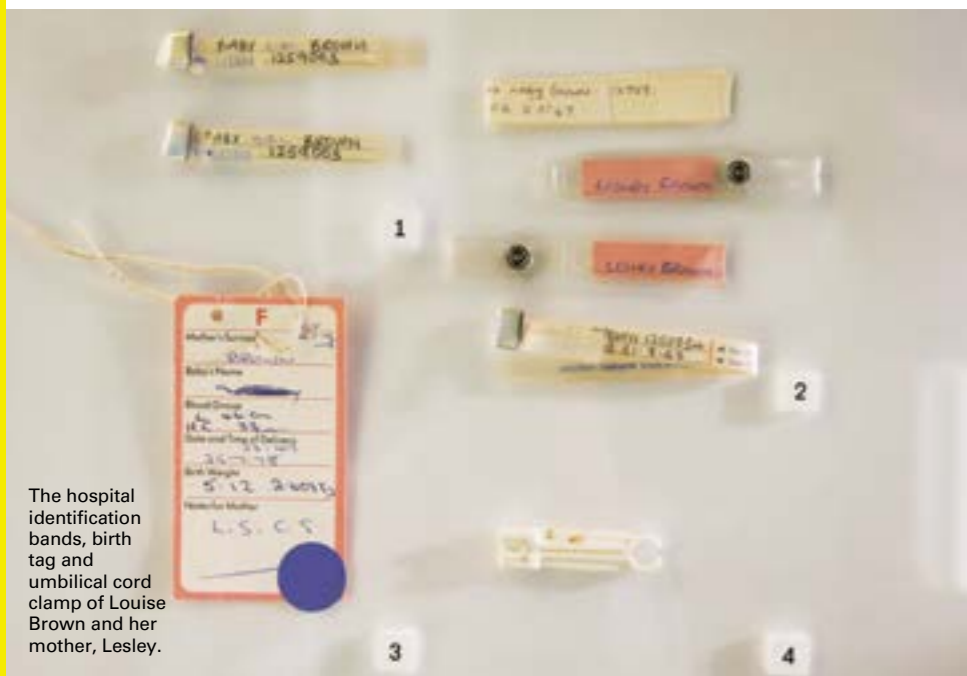


recurrent principles: a wide-ranging curiosity, multidisciplinary training, fruitful collaborations and an element of serendipity. Aristotle made contributions to the fields of biology, botany, chemistry, ethics, history, metaphysics, philosophy, physics, politics, psychology, rhetoric and zoology. He also founded formal logic. Leuwenhoek and Spallanzani were also polymaths. Louise Brown’s birth was made possible by collaboration among many scientists around the world. The story continues to unfold and in this digital age the possibilities it holds are

endless. As an example, a recombinant PLC ζ may be the solution to infertility for many aspiring parents. However, a single mature human sperm contains over 1,700 functional proteins, any of which it might be a potential therapy target, as might be any of the hundreds of egg proteins.

This paper has described management strategies for women who delay childbirth until later in life; health advice for men with infections or harmful lifestyles; new diagnostic tools for male infertility; and the potential for important breakthroughs due to the deployment of molecular biology tools. Polymathy, a trait linked with groundbreaking discoveries, is increasingly discouraged in the current era of specialisation. However, given the explosion of data growth and knowledge across different fields, interdisciplinary collaboration is essential for optimal progress in this, as in any other, field. 

Zara Josephs is an independent researcher, specialising in clinical biochemistry and molecular pathology. To see the references, view the article online at thebiomedicalscientist.net



The hospital identification bands, birth tag and umbilical cord clamp of Louise Brown and her mother, Lesley.