**—Mini Review—**

# *History of the Egg in Embryology*

*Alex Lopata*[1]

[1]*Department of Obstetrics and Gynaecology, University of Melbourne, Victoria, Australia*

***Abstract:*** *From antiquity humans have tried to understand how a new individual is generated after sexual intercourse. A popular notion was that a woman's blood and a man's semen were involved. Aristotle, based on studies of chick embryology, proposed that the mixture produced an egg from which the foetus developed in the uterus. This idea lasted for nearly two thousand years. William Harvey disproved the old notion by finding an empty uterus in a variety of animals soon after mating. Although he did not find any eggs he insightfully proposed "ex ovo omnia", implying that all creatures arise from eggs. This lead to an intensive search for the source of eggs in mammals and resulted in the discovery of the role of the ovaries and fallopian tubes. With the introduction of the microscope in the 17th century sperm were discovered in the semen. Studies involving impregnation of amphibian eggs revealed that single sperm could produce a foetus. Despite this information the mammalian egg remained undiscovered for another 200 years. Finally, Karl Ernst von Baer discovered the ovum by microscopic examination of the ovarian follicle contents of the dog. Studies on fertilization and embryo development in mammals were initiated. With the advent of the cell theory it was recognized that the ovum was a cell, its nucleus was discovered, meiosis was described and the role of the chromosomes in heredity was revealed. Egg and embryo transfer in animals developed quickly and was facilitated by the introduction of tissue culture. The establishment of egg and embryo culture techniques encouraged John Rock and Miriam Menkin to attempt the first studies on human in vitro fertilization and embryo culture. Subsequent refinement of chemically defined culture media set the stage for successful in vitro fertilization, embryo culture and transfer for treating infertility.*

***Key words:*** *Ovum, Egg, Embryology, History, Ancient, Medieval, Modern, Development of concepts*

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e-mail: alex.lopata@gmail.com

One of the earliest descriptions of human conception is found in the ancient Hindu writings (Garbha Upinandas 1416 BCE). The embryo was believed to come into existence after the combination of the mother's blood and semen following sexual intercourse. After the passage of seven nights the embryo was considered to become a vesicle, and an enlarging spherical mass at the end of a fortnight.

In ancient Greece, the writings of Hippocrates (460–370 BCE), although the true author is unknown, suggest that the seed (embryo) comes into being from the semen of both parents. The text proposes that the embryo grows inside a membrane and is nourished by the mother's blood. These writings also advise that the study of embryonic development is possible by opening and examining the incubating eggs of hens, in order to observe daily the progressive growth of the embryo until the chick hatches.

Aristotle (384–322 BCE) can be considered to be the first biologist who designed experiments for studying embryonic development. He systematically observed the development of the chick embryo, and produced accurate descriptions and drawings of the growth of the foetus inside the egg until the chick hatched. He was also the first to describe the development of the beating heart, the blood vessels that arose from it, the development of the umbilical cord and proposed that the blood vessels nourished the growing embryo. Aristotle's study design, involving the opening and examination of sequential stages of bird eggs, can be regarded to be the earliest comparative experiments in embryology. Records and illustrations of these investigations have survived to the present day.

However Aristotle proposed that in mammals, including humans, an egg was formed in the uterine cavity and conception occurred by the action of the male semen on coagulated blood in the uterus, soon after mating. Galen (129–210 ACE) taught that the semen of women originated in the female testes and travelled via the fallopian tubes to the uterus, where it mingled with the male semen to produce an embryo.

The ideas of these two teachers persisted for over a thousand years. In Italy the anatomist Vesalius, and his pupil Fallopius, observed vesicles in the female testes. The latter provided an accurate description of the fallopian tubes, which were named after him, but he made no links between the fluid-filled vesicles and the tubes attached to the uterus. Subsequently Fabricius, a pupil of Fallopius, reintroduced the study of hen's eggs and clearly observed their formation in the organ that he called the ovarium. But because of the ideas of Aristotle, who believed that the egg formed in the uterus, Fabricius did not attempt to challenge the old dogma. It was his English student, William Harvey (1598–1602), who designed experiments to test Aristotle's longstanding teaching on where and how the egg and embryo are formed.

Harvey investigated the validity of the very old idea that an organism arises from a visible accumulation of maternal blood and paternal semen inside the uterus. As he was a physician to the King of England at the time, he used deer obtained from the Royal Park, as his main experimental model. In Harvey's experiments the mated does were separated into two groups away from the bucks. At various stages after mating the does in one group were dissected to examine the contents of their uterus. The other animals were not disturbed until they produced foal, to ensure that all the deer that were exposed to rutting were pregnant. To Harvey's surprise nothing was visible to the naked eye in the uterine cavity or the cornua. His findings were expressed in the following way:

> *Repeated dissections performed in the course of the month of October both before the rutting season was over and after it had passed, never enabled me to discover any blood or semen or trace of anything either in the body of the uterus or in its cornua.*

He performed similar experiments on other animal species, obtaining the same results as before which he described as follows:

> *In the dog, rabbit and several other animals, I have found nothing in the uterus after intercourse. I, therefore, regard it as demonstrated that after fertile intercourse among viviparous as well as oviparous animals, there are no remains in the uterus either of the semen of the male or female emitted in the act, nothing produced by a mixture of these two fluids, as the medical writers maintain, nothing of the menstrual blood present as 'matter' in the way Aristotle would have it; in a word, that there is not necessarily even a trace of the conception to be seen immediately after a fruitful union of the sexes.*

Harvey's studies disproved all of the traditional ideas on conception that prevailed amongst physicians for about 2000 years. His translated words "not necessarily even a trace of the conception to be seen…" indicates that the conception was not visible to the naked eye, while some of his other writings suggest that Harvey was aware of the possibility that the conceptus, in viviparous animals, may be too small to be seen by unaided observations. At about the same time Harvey studied the development of the chick using incubated hen eggs. Thus a clear picture emerged on the embryology of bird eggs, but there was an absence of information on the existence of yet to be seen mammalian eggs. Harvey probably opted for the eventual discovery of mammalian eggs when he postulated "Ex Ovo Omnia", the dictum that has been interpreted to mean that all living creatures come from an egg.

Harvey's book "De Generatione Animalium", appeared in 1651 and it undoubtedly aroused a lot of interest at institutions of learning. To some extent his writings could have delayed advancements in embryology for he pronounced that the ovaries do not have a role in generation, and he failed to recognize the similarity between the ovaries of birds and animals. Despite this potential set back, the anatomists working at Leiden, around 1666, made further progress in discovering the functions of the ovaries in mammals. In particular, Jan Van Horne and his student Jan Swammerdam, reached the conclusion that human "female testes" were similar to the ovaria of birds and produce eggs. At about this time two other former students of the Leiden group, Niels Stensen and Regner de Graaf, developed similar concepts about the significance of the ovaries in mammals [12, 13]. Stensen (1667) introduced the term "ovary" and both he and de Graaf supported the principle that mammalian organisms derive from eggs released by the ovaries. In the book written by de Graaf the entire female genital tract, including the ovaries and fallopian tubes are described and accurately illustrated. He considered the ovarian follicles and their contents to represent eggs. In his view the corpus luteum formed before ovulation and that its growth squeezed out the egg enabling it to be released from the ovary [13].

Earlier, Wharton (1656) suggested that the male semen reaches the mammalian ovary by passing through the uterus and fallopian tubes. He also proposed that after the semen fertilizes ovarian eggs they pass through the fallopian tubes to reach the uterus. However, it was de Graaf (1672) who investigated these speculations by dissecting the rabbit genital tract at various stages after mating. Thus, although de Graaf did not observe actual eggs, he demonstrated that the contents of the follicles enter the fallopian tubes and develop in the uterus. He also found that the number of empty ovarian follicles conformed to the number of conceptuses in the uterus. He discovered that fertilized eggs, which he believed originated in the ovary, became detectable as spherical bodies in the fallopian tube by 3 days, and larger vesicles in the uterus by 4 days after mating [13]. This work completely invalidated the doctrine of an empty uterus, as well as the claim that the ovaries did not play a role in generation, previously promulgated by Harvey. Corner has put forward the view that if a microscope were available to de Graaf during these investigations he would have discovered the definitive mammalian ovum at that time [13].

A few years later in 1677 Antoni van Leeuwenhoek, a draper skilful in making optical lenses, built a microscope containing a glass bead lens and a moving stage, to study human semen. He observed large numbers of living organisms, which he called “animalcules” or “spermatic worms”. He described these findings in a letter, originally written in Dutch then translated for him into Latin, which was published in the *Philosophical Transactions* (1679) of the Royal Society in London. The letter also points out that a medical student (Mr Ham) initially observed these living microscopic creatures in an ejaculate of a man who had gonorrhoea, and that van Leeuwenhoek confirmed the presence of the animalcules. Subsequently, he examined his own semen, obtained from “my conjugal relations”, and found that the microscopic animalcules were present in a healthy man. Cole writes that over the next few years Leeuwenhoek examined and illustrated the structure of the seminal animalcules obtained from a range of animals and became convinced that their role is to impregnate the egg for procreation [12]. Using dogs he then investigated and demonstrated that semen containing living animalcules can be found inside the uterus after mating. He therefore discovered the essential missing link in the physiology of reproduction, which Harvey and de Graaf could not elucidate.

In contrast to the views expressed by Cole [12], an article written by Corner [13], proposes that van Leeuwenhoek (1683) believed that only the sperm contributed to the formation of the embryo. As he could not find eggs in the uterus he rejected their importance in reproduction and even denied the existence of eggs in mammals. Van Leeuwenhoek’s view at the time was that the uterus was a place of incubation, and contributed nourishment, to the seminal animalcules that contained all the precursors required to form an embryo.

Victor Albert von Haller (1708–1777), an eminent physiologist, initially agreed with Harvey that semen does not enter the uterus, but later corrected this view after finding that mated sheep contained semen in the uterine cavity. He also contradicted de Graaf’s proposal that ovarian vesicles are eggs. Yet Haller believed that the ripe vesicle contained a precursor of the embryo, which is discharged into opening of the fallopian tube, when the follicle released its contents. The oviduct was believed to deliver the preformed embryo into the uterus, where it began to grow. Haller was convinced that conception, following impregnation of the egg, occurred in the ovary rather than in the oviduct or the uterus. This is not an outrageous idea since we now know, from the publications of a number of 20th century biologists, that in animals such as primitive insectivores, some bats and the shrew that fertilization occurs while the eggs are in the ovary, and the zygotes enter the oviduct during pronuclear development [4].

Lazzaro Spallanzani, an Italian biologist and priest, in 1785 published a description of his experiments with amphibian eggs. He demonstrated that amphibian eggs were fertilized after they were released from the body of the animal and that only eggs that came in contact with semen would develop [15]. Physiologists in his time believed that semen released a vapour or an aura that could fertilize eggs without direct contact. Spallanzani disproved this idea, and showed that only direct contact of semen with eggs induced their development. He was the first to artificially fertilize frog eggs, and later succeeded in other amphibians and in the dog. In later experiments he showed that semen could be greatly diluted and still induce development of eggs that were exposed to it. Spallanzani also filtered the diluted semen using blotting paper, and showed that the filtrate lost its potency after passing through six filters. It is astonishing, therefore, that he attributed the action of semen on eggs to be due to a chemical or mechanical effect, rather than to the seminal animalcules that were discovered by van Leeuwenhoek in the preceding

century.

A widespread belief at the start of 1800 was that conception occurred in the ovary. In 1824, experimental results reported by the Swiss physiologist Jean-Louis Prévost and the French chemist Jean Baptiste Dumas, disputed this idea. These scientists described the formation of Graafian follicles in the ovaries of the rabbit and the dog, the establishment of corpora lutea after the follicles burst, and the transport and growth of the "ovum" in the uterus. They discarded the notion that ovarian follicles are eggs because nothing of that size could be found in the uterus. Nor was it feasible that intact ovarian follicles could be released and travel through the narrow lumen of the oviduct. Also, the early vesicular embryos that they observed in the uterus were many times smaller than the original follicles. Even 12 days after mating the translucent "ovum" or embryo in the uterus, was considerably smaller than the follicles protruding from the surface of the ovary. They also observed that when a breach formed in some of the larger follicles, a possible "egg" was extruded with the released fluid. However, the real nature of the released body was not defined. After this tiny structure exited from the follicle it was more opaque and smaller than the transparent vesicle detected in the uterus a few days later. Prévost and Dumas were, therefore, on the brink of discovering the real ovum, and yet they did not establish the link of an egg being fertilized in the oviduct, and transported to the uterus, to establish a transparent vesicular embryo a few days later.

Prévost and Dumas investigated the fertilization of amphibian eggs, and they claim to have observed a sperm inside the mucinous coat of the egg. They concluded that a single sperm was needed to fertilize an egg. In addition, they demonstrated the role of seminal animalcules in fertilization in both rabbits and dogs [30]. In view of their discovery of such crucial information, it is a quandary why they failed to postulate a sequence of events that started with mating of rabbits, or dogs, followed by extrusion of follicular contents in each animal, then the entry of the liberated follicular body into the oviduct, subsequent contact of the animalcules (sperm) with the small spherical body within the oviduct, leading to the fertilization of the follicular body and the formation of a barely visible embryo, then development of this opaque structure into a more transparent spherical ovule, and finally the passage of the enlarging vesicular ovule into the uterus. Corner summarised the outcome of the experiments reported by the two diligent investigators as follows [13]:

> *In spite of all their work, Prévost and Dumas had no idea as to the true nature of the mammalian ovum, nor of what occurs between ovulation and the appearance of blastocysts in the tube some days afterward.*

Karl Ernst von Baer recognized and accurately described the mammalian ovum in 1827 (some say in 1826), two or three years after the publication of the investigations by Prévost and Dumas. As this had a profound influence on the further progress of embryology it is worth noting how two authors in the early 20th century described the discovery.

Cole writes [12]: "*The belated discovery by von Baer of the true ovum of Mammals, which, however, he called the ovulum, is not only important in itself, but still more so on account of its bearing on the history of embryology in general*".

Corner says [13]: "*This observation* (ovum of a dog seen through a microscope) *was made about May 1, 1827, and was soon confirmed by the finding of similar bodies in the ovarian follicles of many other species, including the human.* (This) *truth about the ovum was carried to all the laboratories of Europe, and the world of science knew that the problem of two thousand years was solved*."

Previous investigators thought they saw the ovum inside ovarian follicles but they did not describe its morphology in sufficient detail. Microscopic examination of the structure seen within the follicle enabled von Baer to realize that the ovum was hidden within the protruding mass, which he called the discus proligerus, and is now known as the cumulus oophorus. In addition to detailed descriptions of the eggs of different animals, von Baer gave a full account of how to recover and examine them so that other embryologists could confirm his findings. Some historians believe that von Baer failed to observe the nucleus of the ovum [12], but others have suggested that that he would have seen the germinal vesicle but did not comprehend its nature [15]. When the ovum was discovered it was not known as a cell. In 1834 Adolph Bernhardt, a Polish biologist, described the germinal vesicle of the mammalian ovum in his doctoral thesis, a few years before the cell doctrine appeared. The cell theory of Matthias Schleiden (1804–1881) and Theodore Schwann (1810–1882) was propounded in 1838 and soon afterwards it was widely accepted that all living things were

composed of cells and the cell was the structural unit of life. At this time Schwann proposed that the ovum was also a cell, yet more than 20 years elapsed before Karl Gegenbaur, in 1861, demonstrated convincingly that the ova of all vertebrates are cells. William Harvey's dictum that living creatures come from an egg was, therefore, ready to be confirmed more than 200 years after it was conceived.

In 1683 Leeuwenhoeke suggested that one animalcule enters the yolk of the egg, and that this ensures that the embedded animalcule will be nourished as it develops into the fetus in the uterus. When von Baer discovered the ovum he proposed that one animalcule, which he named spermatozoon, attached to the surface of the egg to induce its development. However, more than 150 years after Leeuwenhoeke's perception, the physiologist Allen Thomson (1839) comments: "*Neither experiment nor observation enables us to form the most distant conjecture what the nature of that action may be, which, from the influence of the male product, confers upon the ovum a new and independent life*". Nevertheless progress did take place, for in the next few years, Martin Barry (1843) demonstrated that spermatozoa penetrated through the zona pellucida of fertilized rabbit eggs obtained from the fallopian tube. Yet it is more likely that the discovery of fertilization should be credited to Newport (1853) who reported that he observed the passage of active spermatozoa into the substance of the frog egg. By 1855 Robert Remark showed that embryonic cells originated from the fertilized egg. He introduced biological fixatives and was able to visualize the plasma membrane of the egg and cleaved cells. Remark's work established the continuity between the zygote and developing embryo, and provided the idea of the transmission of hereditary characteristics through the fertilized egg.

During the highly productive period for biology, in the late 19th century, Edouard Van Beneden (1883) discovered chromatin reduction during egg maturation [18], which advanced information on the morphological aspects of heredity. However, it was August Weisman (1883–1884) who realized the significance of Van Beneden's discovery. Weisman pointed out that both female and male gametes must undergo a special division that halves the number of chromosomes in the nucleus, in preparation for fertilization that restored the diploid complement. He called this process meiosis to distinguish it from mitosis. In addition, Wilhelm Roux (1850–1924) suggested that corresponding chromosomes were linearly arranged during meiosis and later separately transferred to daughter cells. Shortly afterwards, Eduard Strasburger (1884), and Oskar Hertwig, independently proposed that the key event in fertilization of the mature egg involved "fusion" of the male and female pronuclei, obtaining further support for the transmission of heritable characteristics by the nucleus. During this intense period of research Walter Flemming (1882), described chromosomes and provided an account of mitosis that has largely stood the test of time. During this golden age for biology, Roux who was considered to be the pioneer of experimental embryology, promoted the use of chemistry and physics, and experimental intervention in cleaving embryos, to study the mechanics of development [29].

The enhanced research activity produced valuable descriptions of fertilization, including oocyte maturation. Among them were those of Van Beneden in the rabbit [32], Hertwig (1876) and Fol (1877, 1879) in sea urchin and starfish, and Van Beneden and Julin (1880) in the bat. Van Beneden also described in detail mammalian egg development to the blastocyst stage [3]. Before the end of the 19th century, Sobotta (1895) published an authoritative description of mouse egg maturation, fertilization and cleavage, using one of the first histological preparations for studying early development.

It is now clear that the discovery of the ovum promoted rapid advances in oocyte morphology and physiology in the second half of the 19th century. The findings, in a large range of animals, also demonstrated that fertilization involved the union of egg and sperm nuclei and lead to the first insights into the cellular basis of inheritance of maternal and paternal characteristics. One of the first attempts to culture and fertilize eggs *in vitro* was made by Schenk [31], although these trials were not successful. Walter Heape (1891) described the first successful recovery and transfer of fertilized eggs between animals, using an Angora rabbit as the donor and a Belgian line recipient, which resulted in the birth of young [19]. Some time later Lewis and Gregory [21], achieved *in vitro* culture and development of fertilized rabbit eggs, and as the cleavage divisions were filmed for the first time, the outcome provided audiences the spectacle of embryonic development.

After the successful egg transfers between rabbits, performed by Heap, a similar experimental model was used by Nicholas [24] in the rat, by Warwick *et al.* [33] in the goat, Fekete and Little [14] in the mouse, Warwick and Berry [34] in sheep, and in 1951 by Willett *et al.* in the cow [31], and Kvasnicki in the pig [20]. These investigations revealed the importance of the close

relationship between the maturity ("age") of the egg and the receptivity ("age") of the uterus. In addition, the studies showed that eggs maintained in culture remained viable and produced normal conceptions. By 1956 embryo culture followed by transfer to the genital tract of the recipient and birth of viable offspring was well established [2].

Van Beneden was the first to describe and accurately illustrate cleavage of the fertilized egg to the blastodermic vesicle (blastocyst) stage in the rabbit [32]. Five years later, Schenk, claimed that he had obtained 2-cell stages in the rabbit and guinea pig after inseminating their eggs *in vitro* [31]. But between 1900 and 1920 Albert Brachet, a student of Van Beneden, and promoter of "causal embryology", clearly pointed out that direct experimentation on eggs and embryos remained inadequate due to lack of effective tissue culture methods. After 1930, Gregory Pincus and his colleagues made valuable progress in culturing fertilized eggs of the rabbit to the late morula stage [25–27]. Using complex culture media such as plasma and embryo extracts, as well as more defined media, these workers were able to maintain the embryos *in vitro* for 2 days, and after transfer to recipients apparently normal offspring were born. At about the same time Lewis and Wright (1935) photographed cleavage stages of mouse embryos [22]. Subsequently, Hammond, using a physiological salt solution containing glucose and egg white, successfully cultured 8-cell and 4-cell mouse embryos to the blastocyst stage [17]. In a further refinement, Adams, used Krebs-Ringer bicarbonate, supplemented with bovine serum albumin, to culture rabbit embryos [1]. Whitten, extended this work to the mouse and showed that 8-cell embryos could be cultured to blastocysts, while adding lactate to the medium, enabled the development of late 2-cell embryos to blastocysts [35, 36].

Before examining further historical developments, related to embryo culture, it is rewarding to consider the pioneering work on *in vitro* fertilization of human eggs reported by Rock and Menkin in 1944 [28]. It is worth keeping in mind that the investigators embarked on groundbreaking research with only their insight to guide them. The work took 6 years during which nearly 800 eggs were recovered from ovarian follicles in women having various surgical procedures. As hormone assays were not available, the operations were planned for the late follicular phase so that oocytes were collected before the expected time of ovulation. Of the total number of oocytes retrieved, 138 were exposed to sperm. During these exploratory studies a number of conditions were varied. These included the type of cultures used for oocytes before and after insemination, the duration of exposure to sperm and the concentration of sperm in the inseminating suspensions. In the successful experiments the eggs that were collected were washed with Locke's solution, underwent maturation culture in serum for 22 to 27 hours, they were then inseminated for one hour in sperm washed in Locke's solution, and finally incubated in serum for 40 to 45 hours. In one experiment, two eggs appeared to cleave to the 2-cell stage, and each blastomere contained a nucleus. In another experiment, using similar methods, two embryos containing three blastomeres were observed. One degenerated but the other was fixed and sectioned. Each blastomere contained a nucleus with features of stained chromatin. This innovative work preceded the discovery of capacitation of sperm, antedated the improved culture technology that was launched in the second half of the 20th century, and was the forerunner of clinical *in vitro* fertilization. I would like to believe that John Rock and Miriam Menkin foresaw the possibilities for infertility treatment, which took place more than 40 years after they started their investigations. The successful outcome of the early work on in vitro fertilization, which Rock and Menkin did not live long enough to experience, is summarised in Table 1.

The potential of embryo culture and *in vitro* fertilization, for experimentation and for clinical applications, became apparent with the introduction of simple chemically defined media developed by Whitten [35, 36]. Shortly afterwards, McLaren and Biggers showed that mouse embryos cultured to blastocysts, and then transferred into the uterus of synchronous recipients, gave birth to ostensibly normal offspring [23]. A year later, Min Cheuh Chang fertilized rabbit eggs *in vitro*, observed normal embryo development, and following transfer of the embryos into the uterus of genetically distinct recipients, obtained visibly normal young with the genetics of the egg donors [11]. Since chemically defined media were used in both of the above studies, the work could be reproduced under different conditions in other laboratories, and applied in different species to determine whether there were any specific requirements for the development of the zygote to the blastocyst in different animals. Further refinements in embryo culture media were made by the comprehensive and meticulous studies of Ralph Brinster, who evaluated the levels of essential ingredients required for normal preimplantation embryonic development [6–10]. In addition, his studies

**Table 1.** A summary of the birth of children conceived by IVF in various countries, arranged in a time sequence in rows 1–10

| Country | Date | Sex | Investigators |
|---|---|---|---|
| 1 England | Jul, 1978 | F (Louise) | Edwards, Steptoe |
| 2 Australia | Jun, 1980 | F (Candice) | Lopata, Johnston |
| 3 USA | Dec, 1981 | F (Elizabeth) | Jones, Veek |
| 4 France | Feb, 1982 | F (Amandine) | Testart, Frydman |
| 5 Germany | Apr, 1982 | M (Oliver) | Kniewald, Trotnow, |
| England | Apr, 1982 | M, M | Craft, Green |
| 6 Austria | Aug, 1982 | M (Zaltan) | Feichtinger, Szalay |
| 7 Sweden | Sep, 1982 | F (Maria) | Hamberger, Wikland |
| 8 Singapore | May, 1983 | M | Ng, Ratnam |
| 9 Japan | Oct, 1983 | F | Suzuki |
| 10 Canada | Dec, 1983 | M (Robert) | Gomel, Poland |

The "Date" column shows the month and year of each birth. The "Sex" column shows the gender of the child born, and her/his name if available. The "Investigators" column shows the surname of the main clinicians/scientists involved with the conception and birth. In row 5, England is listed for a second time because of the birth of the first IVF twins in London.

defined the optimum osmolarity, pH, energy sources, amino acid requirements, and the culture volume and physical support, required for dependable embryo development (Reviewed in 1998 by R. E. Hammer [16]; J. D. Biggers). Of particular interest was the identification of pyruvate as the essential energy source for embryo cleavage in most mammals. The stage was therefore set for the dramatic developments in the understanding, manipulation and application of oocytes and embryos, which lead to the reproductive revolution by the turn of the 20th century.

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