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The rise of embryology in Italy: from the Renaissance to the early 20th century

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In the present paper, the Italian embryologists and their main contributions to this science before 1900 will be shortly reviewed. During the twentieth century, embryology became progressively integrated with cytology and histology and the new sciences of genetics and molecular biology, so that the new discipline of developmental biology arose. The number of investigators directly or indirectly involved in problems concerning developmental biology, the variety of problems and experimental models investigated, became too extensive to be conveniently handled in the present short review (see "Molecularising embryology: Alberto Monroy and the origins of Developmental Biology in Italy" by B. Fantini, in the present issue).

There is no doubt that from the Renaissance to the early 20th century, Italian scientists made important contributions to establishing the morphological bases of human and comparative embryology and to the rise of experimental embryology. Italian embryologists were often at the centre of passionate debates concerning basic problems of early embryology such spontaneous generation, preformism versus epigenesis or ovism versus animalculism. Great scientists like Marcello Malpighi and Lazzaro Spallanzani marked turning points in embryology by introducing the microscopic observation of embryos and controlled experimentation in embryology, respectively. The Stazione Zoologica, established in Naples in 1872 by the German Anton Dohrn, became an international scientific centre in which fundamental experiments for the history of developmental biology were performed (see "Molecularising embryology: Alberto Monroy and the origins of Developmental Biology in Italy" by B. Fantini, in the present issue).

Embryology in the XV and XVI centuries

After the first embryological observations and theories by the great ancient Greeks Hippocrates, Aristotle and Galen, embryology remained asleep for almost two thousand years. In Italy at the beginning of Renaissance, the embryology of Aristotle and Galen was largely accepted and quoted in books like *De Generatione Animalium* and *De Animalibus* by Alberto Magno (1206-1280), in one of the books of the *Summa Theologica* (*De propagatione hominis quantum ad corpus*) by Tommaso d'Aquino (1227-1274) and even in the *Divina Commedia* (in canto XXV of Purgatorio) by Dante Alighieri (1265-1321). The activity of Dante's contemporary, Mondino de Luzzi (1270-1236) brings to the more practical aspects of embryology at this period. Mondino was the most outstanding figure among the Bolognese anatomists in what is really the first period of the revival of biology. He personally dissected human embryos and published in 1316 a book *Anatomia* in which some important observations on the anatomy of the uterus and on the physiology of embryo formation can be found. After Mondino, more than a couple of centuries must pass until the age of the great Italian macro-iconographer embryologists of the sixteenth century, who

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A Leonardo Da Vinci (1452-1519) drawing including a human fetus in the womb. British Royal Collection (Windsor Castle).

did fundamental morphological observations about the developing embryo: the first great step in the history of embryology.

The ancestor of these was Leonardo da Vinci (1432-1519). Leonardo's embryology is contained in the third volume of his notebooks (*Quaderni d'Anatomia*) that remained unknown until early 1900 (Fig. 1). His dissection of the pregnant uterus and its membranes are beautifully depicted. He was acquainted with amnios and chorion, and he knew that the umbilical cord only contained vessels. Concerning the foetus, he writes:

The veins of the child do not ramify in the substance of the uterus of its mother but in the placenta, which takes the place of a shirt in the interior of the uterus which it coats and to which it is connected but not united… The child lies in the uterus surrounded with water, because heavy things weigh less in water than in air and the less so the more viscous and greasy the water is.

Leonardo was the first embryologist to make quantitative observations on embryonic growth; he defined, for instance, the length of a fully-grown embryo as one *braccio* (one arm) and noticed that the liver is relatively much larger in the foetus than in the grown-up man. He also observed that the human body grows daily far more when in the womb of its mother than after birth. The application of the concept of change in weight and size with time was thus first made by Leonardo more than one hundred years before William Harvey.

After Leonardo three great embryologists were born: Ulisse Aldrovandi and Cesare Aranzio in Bologna and Girolamo Fabrizio d'Aquapendente (nr. Viterbo). They were distinguished anatomists and left beautiful and accurate drawings and descriptions of the human embryo and of embryos of several other species as well. Aristotle's ideas begun to be critically discussed and some rejected, but the new theories were often erroneous, confusing and quite fantastic. The religious and social ideas of the age, together with the lack of any experimental methods, techniques and instruments to verify their theories produced the scientific thought of these embryologists.

Aldrovandi (1522-1605) was the first biologist since Aristotle to open the eggs of hens regularly during their incubation period, and to describe in details the stages of its development. From then on chick's egg became the most studied object by embryologists of those centuries. In his *Ornithologiae*, published in 1599, Aldrovandi set out to describe all the known kinds of birds. The book is sumptuously illustrated, but there is only one picture of embryological interest, namely, a chick in the act of hatching.

Giulio Cesare Aranzio (1530-1589) published in 1564 a rather important book of embryology, *De Humano Foetu libellus*. He was the first to argue that the function of the placenta (*jecor uteri*) was to purify the blood supply to the foetus and that the maternal and foetal blood vessels are not connected. He discovered the vessel that connects the umbilical vein to the inferior vena cava and that brings his name.

Girolamo Fabrizio d'Aquapendente (1533-1619) professor of Anatomy in Padua was probably the most important embryologist of this age. In his famous books *De Formatione Ovi et Pulli Pennatorum* and *De Formato Foetu* of 1604 (Fig. 2), however, he introduced a number of grave mistakes and misleading theories. One of his mistakes was that the heart of the foetus has no proper function, but beats only in order to preserve its own life. Moreover, he exhumed the Aristotle's theory that the male semen plays but a secondary role in the generation of the embryo, by activating the egg through the *aura seminalis* but without contacting it. Fabrizio was a good comparative embryologist and it is upon this ground that he deserves praise: his plates were far better than anything before and for a long time afterwards. He dissected embryos of man, hen, rabbit, guinea-pig, mouse, dog, cat, sheep, pig, horse, ox, goat, deer, dogfish and viper, a comparative study which had certainly never been made previously. Fabrizio must also be remembered as teacher of the great William Harvey, who spent five years in Padua attending anatomy lessons by Fabrizio.

Gabriele Falloppia (1523-1562), born in Modena and professor of anatomy in Ferrara, Pisa and Padua, must be mentioned as discoverer of the uterine tube, the female reproductive organ which bears his name, but his service to embryology was only indirect.

Two other scientists of this age who left embryological studies of some importance were Girolamo Cardano (1501-1576) and Costantino Varolio (1543-1575). Cardano's main thesis was about the origin of chick's embryo from the egg, one of the most debated subjects of that time. He attempted to mediate two theories by Aristotle and Hippocrates by maintaining that the limbs of the embryo derived from the yolk, while the rest of the body came from the white. Varolio was professor of Anatomy at Bologna and Rome. He treated of the formation of the embryo in a book appeared in 1551, but quite inadequately. He had certainly opened hen's egg and described the fourth-day embryo as *forma minini faseoli* (in the shape of a very small bean).

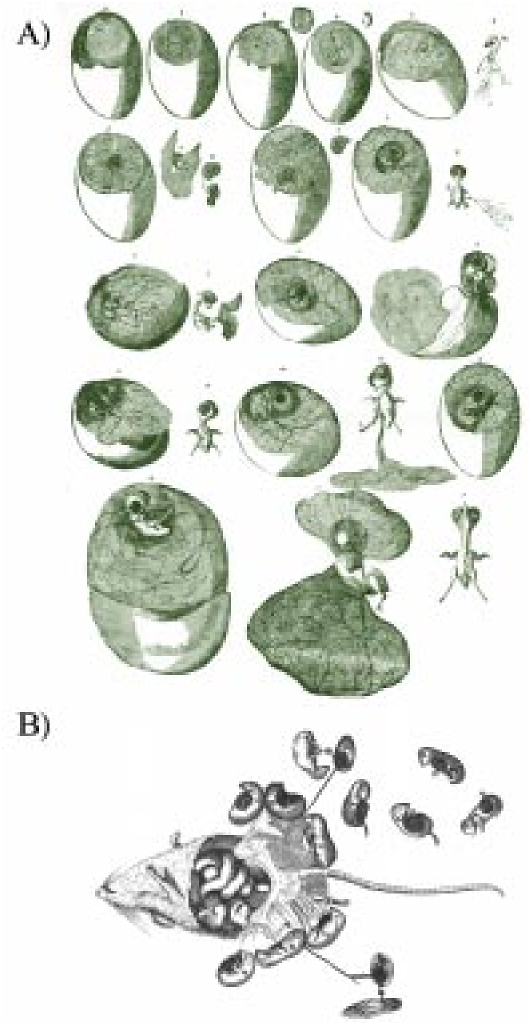

Fig. 2. *Fabrizio d'Aquapendente's drawings of* **(A)** *hen egg development and* **(B)** *mouse embryos (from "De Formatione Ovi et Pulli Pennatorum" and "De Formato Foetu", respectively).*

Embryology in the XVII and XVIII centuries

The last of the macro-iconographic group was Emilio Parisano (1585-1652). Parisano, a Venetian, dealt with embryology in the book *De Subtilitate*, published in 1623. Some of his observations are noteworthy and he should be remembered for his opinion that the heart of the chick begins to beat some time before any red blood appears in it.

Also to be mentioned is the publication in Padua, in 1616, of *De Monstruorum Natura* by Fortunio Liceti (1577-1657). This book marks the beginning of the study of embryo malformations. Liceti describes real and imaginary monsters and searches for the cause of their origin giving fantastic explanations. He believes in the transmission of the acquired characters, since he thinks that a son will show the same mutilations as his parents. Liceti published in 1618 *De spontaneo Viventium Ortu* (see below) in which he supports the theory of spontaneous generation.

Around 1602 Zacharias Jansen in Holland built up a new, more powerful optical composite instrument, that in 1625 Francesco Stelluti, a pupil of Galileo, proposed to call *microscopio*. It was the beginning of a new age in biology and embryology. Galileo introduces the use of microscope in science and the first microscopic observations on small animals and plant seeds were made within the Accademia dei Lincei, established in Rome in 1603. In England, William Harvey (1578-1657) marks the transition from the static to the dynamic conception of embryology, from the study of the embryo as a changing succession of shapes, to the study of it as a causally governed organization of an initial physical complexity. Iconography did not die: on the contrary, the improvement of the microscope gave it a new life, and a micro-iconographic school emerged with the great personality of Marcello Malpighi.

Marcello Malpighi was born in Crevalcore (nr. Ferrara) in 1628 (Fig. 3). He taught Medicine at the University of Bologna, Pisa and Messina. In the 1691 he became the private doctor of Pope Innocenzo XIII in Rome, where he died in 1694. He is considered as the founder of the microscopic animal and plant anatomy. In the year 1672 Malpighi, who had been working for many years previously on various embryological problems with the aid of a simple microscope, published his treatises *De Ovo Incubato* and *De Formatione Pulli in Ovo* (Fig. 4), which he sent to the Royal Society of England. The letters that Malpighi sent to the Royal Society about his embryological observations and published on *Philosophical Transactions* may be considered as one of the first examples of a scientific paper published on a journal with international diffusion. The development of the hen's embryo is described during the very first hours of incubation. Using the microscope, he concentrates his attention on the *cicatricula* (little scar) as the place where development begins and describes for the first time the blastoderm, the neural groove, the optic vesicles, the somites, the earliest blood-vessels and heart. He made also the first observations on the embryology of plants.

It is generally considered that the controversy over preformation *versus* epigenesis began with Malpighi. Preformism in modern terms would correspond to growth without differentiation, all the complexity of the finished form being supposed to be present *ab initio*. In fact, Malpighi was led towards preformism by the fact that development begins after fertilization as the egg passes down the oviduct, and in the most recently laid eggs gastrulation is already over, so that in his research he could never observe the very early stages of egg development. Thus he writes:

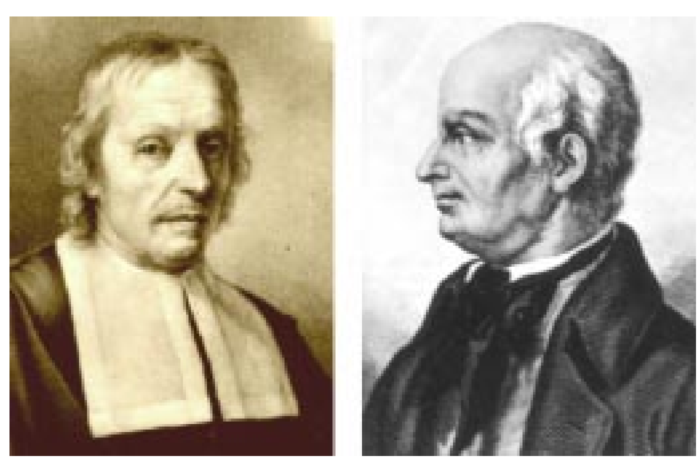

Fig. 3. *Marcello Malpighi (1628-1694) (left) and Lazzaro Spallanzani (1729-1799) (right).*

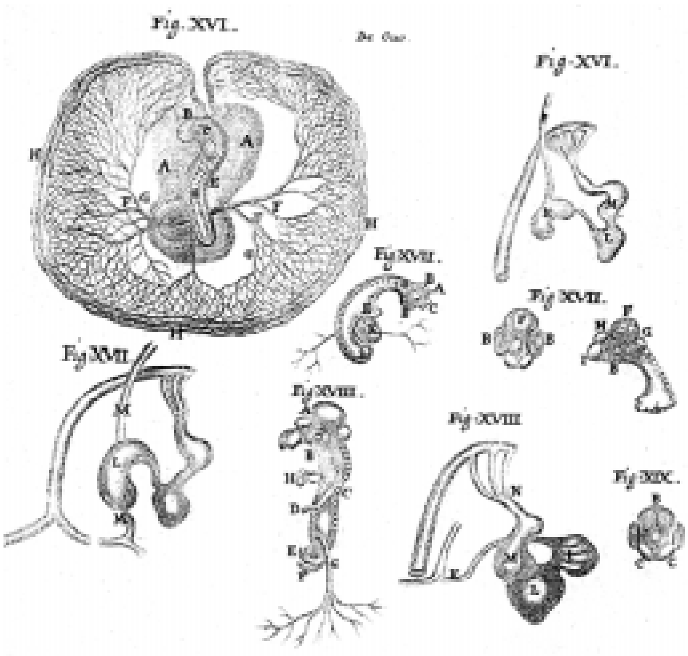

Fig. 4. *Malpighi's drawings of chick embryo development (from "De ovo incubato").*

When we enquire carefully into the production of animals out of their eggs, we always find the animal there, so that our labour is repaid and we see an emerging manifestation of parts successively, but never the first origin of any of them.

Besides to the development of the hen's egg, Malpighi studied the development and the female reproductive apparatus of the silkworm in *Dissertatio Epistolica de Bombice*. He was also a talented draftsman; his copper engravings of the *Bombix* female reproductive apparatus are of excellent quality. In addition to his important contributions to embryology, Malpighi was much interested in the structure and function of reproductive organs. At that time, he was aware of the work of the Dutchmen Régnier de Graaf (1641-1673) and Jan Swammerdam (1637-1680) who in 1672 discovered the ovarian follicles of mammalians (although they mistook them for eggs), and of Antoni van Leeuwenhoek (1632-1723) who in 1677 had described the spermatozoa (*animalculi spermatici*) in human semen. As a result of his interest and microscopic observations, Malpighi was able to make important contributions toward understanding the ovary, describing the corpus luteum and the uterus, reporting on the so-called "Malpighi-Gartner ducts". According to Spallanzani, Malpighi was also the first one to try, without success, artificial insemination in animals:

The first one to think of artificially fertilizing animals was our immortal Italian, Marcello Malpighi, who having taken eggs from the silkworm butterfly, bathed them with the fertilizing fluid of the male. Indeed, the result was not that which this curious naturalist had hoped for, since the eggs so treated remained infertile…

Around the mid XVII century the old controversy about the spontaneous generation of small animals (see, for example, De spontaneo viventium ortu, 1618, by Fortunio Liceti) was challenged by the celebrated experiments of Francesco Redi.

Redi (1626-1696) was born in Arezzo and spent his academic life in Pisa, Rome, Naples, Padua and for the most part in Florence. He was a physician rather than an embryologist, but he must be considered in the present review for the impact that the problem of spontaneous generation had for early embryology. In his book "*Esperienze intorno alla generazione degli insetti*" (Experiments about insect generation) published in 1668, Redi gave the first experimental demonstration that insects do not originate from meat by spontaneous generation. His experiment is too well known to be reported here, but it should be said that his observations received many criticisms. On the other hand, Redi himself continued to admit the spontaneous generation of insects living in the galls of the oak. Eventually, Malpighi and his pupil Antonio Vallisneri demonstrated this also not to be true by observing the eggs and larvae of the insects on the plants.

A pupil of Redi was Stefano Lorenzini (1645-1725), born in Florence, a follower of Malpighi. In1678 he published a description of the anatomy of the fish Torpedo. He recognized the ovary of this fish as homologous with that of hen and studied the early development of the eggs, giving pictures of the blastodisc and of the first stages of neurulation (Fig. 5).

The experiments by Redi and the discovery of eggs of mammals, fish and insects gave support to the theory of ovism that at the end of XVII century was largely accepted. The ovists regarded all embryos as being produced from smaller embryos preformed in the unfertilized eggs. Supporters of such theory were Malpighi and his

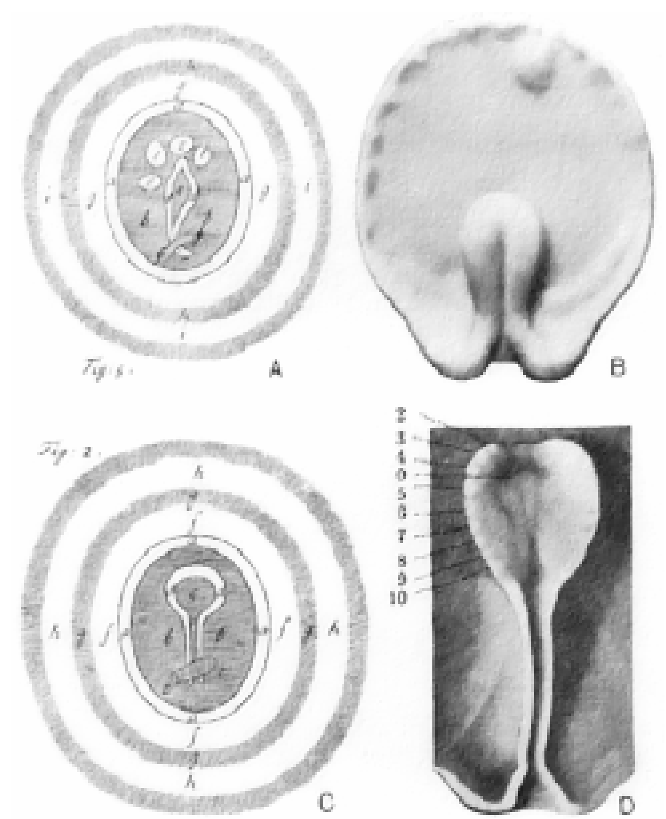

Fig. 5. *Stefano Lorenzini's drawings of the cramp-fish blastodisc and neurulation (from Torpedo).*

pupil Antonio Vallinseri (1661-1730). Vallisneri even argued that the eggs in Eve's ovaries contained minute forms of all human beings to come up to extinction of the species, though he frankly admitted that, in spite of long searching, he had never seen such forms in the eggs of the animals examined. He also fiercely debated with animalculists, who regarded all embryos as being produced from the smaller embryos provided by the male in his spermatozoa, that the existence of so many animalcules were an illusion, since Nature could hardly be so prodigal.

During the XVIII century a significant advance of embryological techniques took place. Hardening of soft embryonic tissues using alcohol (distilled spirits of vinegar) begun to be performed so that they could be better dissected. Dyes were beginning to be used and progress in artificial incubation of hen's eggs were made. At the same time, the increasingly frequent use of controlled experimentation to verify theories and observations lead to the rise of experimental embryology. An eminent scientific personality of this new branch of embryology during the second half of the XVIII century in Italy was Lazzaro Spallanzani (1729-1799).

Spallanzani was born in Scanziano (Reggio Emilia) in 1729 (Fig. 3). In 1754 was appointed professor of philosophy in Modena. In 1769 he moved to the University of Pavia where he remained for the rest of his life as professor of natural sciences. His first biological work, "*Saggio di ossservazioni microscopiche concernenti il sistema della generazione de'signori Needham e Buffon*", (Dissertation about microscopic observations on the generation pattern according to Mr. Needham and Mr. Buffon) published in 1765, was an attack to the view of Buffon and Needham that microscopic protozoa that had been observed in water and in infusions by Leeuwenhoek had formed by spontaneous generation. In a series of well-controlled experiments he showed that gravy, when boiled, did not produce these forms if placed in phials that were immediately sealed by fusing the glass. He writes:

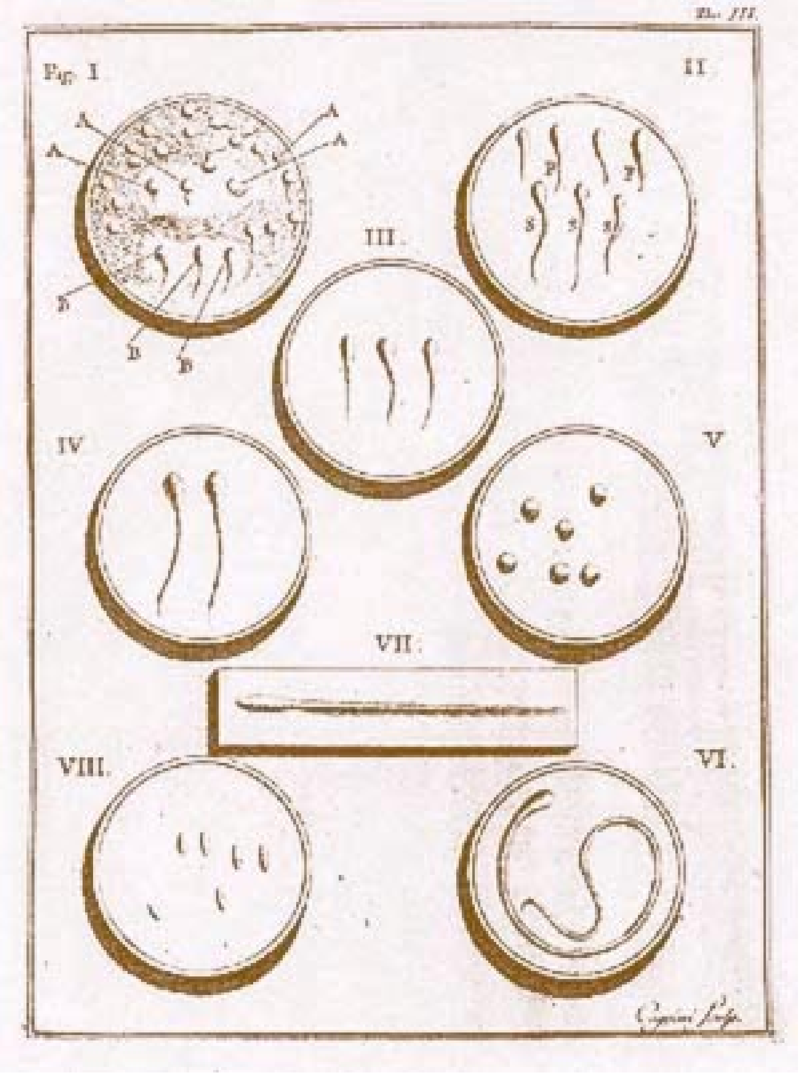

Fig. 6. *Spallanzani's drawings of sperm from various animals (from "Dissertazioni di fisica animale e vegetabile" {Dissertations about animal and plant physics}).*

I cannot see any other conclusion that the origin of these animalcules is from little eggs or seeds or little pre-organized little bodies as you like to say and that we will term germs.

The term "germs" is here used for the first time and will be later used with great success by Louis Pasteur. It is impossible to review here all the experiments and studies carried out by Spallanzani in the following thirty-four years of his life. According to his friend Jean Senebier, Spallanzani performed between eleven and twelve thousand experiments on men and animals, both in vivo and in vitro, in various branches of biology and medicine. Relevant for the history of developmental biology are the results he published in 1768 regarding his regeneration and transplantation experiments. He studied regeneration in a wide range of animals including planarians, snails and amphibians. His transplantation experiments showed great experimental skill and included the successful transplant of the head of one snail onto the body of another. At the request of his friend Charles Bonnet, Spallanzani investigated the male contribution to generation. As the results of earlier experiments with various animals (horse, rabbit, fish, frog and man), Spallanzani supported the prevailing view that spermatozoa were parasites of the semen. His main step forward was his recognition of the semen as the actual agent in fertilization, on precise experimental grounds. Using amphibians, Spallanzani showed that actual contact between egg and semen is essential for the development of a new animal. Moreover, he demonstrated that filtered semen becomes less and less effective as filtration becomes more and more complete. But Spallanzani failed to convince himself that the spermatozoa themselves were the active agents. He concluded:

To me it is the spermatic secretion (and not the spermatozoa) the stimulating fluid that penetrates into the heart of the Egg, resulting in increasing the frequency and strength of its beating and giving rise to a very significant increase of the parts and the following life.

He published these results in 1780 in the book "*Dissertazioni di fisica animale e vegetabile*" (Dissertations about animal and plant

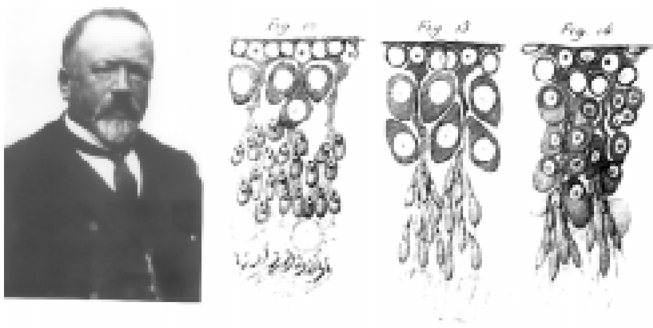

Fig. 7. *Enrico Sertoli (1842-1910) and some of his original drawings (Sertoli, 1878, see text) illustrating branched cells (now called Sertoli cells) with different germ cell associations.*

physics, Fig. 6). Despite his mistake, Spallanzani performed the first successful artificial insemination experiments on lower animals (silkworm, frog, and fish) and a famous one on a bitch.

Sixty-two days after insemination the bitch became mother of three very lively pets, two males and one female. … I may truly say that this success gave me more intellectual satisfaction than any other experiments I had done so far.

The echo that this first successful artificial insemination in a mammal had at that time is comparable to that caused by the cloning of Dolly in our time. These experiments finally disposed of the *aura seminalis* of Fabrizio d'Aquapendente. About Spallanzani's views on embryology, they were largely drawn from his study of the development of the frog's egg (it seems that he sectioned more than two thousand frogs). From these studies onward *Amphibia* will take an important place in the history of experimental embryology. However, in spite of many careful observations, he remained a convinced preformistic ovist and thought he could see the embryo already present in the unfertilized egg.

Embryology from XIX to the early XX century

The scientist who continued the great Italian tradition in embryology during the first half of XIX century was Mauro Rusconi (1776-1849). He was born in Pavia and was a pupil of Spallanzani. Rusconi had a difficult academic career so that he performed most of his research in his private laboratory in Pavia. His scientific activity was more appreciated abroad than in Italy so that in 1831 the Institute of France gave him a gold medal for science. In a famous paper "*Del Proteo anguino di Laurenti*" (On the anguinous Proteus of Laurenti,1819), Rusconi demonstrated that the proteus is an adult animal and not a larval form that maintains gills during development. From this the new order of Perennibranchiata was established. Excellent was also his research on the reproduction of the salamander and the artificial insemination of fish. His most important embryological studies were about the development of the frog's egg (*Déeveloppement de la grenouille commune*, 1826). He observed and gave the right interpretation of the first divisions of the egg, termed to day segmentation, until the formation of something like a *framboise* (mulberry). He writes:

These divisions and subdivisions represent an activity by which nature distributes the elementary molecules of the main systems.

In a sense, this observation anticipates the model put forward fifty years later by Weismann who postulated that the nucleus of the zygote contained a number of special factors or *determinants* that are unequally distributed to daughter cells during segmentation. Moreover, Rusconi described also the pre-gastrulation stages visible without the use of a microscope: the formation of the *falciform sulcus* that brings his name, and that of the blastopore.

Contemporary of Rusconi was Giovan Battista Amici (1786-1863) considered as the founder of plant embryology. Thanks to his skills in making microscopes and to his setting up of a water-immersed objective, he was able to observe small granules of pollen on the stigma of *Pepo macrocarpus* and *Pepo cucubita*. He noticed that pollen produced a short tubule (that he called *budello*) penetrating into the plant ovarium. Most importantly, Amici observed that in the embryonic sac an egg is present before arrival of the pollen and that egg development was initiated by the pollen.

During the second half of XIX century Enrico Sertoli ideally took up Spallanzani's work on biology of reproduction. Sertoli (1842-1910) (Fig. 7) published the first description of his *cellula ramificata* (or "branched cell", later called Sertoli cell) in the seminiferous tubules of the human testis in the year of his graduation in medicine (1865), when he was just 23 years old ("*De l'esistenza di particolari cellule ramificate nei canalicoli seminiferi del testicolo umano*" {On the existence of characteristic branched cells in the seminiferous tubules of the testis} Morgagni, 7, 31-40, 1865) (Fig. 7). Two years later, he published two papers ("*Sulla struttura dei canalicoli seminiferi nel testicolo*" {On the structures of the seminiferous tubules in the testis}. Arch. Sci. Med. 2, 107-147 and 267-295, 1878) in which fundamental observations on spermatogenesis can be found. Sertoli corrected the idea first reported by von Ebner (1871) that spermatozoa arose from the branched cells of the tubules and put forward the idea that these cells have nutritive and/or mechanical functions. Moreover, he showed that spermatozoa derived from round spermatides (or nematoblasts as he called them) describing the stages of spermiohistogenesis and divided the development of spermatocytes (or seminiferous cells) in three stages corresponding to what we call leptotene/zygotene, pachytene and diplotene. He even identified two types of spermatogonia (or germinative cells). Finally, he recognized the presence of a spermatogenic wave along the seminiferous tubule and estimated

Fig. 8. *Angelo Ruffini (1864-1930) in his laboratory at the University of Bologna.*

its length, showing that germ cells often occur in groups, with cytoplasmic bridges between the cells.

The best known Italian embryologist of the second half of the XIX century was Angelo Ruffini (1864-1930) (Fig. 8). He became professor of histology at the University of Bologna in 1894. In 1901 he moved to the University of Sienna where he performed his most important embryological studies. Ruffini was aware that much work was still to be done in the field of histology and embryology using the continually improving histological and microscopic techniques. Nerve endings termed Ruffini's corpuscles are reported in all books of anatomy and histology. However, the most original contribution by Ruffini was about embryology. By using an original method for positioning the egg to be sectioned, he identified in the blastula of the frog's embryo at the pre-gastrulation stage the presumptive regions that give rise to the germ layers, until then believed to differentiate during gastrulation. These observations deeply modified the vision of early embryo development, anticipating the concept of cell determination and cell fate that will lead to the fundamental experiments of Spemann in 1918. Ruffini performed also basic observations on cell movement during gastrulation and neural fold formation. He reported that germ layer invagination is associated with a change in the shape of the cells that become elongated and club-shaped. Moreover, he describes that during this process cells are characterized by active secretion. All these studies were published in 1925 in a voluminous book entitled *Fisogenia*, in which all the fundaments of embryology of that time can also be found.

This review on the origin of embryology in Italy cannot end without mentioning the foundation in 1872 of the Stazione Zoologica in Naples by Anton Dohrn. The Stazione soon became an international centre in which fundamental research on developmental biology was performed, as reported in a companion paper in the present issue. The establishment of this centre marked a turning-point in experimental embryology that, by integrating more and more with cytology and histology and later with the new sciences of genetics and molecular biology will lead during the XX century to the rise of modern developmental biology. It is at the Stazione Zoologica that soon after World War II Alberto Monroy will start his studies on sea urchin development that can be considered as bridge between classic embryology and developmental biology (see "Molecularising embryology: Alberto Monroy and the origins of Developmental Biology in Italy" by B. Fantini, in the present issue).

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