REVIEW

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Lazzaro Spallanzani: pioneer of artificial insemination, multidisciplinary research, and scientific dissemination

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Abstract

Lazzaro Spallanzani (1729-1799) was a father of modern biology, with peculiar traits such as a multidisciplinary approach and penchant for scientific dissemination. Spallanzani consistently contributed to modern reproductive medicine by implementing experimental methods for the first historically successful artificial insemination. Nevertheless, he participated in discoveries pertaining to blood circulation, digestion, and respiration. Widely known in Europe in the eighteenth century, his fame prolonged to the following century, not exclusively through scientific acknowledgments, but even in literature. Nowadays, the figure of Spallanzani experience a kind of neglect and it would appear essential to maintain his work in the light of the history of medicine.

Keywords: Lazzaro Spallanzani; biology; history of medicine; artificial insemination; artificial fecundation; assisted reproductive medicine; in vitro fertilisation

Background

Lazzaro Spallanzani (1729-1799) can be regarded as true pioneer of modern biology and a precursor of microbiology. Spallanzani was an Italian naturalist, Professor of logic, metaphysics and greek at the University of Reggio (1754), and then Professor of natural history at the University of Modena and Pavia, Europe-famous researcher of the Grand Siècle (Enlightenment), as well as a key figure in the scientific renaissance of the eighteenth century. This brilliant scientist was even ordained to the priesthood (1762) and therefore well-known as Abbé Spallanzani. Though commonly regarded as a leading figure in the foundation of modern biology, his experimental research was essential to medicine development as well. Amédéé Dechambre (1812-1866), French physician and medical writer [1], highlighted how no other scientist served medicine, without being a physician, more than Spallanzani did with his work [2]. Although scientific literature has already portrayed Spallanzani's work, it would be worth considering anew his contribution to modern biology and reproductive medicine, by offering the reader a thorough analysis of it. More specifically, it would seem interesting to shed light on some features of Spallanzani's work formerly less covered, such as his passion for scientific dissemination.

Life and education

Lazzaro Spallanzani was born in Scandiano, a small town in Italy, on January 12, 1729, from lawyer Nicola Spallanzani and Lucia Zigliani. Formerly, Spallanzani received classical education (rhetoric and philosophy) at a Jesuit seminary in Reggio Emilia (Italy), encouraged by his father, who longed for his son to become a lawyer as well. Then, Spallanzani enrolled in the Faculty of Law at the University of Bologna (Italy) in 1749, but this educational path did not last long. Indeed, in Bologna he met a distant relative, Laura Bassi (1711-1778), who was the first woman to earn a doctorate in science and the second woman in the world to hold the Doctor of Philosophy degree (Ph.D.) [3, 4]. Her fame was such that the term 'doctrix' (latin female gender noun for 'doctor') was coined and Bassi became a member of the Institute of Bologna Academy of Sciences (one of the most famous scientific societies in Europe), as well as full professor in physics in Bologna (first woman in any European university) [5, 6, 7]. The working cabinet of Bassi provided Spallanzani with material and conceptual instruments to develop his scientific curiosity through Newtonian physics seminars and encounters with the leading cultural figures of Bologna [5]. Law education no longer held a fascination for Spallanzani, who began to learn astronomy, mathematics, botany, and progressively abandoned his father's footsteps. In 1753, he definitively left his legal studies, and four years later he became professor of physics and mathematics in Reggio Emilia. In 1762 he received the priestly order and a year later became lecturer of philosophy at the University of Modena. Acclaimed throughout Europe due to his visionary experiments, Spallanzani was elected a member of the London Royal Society on 2 June 1768, one of the highest honors in science then and now. In 1770 he resigned from the University of Modena, for Empress Maria Theresa of Austria wanted him In Pavia, where Spallanzani was appointed to the chair of Natural History at the University. In Pavia, Spallanzani contributed to the foundation of the Natural History Museum on the advice of Empress Maria Theresa and her son, Joseph II. Spallanzani took over the role as museum director and retained his professorship until his death, which occurred in 1799 in Pavia. Along with these appointments, from 1770 to 1799 Spallanzani dedicated several months to study tours across Italy and abroad, steadily committed to saving specimens for the Natural History Museum in Pavia and performing experiments on diverse species collected along his trip. Nevertheless, Spallanzani was fascinated by human customs and tradition, as his tour to Constantinople made plain; Indeed, on the return voyage, his inquisitiveness was equally attracted by natural phenomena, unknown animal species, and human habits [5].

Historical scientific context and experimental method

Western history of science, and medicine, had been informed by classical paradigms until the Enlightenment. This means that philosophy and science were gravely interwoven, and scientists until the eighteenth century were more concerned with imaginative metaphysics explaining natural phenomena rather than meticulous examination. Roots of a new paradigm can be traced back to Copernicus (1473-1543), who undermined the Aristotelian-Ptolemaic conceptualization of the cosmos, and to the questioning of Galen's physiology in the seventeenth century [8].

The eighteenth century was stage for paramount innovation in scientific methodology, through a novel approach to measure biological phenomena (e.g. blood circulation), which culminated in pervasive use of microscopes. Within this context, we can locate Spallanzani, who embarked on his experimental path formerly attracted by circulation of blood, particularly interested in the observation of microparticles enclosed therein. Indeed, even if the fame of Spallanzani is mainly associated with his findings related to artificial insemination, he was the first describing blood leukocytes, preceding English microscopist William Hewson (1739-1774) [8].

Spallanzani wrote in his journals: "I have discovered a species of globules which are smaller and present in far smaller numbers than the first ones. Since I doubted of my discovery, I wanted to make sure of it. I feared that the observation might derive from an optical effect, since the normal globules could be seen from the pointed end and thus be judged smaller and of a different nature. But after repeated, diligent and minute observations, I could be certain that the two species of globules must absolutely be distinguished" [9]. These lines show two core factors of Spallanzani's work: His propensity to measure and observe biological phenomena, putting aside philosophical frameworks in relation to them, and his distinguishing trait of exquisite experimental scientist committed to reiterated attempts, designed to minimise errors and establish a range of benchmarks. The experimental method as a primary mechanism for investigating biological phenomena constitutes a peculiar feature of naturalists and scientists in the eighteenth century, but Spallanzani embodied astonishingly this approach by making it an absolute discipline [8]. Spallanzani wrote: "This is my fundamental method, whatever I tackle, even the most disparate things, so long as they have material causes: to take no account of the opinions, however authoritative and respectable, of those who have defined them before, but to dedicate oneself to a practical examination of the facts" [10]. Spallanzani reiterated his observations several times, to such an extent that John Hunter (1728-1793), widely known British surgeon, expressed harsh criticism against this method, by maintaining that scientific truth can be proven by a single observation, following a plain analogy between a handful of conclusive observed facts. According to Hunter, it would have been futile from a scientific point of view, and simpering for the reader, to back each elucidation with tailor-made experiments [5].

Spallanzani's inquisitiveness led him to never become a taxonomist: He had never been concerned with classificatory questions, neither been a theorist. Theoretical investigation on scientific observation, so crucial over the centuries and still among his colleagues, never enticed him. Undeniably, Spallanzani pursued the functional meaning of his observations, rather than drawing from them classificatory findings [5].

Roots of modern multidisciplinary approach and scientific dissemination

Notwithstanding that most authors reckon Lazzaro Spallanzani as a founder of modern biology [5, 8, 11-13], some sustain his experimental work must be assessed in the light of his time. Precisely, Spallanzani nourished polyhedral interests in research, implementing observations in many fields, such as human and animal physiology, botany, palaeontology, chemistry of gases, meteorology, physics, entomology, etc. [5, 14, 15]. While it is true that naturalists in the eighteenth century generally developed multifaceted attentiveness to diverse fields, and therefore this was not a peculiar feature of Spallanzani's work, it must be considered how this scientist distinguished himself for a specific quality. In observing natural phenomena comprehensively, from several disciplines' viewpoints, Spallanzani pursued a thorough knowledge of nature. In other words, he sought an exhaustive conception of nature as the whole set of interrelations among natural phenomena and, consequently, among disciplines. Actually, Spallanzani maintained laws of nature as universal [2, 13], being a pioneer even in comparative biology, by arguing that organic nature could not be studied with isolated test subjects belonging to a single species. According to Spallanzani neither the naturalist nor the philosopher should have analysed exclusively one "living machine" to attempt to penetrate scientific truth on a specific phenomenon [2].

Hence, Spallanzani can be deemed as well as a pioneer of contemporary multidisciplinary, given the peculiar and authentic perspective leading his experimental work. This naturalist deeply concerned with physiology, and contributed greatly to modern medicine, did not nurture a conceptual view of humanities and hard sciences adamantly detached. This vision, rooted in the eighteenth century, was strengthened in the nineteenth and twentieth centuries, leading to a biased understanding of many biological, medical, and psychological phenomena. This "false alternative" [16], wherein humanities thwart hard sciences, was utterly absent in Spallanzani's observational analyses. This brilliant naturalist had an indisputably distinctive way of looking at the natural realm as a thorough, complex, entity.

Furthermore, Spallanzani investigated natural phenomena "plainly [...] without any kind of inclusion from our mind" [17], namely committed in avoiding inferences not unswervingly stemming from experimental observation. In the aftermath of an experiment, philosophical investigation supervened as thoughtful question on the meaning of the interrelation of elements observed. In other words, Spallanzani, though his ecclesiastical role, was biased neither by theology nor by philosophy. Undeniably, as will be seen in what follows, Spallanzani performed experiments and dedicated to challenging topics for the Catholic Church [5], without neglecting his unrestrainable all-round power of observation.

In addition to this, Spallanzani was a truly pioneer of scientific dissemination, dedicated to elucidate complex experimental findings to the general public and notable for being an excellent speaker, and orator, accurate and effective in his speech [5]. Indeed, Spallanzani did not confine his teaching activity to institutional boundaries: Not exclusively students, but even common people, high-ranking officials, and diplomats were occasionally involved in his description of natural phenomena. A glaring example of this propensity, and curios anecdote, was the occasion in which, during his study travel in Constantinople, Spallanzani noticed some frogs copulating in a body of water. In the throes of his authentic scientific passion, Spallanzani proceeded to explain how frog copulation worked to his audience comprising women, diplomats, and high-ranked officers. Much to the dismay of Spallanzani, the audience proved to be unresponsive and unaware obout this topic. Presumably, sexuality, even if frog sexuality, was an uncomfortable topic to be discussed in public, all the more by a clergyman. Nevertheless, the naturalist was more astonished by genuine ignorance of his audience, rather than from its embarrass or discomfort in relation to the specific topic [5, 14]. Undoubtedly, Spallanzani was genuinely driven by an unbridled passion for observation, discovery, and investigation. This peculiar characteristic has been described by the leading scholar on Spallanzani, as "the lust of knowledge" [5]. Eager to observe, longing for investigation, and anxious to record findings, Spallanzani lived with an authentic burst for science for all his academic and personal life, intensely willing to involve general public in his research work. Indeed, his scientific excitement had never been confined to academia or institutions. Therefore, it can be argued that he was ahead of his time even in this field, as a pioneer of contemporary scientific dissemination.

Theories of generation: Ovism, Animalculism, and Epigenesis

Pioneer of a multidisciplinary approach to research and scientific dissemination, Lazzaro Spallanzani owes his reputation first and foremost to the observations on reproduction and the results of artificial insemination. As aforementioned, in the field of human physiology the brilliant naturalist performed experiments even on blood circulation, then committed to the study of digestion and respiration. Nevertheless, his outstanding observation on reproduction, gained through massive experimentation on diverse amphibian species, is undoubtedly the most relevant part of his inheritance to modern medicine and biology.

In 1765, Spallanzani developed his interest in reproduction, which in those days referred to as theory of generation (*Theoria Generationis*). Reproduction of organisms was already a core topic for philosophers, physicians, and naturalists. In the eighteenth century in Europe, animal and human reproduction was hostage to intrinsic involvement of philosophy and theology, through two main hypotheses. On the one hand, Aristotelian view of sperm infusing soul into female menstrual blood, lifeless prior to that moment [18]. On the other hand, Galen (129-216) suggested equal participation of the male and female seeds in the creation of the embryo. These two postulates were enriched by philosophical restrictions hindering an effective observation of reality, hindering for centuries genuine progress of scientific knowledge. Until William Harvey (1587-1657), English physician of indisputable fame, while preserving an Aristotelian view, published the first work on viviparous reproduction grounded on concrete facts [19]. Harvey placed the origin of a new organism in the egg, maintaining sperm performed fertilising action through an intangible principle and suggesting embryonic development was due to consequential and progressive additions stemming firstly from female blood, then deriving from uterine walls [8]. Later in the decades, this specific view was called "ovism" (from *ovum*, latin for egg) and it was supplemented with the observations of Johann van Horne (1621-1670), Niels Stensen (1638-1686), and Renier de Graaf (1641-1673). Notwithstanding, the egg was effectively seen and discovered later by Karl Ernst von Baer (1792-1876).

Ovism, as a trend of thought in the field of reproduction, had its orthodox version in the so-called 'preformism', namely notion of a thoroughly pre-formed, and miniaturised, version of the whole organism already existent within the egg. In other words, egg contained a miniaturised version of future organism, suspended and without life till the animating action of sperm is performed, bringing to life the inert and unanimated miniaturised entity [2, 8, 13]. Furthermore, Harvey, through dissection of deers after coitus, had not noticed sperm in the uterus of his test subjects, consequently assuming that any physical action of the sperm must be excluded. Instead, theory of "aura spermatica" (immaterial fertilising principle of the sperm) was proposed: Sperm had an incorporeal and spiritual ability to animate the miniaturised organism (germ) within the egg [2, 8].

This view was then thwarted by a different hypothesis, grounded on the discoveries of Antonie Philips van Leeuwenhoek (1632-1723), a Dutch scientist and microscopist, who observed some miniature entities in human sperm. Leeuwenhoek called these creatures animalcules, sustaining how viviparous eggs were inexistent; therefore, animalcules were the ones directly implanting in the uterine wall [2, 8, 13]. By a consequence, a new version of preformism was proposed: Miniaturised pre-formed organisms (homunculi) were contained in sperm, as well as preformist ovists considered them existent in the egg.

Animalculism and Ovism confronted for decades, while a new hypothesis stemmed by observations of naturalists such as globally well-known Georges-Louis Leclerc, Comte de Buffon (widely known as Buffon, 1707-1788). Buffon and other naturalists, like John Turberville Needham (1713-1781) sustained preformism was inconsistent with vigorous evidence, such as the inheritance of physical features from both parents (e.g., kittens with the same colours of both the female and the male which generated them). Nevertheless, this view, called epigenesis, was intrinsically undermined by its excessive simplification, insofar as it was founded on the idea that the embryo is generated by feminine and masculine amorphous fluids [2]. In particular, these fluids could not be regarded as properly animal, since they were considered casual clusters of organic molecules. Epigenesists were right in challenging preformist theories, but they unduly simplified the process of generation, far too complex than the encounter of two general body fluids. Indeed, role and function of spermatozoa, penetrating eggs, were discovered only in 1875 by Oscar Hertwig (1849-192299 in sea-urchin eggs [8]. As well as an effective comprehension of roles of both gametes was feasible only after 1839, when Theodor Schwann formulated Cell Theory, under which germs of a certain kind, all else being equal, would produce adult organisms of the same kind, and vice versa [20].

Artificial insemination: preformism and spermatic worms of amphibians

In this context, Spallanzani developed his interest in animal reproduction. As aforementioned, this naturalist nurtured polyhedral interests in research. Thus, after having published a critical essay towards Buffon's and Needham's theories [21] in 1765, Spallanzani was still investigating several fields, including entomology. Upon suggestion of his friend Charles Bonnet (1720-1793), a Swiss naturalist who discovered parthenogenesis (reproduction without fertilisation) and developed the catastrophe theory of evolution, Spallanzani devoted his attention reproduction or regenerations. Bonnet regarded regeneration as a core topic for naturalists and science in general, but particularly he wished for his observation to be pursued by Spallanzani, since Bonnet had developed blindness [13]. Furthermore, Bonnet wished for preformism to prevail over epigenesis, throughout an experimental objection to Needham and Buffon. Although Spallanzani had been preserving his observations by any kind of philosophical bias till that time, in a letter to Bonnet in April 1766, he asserted to have read Needham's works on generation and that he would have proven how epigenesis was inconsistent with natural facts. This was a kind of statement of faith to his close friend Bonnet, since Spallanzani wrote those lines before having performed any experiment or having collected any data in the field [13]. Hereinafter, Spallanzani, even without being a relentless supporter of preformism, was biased by preformist view and thus psychologically conditioned to interpret ambiguous or indifferent data in preformist way. Indeed, Spallanzani had confronted two main dilemmas in investigating epigenesis [13]. The first: What kind of force, of what nature and what origin, could perform the fertilising action on living matter? Then, given its complexity, how could any organism inflect times and means of its own reproduction.

Heretofore, René Antoine Ferchault de Réaumur (1683-1757), a French entomologist, had completed several studies on amphibians' and crayfishes' reproduction, with outcomes exclusively on the seconds. Réaumur attempted to fertilise amphibians' eggs several times, even with the aid of Jean-Antoine Nollet (known as Abbé Nollet, 1700-1770). From 1736 to 1740, Réaumur attempted repeatedly by tailor-making some drawers of different materials for male frogs, beginning with bladder tissue and ending with taffeta. None of his experiments succeeded, since male frogs always dumped their peculiar undergarment [2, 5, 8]. Réaumur was determined to collect, through this artifice, amphibians' semen in order to artificially fertilise eggs.

From 1767 to 1768, Spallanzani's journals prove his commitment to multidisciplinary research involving reproduction, blood circulation, and respiration. Definitively committed to reproduction, in those years Spallanzani performed the first embryologic refined observations without microscope of the history of biomedicine and science [13]. Spallanzani decided to analyse reproduction on amphibians for at least four main reasons: (1) amphibians' reproduction occurred outside of their bodies (as the naturalist demonstrated through a series of observations) [2, 22], (2) their reproductive circle was short, (3) amphibians were easy to obtain and (4) quite inexpensive to breed. Hence, the brilliant naturalist observed amphibians' eggs remarking how they had a spheric shape, with a black particle in the middle; Thus, Spallanzani inferred the particle was the egg itself and, biased by preformist view, that the particle enclosed a miniaturised tadpole. Actually, even if biased with preformism, Spallanzani performed sharp observations in discovering how the black particle and the rest of the egg had quite different substances. Namely, Spallanzani discovered the nucleus of the egg, though he could not define it as such [13]. At the end of 1767, the naturalist published a booklet with first results of his observation of extracorporeal fertilisation of eggs, existence of seminal vesicles in male amphibians, and visual analysis of virgin and fertilised eggs [22]. The Royal Society received copies of this booklet, meant to become the foundation stone of upcoming experimental work on artificial insemination, even if discoveries on eggs and fertilisation were not the core topic of this manuscript. In fact, this Prodromo was dedicated to regeneration of beheaded snails, whose heads regenerated and fascinated many European intellectuals, including Voltaire (1694-1778) and Lavoisier (1743-1794), French chemist with an essential role in the eighteenth-century chemical revolution, as well as in the history of chemistry and biology.

From 1765 to 1781, Spallanzani devoted much time to observing amphibians' fertilisation, interested in detecting universal natural laws governing reproduction as a comprehensive phenomenon at the core of the "generation mystery" challenging different disciplines. Prior to him, no other naturalist, biologist or physician, had never committed to a so up-close and systematic study of reproduction [13]. In opposition to animalculism, Spallanzani used to refer its supporters as vermicellai, an ironical moniker deriving from the vermicelli spermatici (minute spermatic worms) and to sustain spermatic worms were parasites in the seed, likewise transmitted from one generation to another [8]. In 1771, Spallanzani began to investigate spermatic worms through both spontaneous emission and collecting them by dissecting testicles. Spallanzani observed spermatic worms in different seasons, while Buffon has attempted exclusively during matins season. The Italian naturalist noticed how spermatic worms were already existent in emitted semen both during mating and in the fresh amphibian's corpse. These inferences stemmed even from the observation of male specimen of newt after starvation, when their vessel walls thinned enough to be translucent and taking advantage of the peculiar large size of this species' spermatic worms [2].

Artificial insemination: the successful experiments

Given the observations performed on amphibians, Spallanzani was then conceptually equipped and minded to attempt artificial insemination on these species. The Italian naturalist was aware of the fact that tadpoles developed from laid eggs fertilised outside of the female's body and that seed enclosed undoubtedly active worms. Therefore, in 1777, Spallanzani decided to replicate Réaumur's experiments with garments, enhancing his chances of success by repeating experiments several times, with great patience and proficiency in using the microscope. Hence, Spallanzani implemented drawers from blade tissue and collected some drops of frogs' semen, while retrieving virgin eggs from female specimens. By moistening eggs with semen, Spallanzani obtained the first artificial insemination in the history of biology and medicine [2, 5, 13].

Afterwards, Spallanzani regularly repeated this experiment, by modifying some parameters, such as the means of semen's collection, often retrieving seminal vesicles, then rupturing and squeezing them to extract spermatic fluid. Subsequently, the Italian naturalist repeated his test on diverse species of amphibians (frogs, salamanders, toads, newts, etc.). The idea underlying artificial insemination experiments was nothing new or excessively elaborate to realise, but Spallanzani's merit was to succeed in the attempt, and more specifically to have achieved successful result in a plain and demonstrative manner [2].

Given the successful results obtained with amphibians, Spallanzani devoted time to attempting artificial insemination in mammals, with a female poodle. To ensure its fecundity, Spallanzani selected a non-primiparous specimen and kept it segregated for thirteen days,

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when the female dog gave evidence of being in heat. After ten more days, the test subject showed mating needs and the Abbé retrieved dog semen by "spontaneous emission", then injected it into the female's uterus through a peaked syringe. After two days, the heat was ended, then in twenty-six days pregnancy was manifest and after thirty-six more days (sixty-two after the injection), the test subjects delivered three perfectly healthy and lively offspring [2]. Spallanzani wrote: "Thus, I succeeded to fertilise that Quadruped, and I was so delighted that it truly was one of the greatest joys of my life, from when I practice experimental Philosophy" [23].

From 1780 to 1781, Spallanzani reiterated artificial insemination experiments on amphibians, sometimes indulging in excessive cruelty towards specimens involves. As an example, on 22 April 1780 the naturalist beheaded a male frog during mating, through a cut under its eyes, to observe if the copulation persevered. On 2 May 1781, Spallanzani tortured with fire a male frog during mating and noticed that male specimen sticked to the female till his thigh caught fire. Turning away the fire source, male began to mate again. Not being satisfied, Spallanzani combined decapitation and fire, obtaining an unaffected result: In all the experiments, male frogs were able to fecund females until their final moments [5]. These cruel experiments led the naturalist to detect not sexual frenzy in amphibians, but an unrestrainable instinct to fecundate.

In addition to this, Spallanzani was even committed to contesting aura spermatica (aura seminalis) theory, by investigating which part of semen provided fertility power. For the purpose of clarifying if a vapor, emanating from the sperm, could hold fertility action, the naturalist placed toad sperm on a watch glass, then placed a dozen of toad eggs in another glass, turned upside down to cover the first. Sperm and eggs were few millimetres apart, and after five hours the eggs were covered by a sort of dew, stemmed from the evaporated seminal fluid, but none developed. Spallanzani repeated the experiment with different arrangements (e.g., connecting glasses, allow air to circulate in the system, etc.), but in the end, spermatic aura was proven inexistent [13].

Besides, the Italian naturalist tackled investigations on sperm diluted in water, still seeking indisputable response on fertilizing action of semen. From 1781 onwards, Spallanzani observed how fertilizing force decreased steadily in relation to the thickness and the number of filters applied to water. Spallanzani inferred, after counterchecking by rinsing filters and employing this water to fertilise eggs, that fertilising fraction of sperm was trapped in filters. Nevertheless, the scientist neglected to analyse this water with a microscope; thus, he could not see spermatic worms to resolve the dispute on their existence and function [2, 8, 13].

Finally, Spallanzani proposed his theory on the role of sperm, revealing himself as a better investigator, rather than a theoretician. According to his observations, eggs had countless "little mouths" aimed at aspirate semen. For its part, sperm was characterised by stimulating property, able to animate the miniaturised foetus, inert and lifeless. Eggs enclosed vital principle, due to which could accrue before fertilisation, even if the egg had "infinitely weak" life, almost vegetative and unsuitable for development without spermatic action, targeted to subtly stimulate foetus' heart to irrigate blood vessels [2].

Artificial insemination: the results

Preliminarily, it would appear appropriate to emphasise that Spallanzani did not appreciate artificial insemination in itself, but rather as a means of research, as a method whereby acquire knowledge in the extensive field of reproduction (generation). Spallanzani was committed to seeking undeniable and universal natural laws on this topic and reproductive medicine, in particular human medicine, was away from his interests. Nevertheless, his experimental results were utterly beneficial to the development of further biology and medicine.

First and foremost, Spallanzani clarified animal nature of sperm particles, which were still questioned prior to his observation, and their permanent presence in fresh sperm. In other words, the naturalist proved spermatic worms existed before semen was emitted, closely approximating to ontogenesis [2].

Then, artificial inseminations performed by the Italian naturalist demonstrated not exclusively its own feasibility, but even similarity in the number of fertilised eggs, compared with natural insemination. Spallanzani went even further obtaining promising results in sperm and eggs preservation, by observing fertilising property of semen preserved until nine hours at room temperature, while until twenty-five hours in an icehouse [2].

Furthermore, as aforementioned, Spallanzani efficiently objected to aura spermatica theory, by emphasizing concrete fraction of sperm role, as well as essential contact between egg and sperm to obtain fecundation. Indeed, the naturalist observed infinitesimal part of semen could fertilise a great number of eggs, even after aqueous component of semen evaporated or was diluted in water [13].

In addition to this, Spallanzani was a precursor of localised fertilisation, by gaining insight into excessive fertilising force in semen, known in the twentieth century as billions of spermatozoa useless to fecundation during mating.

The whole of these results appears impressive for its innovativeness in the eighteenth century and is essential to our contemporary understanding of reproductive biology and medicine. Spallanzani was the first to systematically address reproductive topics through experimental methods, acquire knowledge from lower classes animals, then move to mammals. The brilliant naturalist was even the first to successfully performing an artificial insemination in laboratory, though it seems that Arabs already implemented artificial insemination in horsed during Middle Ages (A.H. 700) [8].

Inheritance of Spallanzani

The Italian naturalist undoubtedly marked history of physiology, biology, and medicine. Amédéé Dechambre (1812-1866), underlined no other scientist served medicine, without being a physician, more than Spallanzani did with his work [2]. Jean Edmond Cyrus Rostand (1894-1977), French biologist and historian of science, biographer of Spallanzani, wrote "Whenever an artificial digestion or insemination will be performed in a laboratory, it will be paid homage to the great Italian biologist" [2].

Indeed, experimental results in the field of artificial insemination were afterwards replicated and heightened by various scientists who actively contributed to the implementation of these techniques in reproductive medicine. Nevertheless, Spallanzani's contribution was decisive in paving the way for modern and contemporary reproductive medicine. In 1790, John Hunter (1728–1793), a Scottish physician, surgeon and anatomist, undertake for several year fertilisation experiments in London. After his death, Everard Home (1756-1832), executor and brother-in-law of Hunter, brought to light a record of an experiment performed around 1790, which culminated in the alleged pregnancy of a woman whose husband was affected by penis malformation (hypospadias) [2, 24]. Hunter recommended the couple have intercourse, ensuring the woman was sexually stimulated, then retrieve husband's sperm in a warmed syringe and inject directly into the woman's vagina [25]. Allegedly, this stratagem worked and Home revealed the details to the Royal Society in 1799, the year when Spallanzani died [25]. Afterwards, in 1866, James Marion Sims (1813-1883), American physician widely known as "father of gynaecology," successfully realised first intrauterine sperm injections [2, 24]. Furthermore, in 1821 two French biologists, Jean-Louis Prévost (1790–1850) and Jean-Baptiste-André Dumas (1800–1884), observed with a microscope the water wherin sperm filters had been rinsed and noticed spermatozoa, which they observed also in the gelatinous coats of amphibian eggs [8, 26].

Even Louis Pasteur, French chemist and microbiologist, widely known for his discoveries of vaccination, pasteurization, and microbial fermentation, determined to hang a portrait of Lazzaro Spallanzani above the fireplace of the Institute Pasteur reception room [27].

In addition to scientific acknowledgements, Spallanzani was honoured even in literature, becoming protagonist of “The Sandman,” a short story by Ernst Theodor Amadeus Hoffmann (1776-1822), published as first in an 1817 book titled “The Night Pieces”. In “The Sandman,” Spallanzani was depicted as scientific genius, kind of a hero, with a similar role to that granted to Einstein in contemporary cinematography and literature [16]. Finally, also Victor Hugo (1802-1885), a French romantic writer, mentioned Spallanzani in his book “The Last Day of a Condemned Man” (1829).

These tributes and acknowledgements ascertain Spallanzani enjoyed widespread fame in life and posthumously. Rostand wrote “The name of the Abbé Spallanzani is one of the few that in history of natural sciences permanently recurs; according to the expression of Quatrefages ‘it is quite impossible not mentioning him when discussing physiology’” [2]. Furthermore, Rostand depicted Spallanzani as a kind of magician to his contemporaries, with impressive fame throughout Europe. Indeed, Voltaire sustained Spallanzani as the first natural naturalist in Europe [28] and it was doubtless so, insofar as the Italian scientist ushered in medical and biological technique some core elements, still essential nowadays.

Conclusion

Lazzaro Spallanzani (1729-1799) can be regarded justifiably as the founder of modern biology, who contributed and served medicine than any more other did without being a physician [2]. Contemporary reproductive medicine, as well as biomedicine and biotechnology in that field, owes much to the Abbé, in terms of conceptual foundations, asides from technological progress that occurred over the following centuries. Even if investigative questions leading Spallanzani to artificial insemination were not innovative, what was uncommon and transformational was the experimental method he implemented. His peculiar outlook on problems, uninvolved in theoretical problems, although fascinated by philosophical topics such as generation and regeneration (that is, life-death continuum), was absolutely original for his time. Unless his unvaluable contribution, artificial insemination, at least in Europe, would have probably remained devoid of successful practical results for years or decades. Even in those experiments wherein Spallanzani neglected relevant elements, as for spermatic water filtered and not observed under a microscope, he paved the way for other naturalists, microscopists, and biologist. It is relevant not to forget Spallanzani was quite a self-taught observer, except for advice received by Bonnet and other naturalists through correspondence. Spallanzani was educated in physics, mathematics, but was a self-educated microscopist and precursor biologist in modern terms.

Nevertheless, Spallanzani has been overlooked, and quite forgotten, after the twentieth century, maybe for him being a clergyman: Indeed, in nineteenth-century Europe, science was assumed to be absolutely counterposed to faith. In this context, the widespread reputation of the Italian naturalist suffered a though backlash, although he had never been influenced by theological concerns in his observational and experimental activity. By contrast, until his last day, Spallanzani was concerned with medical and physiological questions like respiration (performing an experiment on fishes the day before he died), catalepsy, and apoplexy.

Furthermore, Spallanzani was a truly pioneer of scientific dissemination, dedicated to elucidate complex experimental findings to the general public and notable for being an excellent speaker, and orator, accurate and effective in his speech. Genuinely driven by an unbridled passion for observation, discovery, and investigation, Spallanzani nurtured “the lust of knowledge” [5], through a multidisciplinary lens of analysis. Indeed, the Abbé sought interconnections between phenomena and disciplines, inspired by the desire to prove and put before the public these intertwined threads belonging to science, as well as to humanities.

As Rostand underlined, Spallanzani detected, or at least perceived, significant truths in all research fields he addressed, tough he was rarely the first to identify the starting question. In other words, Spallanzani often walk conceptual paths already paved by other naturalists, but he substantially contributed to pursuing those paths by implementing the experimental method and inferring different experimental stages, suitable to make new question arise. The contribution of Spallanzani to contemporary reproductive medicine, cannot be withheld unless denying the interconnection between biology and medicine, as well as the key role of experimental method to the understanding of physiology and pathology.

Lazzaro Spallanzani was a true pioneer of artificial insemination, multidisciplinary research, and scientific dissemination. The Abbé appears still nowadays modern in his conceptual approach, even if features like multidisciplinary and scientific dissemination are obviously not directly pertaining to his epoch. Nonetheless, these traits can be undoubtedly associated with the figure of the Italian naturalist, driven by an unrestrainable desire for observation and discovery, and an essential cornerstone of contemporary reproductive medicine.

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