

# Early and late stages of Developmental Biology in Argentina

SARA S. SÁNCHEZ<sup>\*,1,2</sup> and STELLA M. HONORÉ<sup>1,2</sup>

<sup>1</sup>Instituto Superior de Investigaciones Biológicas (INSIBIO, CONICET-UNT) and <sup>2</sup>Instituto de Biología "Dr. Francisco D. Barbieri", Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina

**ABSTRACT** The history of science in Argentina is based on the enormous contribution that the great immigration of the 19th and 20th centuries produced in the country. The scientific and philosophical ideas and the role played especially by Italian scientists who arrived in the country produced a great impact on the different disciplines including Development Biology in emerging universities. The University of Tucumán pioneered the study of experimental biology, making important contributions to reproductive biology and to the early development of amphibians. The contribution of the Italian embryologist Armando Pisanó and the Argentinian Francisco D. Barbieri expanded the field to other universities and research centers located in Córdoba, La Plata, Bahía Blanca and Rosario. Given its strategic position, laboratories located in the city of Buenos Aires reached technological advances faster than others. Indeed, these laboratories saw the evolution from experimental biology to developmental genetics, renewing interest in this area. Currently, Developmental Biology brings together young researchers eager to consolidate regional and global collaboration networks that seek to help solve specific problems such as fertility, epigenetics, stem cells and tissue engineering.

**KEY WORDS:** *history, experimental embryology, developmental biology, Argentina*


The history of science in Argentina would be unimaginable without the contribution of the great immigration of the late 19th and early 20th centuries. The migratory avalanche coming mainly from southern Europe constituted the largest human contingent that entered the country during that period. The rapid transformation of Argentina from then on was significant and included numerous areas -habits, way of life and conception of the world, economy, letters, science, art, language, religion and politics (UNT, 2002). Immigrants came mainly from Europe. The scientific and philosophical ideas and the role that foreign scientists who arrived in the country played in the academic research community marked a change of paradigms in the knowledge and the development of many scientific disciplines, including experimental embryology, which would lead to developmental biology. In this sense, it is important to know what the historical framework in which the transformations in this area of knowledge were taking place in Europe was, especially in Italy, at the end of the 19th century.

## The influence of Italian laboratories

Most studies in embryology in Italy were conducted in the south of the country, in Palermo and Naples, where the Stazione Zoologica di Napoli played an important role in the formation of several generations of embryologists and developmental biologists

(Fantini, 2000a). Among them were Giuseppe Reverberi (1901-1988), Silvio Ranzi (1902-1996), Giuseppe Montalenti (1904-1990), and Alberto Monroy (1913–1986). They made important contributions to embryogenesis from a morphological, physiological and metabolic point of view using models of echinoderms, amphibians, agnates, ascites, birds and insects (Fantini, 2000 a,b). The Stazione Zoologica di Napoli, was a central point of an ever-growing international scientific network, promoting independent research, exchange and diffusion of novel practices and techniques that

*Abbreviations used in this paper:* CONICET, National Council for Scientific and Technical Research; FIL, Leloir Institute Foundation; IAL, Institute of Agrobiotechnology of Litoral; IBBEA, Institute of Biodiversity and Experimental and Applied Biology; IBCN, Institute of Cell Biology and Neurosciences; IBR Institute of Cellular and Molecular Biology of Rosario; IIB-Intech Biotechnological Research Institute-Chascomús Institute of Technology; IIBYT, Institute of Biological and Technological Research; IMBICE, Multidisciplinary Institute of Cellular Biology; INGEBI, Research Institute in Genetic Engineering and Molecular Biology; INIBIBB, Biochemical Research Institute of Bahía Blanca; INSIBIO, Higher Institute of Biological Research; UNC, National University of Córdoba; UNCuyo, National University of Cuyo; UNER National University of Entre Rios; UNL National University of Litoral; UNMDP, National University of Mar del Plata; UNS, National University of the South; UNSAM, National University of San Martín; UNT, National University of Tucumán.

\*Address correspondence to: Sara S. Sánchez. Instituto Superior de Investigaciones Biológicas (INSIBIO, CONICET-UNT), Chacabuco 461, T4000LI, San Miguel de Tucumán, Tucumán, Argentina. Tel/Fax: 54-381-4107214 ext 111. E-mail: sara.sanchez@fbqf.unt.edu.ar -  <https://orcid.org/0000-0001-6698-3095>

Submitted: 5 January, 2020. Accepted: 28 January, 2020. Published online: 20 August, 2020.

brought together numerous Nobel Prizes (Groeben *and* De Sio, 2006). The most important biologists of the time as Hans Adolf Eduard Driesch (1867-1941), Otto Warburg (1883-1970), Kurt Herbst (1866-1946), Hans Spemann (1869-1941), Charles Otis Whitman (1842-1910) and Charles Manning Child (1869-1954), spent long research stays at the Stazione Zoologica, initially focused only on morphological studies, later on a new experimental methodology (De Felici and Siracusa, 2000; Fantini, 2000). In fact, it was in Naples that the proponents of the new experimental approach to development problems made some of their most important discoveries, thus beginning a new era in the study of development. In the 1940s, Naples was one of the main centers of experimental embryology: research traditions in systematics, cytology, physiology and biochemistry survived and genetics and biophysics became rooted there.

### **Italian immigration in Argentina**

The political changes that shook Europe in the second half of the 19th century led a large part of the Italian population to emigrate not only to Argentina but also to other countries in the Americas such as Brazil, Uruguay, and the United States. Most emigrants were academics, managers, businessmen, diplomats, merchants and career officers in search of a place for both material and cultural survival and where their children could grow and study freely (Terracini, 1989). Italian professionals were welcomed by Argentine universities, which offered generous contracts to incorporate teachers and researchers, thus promoting the internationalization of the institutions. Italian scientists received from the Argentine environment not only hospitality and affection, but also a remarkable cultural stimulus. It was a fortunate encounter between local interests and European energies, a forced but extremely fertile adaptation.

### **Experimental embryology in Argentina**

The history of science in Argentina cannot be separated from the events that took place in the recently created university institutions. The first national university in northwestern Argentina, the National University of Tucumán, UNT, was created in 1914; the National University of Córdoba had already been founded in 1613 and the University of Buenos Aires in 1821. From the beginning, these institutions suffered from the difficulties of the surrounding environment and the contemporary historical period. During the first decades of the 19th century, the universities had as their main objective the training of professionals who would form the ruling class and serve the needs of a country that exported raw materials, while scientific knowledge played only a minor role. In the mid-nineteenth century, according to the enlightened and encyclopedic conception of science that prevailed in the country, the greatest activity of research in the area of biology was carried out in natural science museums and dedicated to the classification and survey of plant and animal species. This state of things began to change at the end of that century with the emergence of the first experimental laboratories connected with the teaching of medicine (Tagashira, 2009).

During its first four decades, the UNT carried out an important scientific activity where important government projects were executed to establish academic models committed to the geographical

region and to the development of knowledge. The most memorable academic administration was that of Horacio Descole, who chaired the UNT between 1846 and 1951. Descole was a dynamic, creative and very ambitious scientist who focused his management on the development of new disciplines and institutes and setting up of undergraduate and graduate degrees. His policy of adding to each Institute an academic with the capacity to strengthen scientific research led to the hiring of outstanding Argentinian and foreign researchers in mathematics, architecture, philosophy and natural sciences in special.

### **José Miguel Alfredo María Cei (1918-2007) and Armando Pisanó (1914-2008)**

As a result of Descole's efforts to the development of natural sciences in Tucumán, a group of leading scientists were transferred to the UNT and joined either the School of Pharmacy and Biochemistry or an academic center -the current Institute Miguel Lillo-, which became the most important natural sciences institution in the country during the third decade of the 20th century (Willink, 1999). José Miguel Alfredo María Cei (1918-2007) and the Armando Pisanó (1914-2008) were among the Italian biologists who arrived in Argentina after 1945.

José Cei, born in San Miniato (Pisa, Italy) in 1918, finished his university studies at the Faculty of Natural Sciences-Biology branch- and the Faculty of Medicine of Florence and Pisa. In 1940, he obtained his Doctorate in Biological Sciences with specialization in Zoology and worked at the University of Florence from 1942 to 1947. In 1945, he participated in the US University Training Command in Florence, conducting research on sexual and hormonal cycles in vertebrates, the comparative anatomy of the organs of vision in mammals and general studies on biogeography and evolution. In World War II, he was transferred to North Africa, where he conducted anthropological studies with the various ethnic group's native to Libya, Somalia and Ethiopia. In 1948, he joined the teaching staff of the Faculties of Biological Sciences and Pharmacy and Biochemistry (UNT) as a Professor in the Department of General Biology and Director of the Zoology area of the UNT. In 1950, he was part of the group of founders of the School of Medicine, which later became the Tucumán Faculty of Medicine. Until 1954, he was also the head of the Institute of General Biology and Experimental Embryology (the current Institute of Biology). His scientific contributions during this period focused on amphibian sexual cycles and biology. He also published a Biology text for university students.

From 1955 to 1980, Cei was a professor at the School of Medicine and the Director of the Institute of Biology of the National University of Cuyo (UNCuyo). He was a visiting professor in Angola, Chile, Italy, Portugal and the United States and specialized in amphibian systematics, ecology and biogeography. Cei's extensive scientific production was an extraordinary contribution to the knowledge and development of Herpetology. A tireless traveler and a privileged explorer of nature, Cei discovered and described numerous amphibian and reptile species in the search for links that would allow us to decipher the great enigma of evolution, the current focus of evolutionary developmental biology.

In 1948, the embryologist Armando Pisanó (Fig. 1) joined the UNT zoology research team. Born in Catania, Italy, in 1914, Pisanó earned his PhD in Natural Sciences from the National University of Palermo, Italy, in 1936. In 1938, the Italian Ministry of Public



**Fig. 1.** Armando Pisanó (1914- 2008) brought experimental embryology to Latin America (left). Francisco D. Barbieri (1930-1993) spread and stimulated the Development Biology in Argentina (right).

Instruction granted him the title of Professor in Natural Sciences, Chemistry and Geography in a National Contest. He completed his scientific training at the Universities of Palermo and Bologna and at the prestigious Stazione Zoologica di Napoli (Pisanó 1939, 1942). While still very young, he became a Professor of Comparative Anatomy and Physiology at the University of Palermo and of General Biology and Zoology at the University of Siena. The UNT hired him as an extraordinary professor of Embryology for medical students at the professor of embryology at the Institute of General Biology and Experimental Embryology of the Faculty of Biochemistry, Chemistry and Pharmacy. He stayed in Tucumán until 1955, when he left in order to continue his scientific career at the Department of Scientific Research of the UNCuyo and later at the Institute of Marine Biology of the National University of La Plata. He also worked as a visiting professor in universities in Latin America, for instance in Colombia and Venezuela, and made numerous study trips abroad which allowed him to establish links with laboratories in Italy, France, the Netherlands, England, Chile, Colombia, Paraguay and various African countries. He even conducted his research work at the Esperanza Base in Argentine Antarctica (Pisanó, 1975), where he wrote "Topics on Embryology", one of his works where he develops several topics concerning comparative and experimental embryology.

His organizational ability was shown when he participated in the organization and creation of the Department of Biology for embryological studies at the University of Quindío, Colombia, as well as in the Pontificia Universidad Javeriana de Bogotá. In 1972, at his suggestion, the Embryology Research Laboratory (LABINE), in Buenos Aires was created by the National Council for Scientific and Technical Research (CONICET), where he continued to carry out his research work. Among his successors are mentioned Dante Paz, Leandro A. Miranda, Enrique Mariano, Jorge Herkovits, Marta Mariano and Clara L. Valdez Toledo.

Few researchers have been able to preserve and transmit their enthusiasm for naturalistic research as Pisanó did, who, though attentive to new trends, always considered it essential to have a broad vision of nature, loving it and not considering it an abstract topic for research. His total dedication to embryological

studies and his intense driving action in all these activities were reflected throughout his work. He co-authored several books and monographs on Comparative Anatomy and Embryology and on Biology of Amphibian Development.

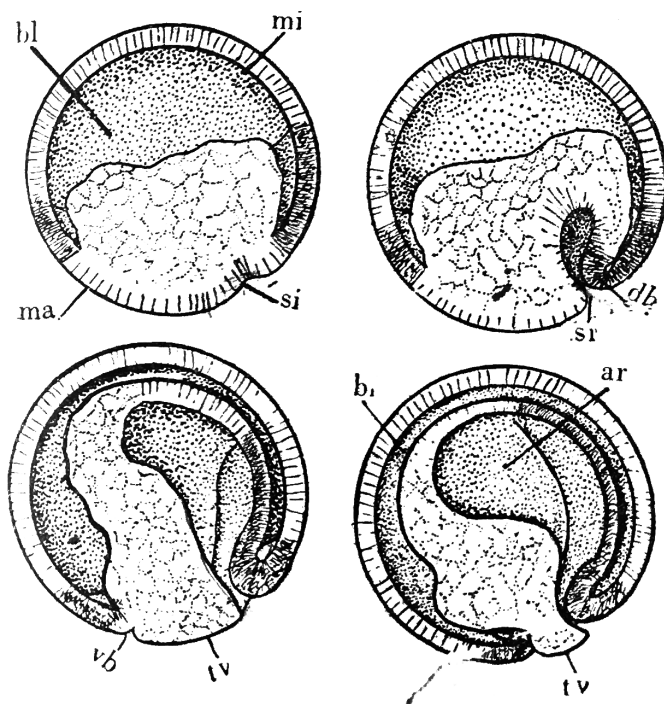
Pisanó's enthusiasm and dedication to the study of biological sciences was transmitted to several generations of students, as reflected in the large number of fellows and thesis candidates whom he directed at the National Universities of Tucumán, of Buenos Aires and of the Northeast and in the Latin American Universities of Colombia and Venezuela as well as in his numerous lectures aimed not only at specialists but also to a wider and more varied audience.

### Francisco D. Barbieri (1930-1993)

The teachings of Cei and Pisanó attracted general biology students interested in the study of nature. In 1949, one of them, Francisco Barbieri (1930-1993) (Fig. 1), was appointed assistant student at the Institute of Zoology of the UNT, and started his career as a researcher conducting cytological studies in native amphibian species (Barbieri 1950, 1954).

At that time, the teaching of biology and developmental anatomy were not clearly outlined concerning their objectives or their teaching methodologies. Pisanó's enthusiasm led Barbieri to the study of embryology, and he collaborated in the writing of the chapter of Elements of Embryology in the General Biology textbook (Fig. 2), one of the first books in Spanish written by Cei and Pisanó for the students of Biochemistry at the UNT (Cei, 1953). In 1952, Barbieri graduated with a biochemistry degree and, in 1954, he earned a PhD in Biochemistry. His histophysiological studies in the field of systematic biology, which he started in 1950, allowed him to define two forms within the same batrachian species, *Leptodactylus ocellatus* and *Leptodactylus chaquensis* Cei (Barbieri, 1950). From then on, Barbieri started a series of research stays in Italian laboratories such as the Istituto di Genetica Lazzaro Spallanzani in Pavia, the Istituto di Anatomia comparata in Florence, Palermo and Torino, the Istituto di Genetica and Institute of Histology in Naples and at the Stazione Zoologica di Napoli, where he came in contact with prestigious researchers who influenced his future research that would pave the way for experimental biology in Argentina. Having been awarded a fellowship by the International Rotary Foundation, he conducted a research stay at the Animal Morphology Laboratory of the Free University of Brussels, Belgium, under the direction of Prof. Jean Louis Auguste Brachet (1909-1988). Brachet, the son of the famous embryologist Albert Brachet (1869-1930), after extensive activity in the field of cell differentiation, in 1933 demonstrated that RNA plays an active role in protein synthesis. Brachet's interest and enthusiasm in elucidating the role of nucleic acids and their metabolism during embryonic development and regeneration attracted a number of Belgian and foreign researchers, Barbieri among them. Having learnt more precise methods, Barbieri reconsidered some aspects of the metabolism of nucleic acids during avian embryo development. In Brachet's laboratory, Barbieri studied the role of DNA synthesis in initial development, following the incorporation of adenine-C14 into the chick embryo. The autoradiographic method allowed him to observe that adenine was incorporated mainly in the neural tube and then in the somites, according to two gradients: caudo-cephalic and ventro-dorsal. Using autoradiographic techniques, he was able to determine that low doses of UV light





**Fig. 2.** Pisanó's drawings of amphibian gastrulation (toad) (from the chapter "Embriología" in the book "Biología General," by José. M. Cei and Armando Pisanó, 1951).

inhibited morphogenesis, preventing the closure of neural folds in the chick embryo as a result of an alteration in the metabolism of nucleic acids, and not by an alteration of steroids - especially vitamin D precursors - as had been suggested by James O. Davis (Davis, 1944). Years after his return to Argentina, these works were published in the *Journal of Embryology and Experimental Morphology* (now *Development*) (Barbieri, 1960; 1961) and led to new studies on the morphogenesis of the neural tube (Brauckmann and Barbieri 1957), the interaction between the notochord and the neuroectoblast in chick embryos (Torres and Barbieri 1987) and other studies on the biosynthesis of deoxyribonucleic acid in amphibians (Barbieri and Fernandez 1962). During his stay in the Netherlands (Belgium, 1956), Barbieri also worked at the Institute of Anatomy of the Free University of Brussels led by Prof. Albert Dalcq. Dalcq was interested in elucidating the role of calcium cations in the maturation, activation and fertilization of sea urchin eggs in order to lay the physiological basis of fertilization and parthenogenesis. His studies on morphogenesis in amphibians gave rise to a new concept about morphogenetic potential during embryo development. Barbieri's stay in Albert Dalcq's laboratory in 1957 had introduced him to the experimental manipulation of amphibian embryos. Upon his arrival in Brussels, Barbieri was already an expert in experimental embryology methods and began studies of reproductive biology and various aspects of embryonic development in an indigenous amphibian, the common toad *Bufo arenarum* (now *Rhinella arenarum*). By 1958, he had already published several articles on endocrine control of embryo development and regeneration.

During those years, his interest had been focused on the study of the energy metabolism of *B. arenarum* eggs, which, like

other amphibian eggs, could be cleaved at a normal rate in the absence of oxygen or in the presence of cyanide - an inhibitor of the respiratory chain - during the first days after fertilization the egg. Together with his disciples Arnaldo Legname (1927-2017) and Hortensia Salomón (1936-2007), Barbieri showed that *R. arenarum* eggs displayed an intense glycolytic activity under anaerobic conditions, which was inhibited when the eggs returned to oxygen (Pasteur effect) (Barbieri and Legname 1957; Barbieri and Salomon 1963). They also observed an increase in oxygen consumption during the first 2h of recovery (payment of oxygen debt) which was not involved in the lactate oxidation (Barbieri and Valdez Toledo de Moreno 1966). These results supported Brachet's idea concerning the presence of an 'oxidative reserve' in vertebrate eggs since a similar respiratory response had been described in eggs of *Rana fusca* (Brachet, 1934), *R. pipiens* (Cohen, 1955) and *R. arenarum* (1964).

In 1958, Barbieri was appointed to the Chair of Animal Histology and then to that of Embryology and Histology at the Faculty of Natural Sciences and the Miguel Lillo Institute. In 1960, he became a professor of General Biology and, in 1971, a professor of Comparative Anatomy in the Faculty of Biochemistry.

One of the most important contributions to the experimental analysis of nuclear differentiation at the time had been the method developed by Briggs and King for the transplantation of embryonic nuclei of amphibians (Briggs and King 1952). In 1967, Barbieri extended the nuclear transplantation experiments to the *R. arenarum* toad, transplanting blast nuclei to enucleated oocytes of the same species in different culture media, managing to develop embryos until the beginning of the tadpole stage (Legname and Barbieri 1967, 1968). Years later, Barbieri, along with his disciple Mario Manes, studied the determination of the bilateral symmetry plane of *R. arenarum* at the time of fertilization. They found that the injection of sperm homogenate, without requiring the intact structure of the male gamete, is enough to activate the oocyte and determine the bilateral symmetry plane of the future embryo. The results suggested that microtubules could play several roles in gray crescent formation and pronuclear movement and orientation in the oocyte (Manes and Barbieri 1976; 1977; Manes *et al.*, 1978).

Barbieri was also interested in aspects of amphibian reproduction. Together with Vilecco, observed that diffusible components of the jelly surrounding *R. arenarum* oocytes ("egg water") were necessary for fertilization to take place (Barbieri, 1976). Thus, with the help of Raisman, Barbieri determined that this factor was produced by dialyzable and heat stable oviductal cells (Barbieri and Vilecco 1966; Barbieri *et al.*, 1977), suggesting that this factor induce a pH increase in the inseminating medium (Barbieri and Cabada 1969). This hypothesis was soon ruled out, although studies that attempted to determine the molecular identity of the diffusible factor and its mechanism of action continued. In 1977, Barbieri and Miceli observed that the epithelium of the most cephalic region of the oviduct, the pars recta, secreted a fluid that allowed the fertilization of coelomic oocytes. The factor involved, a protein displaying a molecular weight of 47000, was purified and called oviductal pars recta factor. It was hypothesized that this protein was a  $\text{Ca}^{2+}$ -dependent trypsin-like enzyme which effect on the egg yolk envelope increase the susceptibility to sperm lysine by facilitating the penetration of the vitelline envelope by sperm. Later studies showed that the secretion of the pars recta factor is hormonally reduced (Miceli *et al.*, 1987).



**Fig. 3. Asa A. Humphries, Jr. (left) and Francisco D. Barbieri (right) at the Bellagio Study and Conference Center, The Rockefeller Foundation, Villa Serbelloni on the shores of Lake Como in Bellagio, Italy, 1983.**

In 1978, at Barbieri's suggestion, the Department of Developmental Biology was created at the Faculty of Biochemistry, Chemistry and Pharmacy. This was the first research unit under the CONICET and the UNT. In 1980, this Department led to the creation of the Higher Institute of Biological Research (INSIBIO, CONICET-UNT)

In 1983, Barbieri was hired by the Rockefeller Foundation to work on amphibian fertilization together with Asa A. Humphries, JR. (Fig. 3), who, at that time, was Vice President and Dean of the Transylvania University, Lexington, Kentucky. The meeting took place at the Bellagio Study and Conference Center, which was the venue for numerous meetings for academics from all over the world where they reviewed subjects of international importance for the development and strengthening of universities and institutes in developing countries. Humphries was the PhD mentor of Eugenia del Pino at Emory University, Atlanta, GA (see article by del Pino in this issue).

Towards the end of the 20th century, the study of developmental biology sought explanations based on cellular or tissue structure and function, considering the interactions between its components. Thus, a modern vision in this discipline, similar to the one outlined in the world, appeared in the UNT. In the 80s, research in Barbieri's laboratory was oriented to the study of cell interactions during primary embryonic induction in *R. arenarum*, analyzing the role of the cell surface and the extracellular matrix during the determination of the neuroectoblast in gastrulation. A first approach aimed at determining whether primary induction was accompanied by a modification on the surface of epiblast cells.

Thus, it was observed that epiblast cells change the composition of surface glycoproteins as a result of neural induction (Barbieri *et al.*, 1980). Whether this alteration of the surface was causally related to the inductive action of the primary organizer remained an open question. With radiochemical studies, synthesis of polyadenylated RNA was observed at the interaction interface of cordomesoblast with the epiblast during neural induction (Sánchez *et al.*, 1983). Years later, the identification by recombinant DNA techniques of the signals released by the Spemann organizer (cordina, cerberus, noggin, follistatin, nodal, etc.) and their mechanism of action by blocking the ventralizing signals verified this hypothesis (De Robertis, 2009).

In a second approach, Barbieri and Sánchez determined that the inhibition of glycosylation of proteins in the blastocoel of *R. arenarum* embryos produced an alteration in morphogenetic movements, leading to embryo exogastrulation (Sánchez and Barbieri 1983). One of these molecules in close correlation with cell migration was identified as fibronectin (Sánchez *et al.*, 1984). Sánchez observed that when epiblast cells were cultured with dorsal extracellular matrix (located mainly at the interface between the dorsal lip of the blastopore and the overlying ectoblast), they differentiated into neural, mesenchymal and pigmentary cells. In contrast, cells grown in saline solution or with extracellular matrix of the ventral region differentiated into ciliated and secretory (non-neural) cells. These results evidenced a differential biological activity of the extracellular material located in the dorsal and ventral regions of *R. arenarum* (Sánchez and Barbieri 1988). This once again confirmed the regulatory role of surface and extracellular matrix proteins in the migration of cordomesoblastic cells during gastrulation.

The energy and passion of Barbieri for the enigmas of development led him to organize and promote numerous postgraduate courses and conferences, spreading his knowledge of development biology in different universities of the country such as the National University of Salta (1959), the National University of Buenos Aires (1961, 1978, 1980) and the Universidad del Nordeste (1966). Thus, he encouraged interest in the study of embryology in Argentina as well as in neighboring countries such as Chile (Santiago de Chile 1971, Concepción, 1981). His integration with Chilean groups led him to organize international courses sponsored by UNDP/UNESCO between 1978 and 1985, with the participation of prestigious researchers in the area of development biology (Figs. 4,5). Two outstanding figures among these researchers were Luis Izquierdo (1928-1992), devoted to the early development of the mammalian embryo, and Juan Andrés Fernández Hidalgo (?-2018), who studied cytoskeleton dynamics and their role in the formation of cytoplasmic domains during early embryogenesis of zebrafish and *Theromyzon rude*. The participation of Claudio Barros (1936-2008), from Chile, was very important given his interest in the different steps of sperm-egg interactions during mammalian fertilization, particularly during the acrosome reaction. Many of the participants in these courses spread the concerns of developmental biology to numerous scientific research centers in Argentina and Latin America. Barbieri's passion in the study of the morphogenetic movements of gastrulation has left its mark on renowned researchers such as Eugenia del Pino (Ecuador) and Roberto Mayor (Chile). In 1973, thanks to Barbieri's effort, the VI Argentine Congress of Biology (Fig. 6) was held in Tucumán, with the participation of the prestigious developmental embryolo-



gist Sir John B. Gurdon (Nobel Prize in Medicine in 2012), who would lay the foundations of cloning in experiments conducted on frogs in 1962, and of embryologist Rubén Adler, who had begun his studies on neurodevelopment. Luis F. Leloir (Nobel Prize in Chemistry in 1970), also participated in the event.

**Barbieri's successors in Tucumán**

Several young researchers who had been Barbieri's students continued investigating aspects of reproduction and embryogenesis, forming new research groups within the Department of Developmental Biology in Tucumán and in other provinces in Argentina. By the end of the 80s, the Biology Institute already had numerous lines of research, the reproduction and embryology of amphibians being the primary focus of research.

Arnaldo Legname (1925-2017) and Hortensia Salomón de Legname (1936-2007), who studied in greater depth the intermediate metabolism during the embryo development of *R. arenerum*, characterized a pregastrular embryonic metabolism where carbohydrates are broken down mainly through the cycle of pentose phosphate with a relatively low participation of the Embden Meyerhof glycolytic pathway. As ontogenesis progresses, this metabolic pathway increases its participation, until in tadpoles, and especially in adult tissue, almost the whole carbohydrate content is metabolized through glycolysis. They also demonstrated that during the segmentation of *Rhinella* embryos this pentose cycle is mainly associated with the glutamic-aspartic cycle, producing intermediates such as fumarate or malate, which would be used as precursors of pyrimidine and purine salts (Salomón de Legname,

1969; Salomón de Legname *et al.*, 1975). As embryonic development progresses, the participation of the glutamic-aspartic cycle decreases by focusing on the classic tricarboxylic acid cycle (Salomon de Legname *et al.*, 1971). Although coelomic oocytes have a metabolism similar to that of adult tissues, during their passage through the oviduct they undergo biochemical and metabolic transformations influenced by pituitary hormones that give them an embryonic metabolism (Salomón de Legname *et al.*, 1979). One of the members of the labora-



**Fig. 4 (top).** Participants of the International Training Course "Biology of Fertilization" sponsored by UNDP / UNESCO. Tucumán, Argentina, 1978. Marta Bühler (1), Claudio E. Barros (2), Enrique del Pino (3), Nestor G. Carri (4), Francisco D. Barbieri (5), Arnaldo H. Legname (6), Armando Pisanó (7), Marcelo O. Cabada (8), Marta I. Gomez (9), Jorge Valz-Gianinet (10), Hortensia Salomón de Legname (11) and Eugenia del Pino (12).



**Fig. 5 (bottom).** Participants of the International Training Course "Initial embryonic development in vertebrates" sponsored by UNDP / UNESCO. Tucumán, Argentina, 1980. Else Brauckmann (1), Clara L. Valdez-Toledo (2), Juan A. Fernández Hidalgo (3), Claudio E. Barros (4), Arnaldo H. Legname (5), Francisco D. Barbieri (6), Armando Pisanó (7), Marcelo O. Cabada (8), Nestor G. Carri (9), Sara S. Sánchez (10), Alicia N. Sánchez Riera (11), Mario E. Manes (12), Marta I. Gomez (13), Marta I. Bühler (14), Luisa Peralta (15), Teresa Petrino (16), Liliana I. Zelarayán (17), Luis Izquierdo (18), Jorge Herkovitz (19), Silvia N. Fernández (20), Dora C. Miceli (21), and Inés C. Ramos (22).

tory, Marta Bühler, continued with the study of the processes that take place during maturation, activation of amphibian oocytes and ability to initiate development. They analyzed seasonal changes in the oxidative metabolism and the signaling pathways involved in these processes (Zelarayan *et al.*, 1995; Toranzo *et al.*, 2010). In recent years, they extended their studies to mammals by adopting the *Chinchilla lanigera* Grey model to study the signaling pathways that participate in the capacitation and acrosomal reaction of epididymal sperm, as well as the location and importance of some sperm proteins involved in gamete interaction (Gramajo-Bühler *et al.*, 2014; Zapata *et al.*, 2016).

Jorge Raisman, while working toward his doctorate degree with Barbieri, made a research stay at the Stazione Zoologica di Napoli under the direction of Alberto Monroy. In 1972, he worked at the Emory University in Atlanta, Georgia, US, in the laboratory of Asa Alan Humphries, Jr., where he studied the role of the jelly coat in amphibian fertilization. On his return, after having earned his Doctor's degree, he continued to work on morphological and biochemical aspects of *R. arenarum* fertilization (Miceli *et al.*, 1977).

Marcelo Cabada, during a stay in the laboratory of Chris Darnbrough at the University of Edinburgh, Scotland, UK, demonstrated the existence of two different classes of "maternal" poly (A) + RNA in *Xenopus levis* oocytes during different oogenesis periods (Cabada *et al.*, 1977). Upon his return to Tucumán, Argentina, he continued with studies on the metabolism of oocyte RNA during the maturation stage. He found that progesterone does not modify RNA synthesis (Sánchez Riera *et al.*, 1988a), and that follicle cells transfer RNA to the oocyte (Sánchez Riera *et al.*, 1988b). He continued studying different factors participating in gamete interaction during *R. arenarum* fertilization such as the effect of sperm concentration on the fertilization rate (Cabada, 1975), the influence of different lipids (cholesterol and phosphatidylcholine) on the acrosome reaction (Díaz Fondevila *et al.*, 1988), the role of glycosidic residues of the vitelline envelope in gamete interaction and the exocytosis of oocyte cortical granules (del Pino and Cabada, 1987).

Dora Miceli, from the Barbieri laboratory, continued studying the function of oviductal molecules involved in fertilization and embryo development of amphibians and various mammalian species (hamster, rat, sow, cow, llama and vicuña). Following her theory about the participation of proteases in the oviductal environment, she and her team evaluated the activity and expression of components of the plasminogen/plasmin system, enzymes that belong to the serine protease family in the mammalian oviduct (Roldán-Olarte *et al.*, 2012). They studied different matrix metalloproteases (MMPs) and their inhibitors, which participate in fertilization and early embryo development of the llama, a camelid native to the Andes mountain range often found in northwestern Argentina (Zampini *et al.*, 2017). Another line of research of the laboratory focused on the participation of members of the TGF- $\beta$  family in the regulation of oviductal function during preimplantation embryo development and early



**Fig. 6.** Prof. Francisco D. Barbieri (top left), Prof. Luis F. Leloir (Nobel Prize, 1970) (top right), Prof. Sir John B. Gurdon (Nobel Prize, 2012) (bottom left) and Prof. Ruben Adler (bottom right) during the VI Argentine Congress of Biology, held on Tucumán in 1973.

pregnancy in rodents and cattle (Valdecantos *et al.*, 2004, García *et al.*, 2015). At present, the research group continues to study the molecular signals that participate in maternal-embryo interaction.

Sara Sánchez continued analyzing the participation of surface molecules and extracellular matrix during the migratory processes that take place in the embryonic development of *R. arenarum*. She identified and characterized six extracellular matrix proteins (EP1-EP6) that are synthesized de novo during gastrulation (Sánchez *et al.*, 1988). In those days, cell lineage studies represented a strong component of developmental biology in France. During her stay at the Laboratoire de Biologie Expérimentale of Jean-Claude Boucaut at the Université Pierre et Marie Curie, Paris, Sánchez studied morphogenetic movements during the early gastrula stage of *Pleurodeles walt*, establishing the map of the fate of superficial and deep circumblastoporal cells (Delarue *et al.*, 1992). Upon her return to Argentina, she and her team determined that the blocking of EP3-EP4 with specific antibodies injected into the blastocoel causes severe alterations in the gastrulation process, affecting the convergence extension processes of the dorsal marginal zone and the epiboly of the blastocoel roof (Genta *et al.*, 1997). Similar effects had been described for fibronectin in amphibians (Boucaut *et al.*, 1985). Using inhibitors of glycoprotein and glycolipid synthesis, the importance of these molecules in the processes of radial intercalation and migration was also established (Peralta *et al.*, 1995). Sánchez and her team also determined that the inhibition of glycosphingolipid synthesis or its blocking by antibodies causes alterations in the contacts of mesodermal cells, affecting cytoplas-



mic processes (filopodia and lamellipodia), inhibiting gastrulation movements (Aybar *et al.*, 2000a; 2000b). Following the study of extracellular proteins, the expression of a vitronectin-like adhesion protein that is stored in vitelline platelets during *R. arenarum*'s oogenesis was determined. Years later, this protein was cloned and its implication in organogenesis was suggested. The need to use molecular biology techniques to try to solve these biological approaches led to the substitution of the native experimental model of the amphibian *R. arenarum* for *Xenopus laevis*, an organism which genome was at least partially known at the time. The Department of Developmental Biology created its first *Xenopus* bioterium and, using *Xenopus laevis* as a model organism, the enzymes *Xlclg1* and *Xlclg3*, responsible for ganglioside synthesis, were cloned, studied, and found to be involved in convergent extension movements during *Xenopus* early development (Luque *et al.*, 2008).

The economic instability in 2000 caused biomedical research and biology in Argentina fall behind in the spectacular worldwide advance that the use of molecular biology represented in numerous scientific research fields. The new technologies had been applied to only a few either areas, while other fields such as cellular and molecular neurobiology, developmental genetics, structural biology, genomics and computational biology were represented poorly or not at all (Sabatini *et al.*, 2000).

Given the recent interest in studying the genes and signaling pathways that control the development processes, young researchers from the Sánchez group conducted different research stays at the Millennium Nucleus in Developmental Biology, the Faculty of Science, University of Chile, with Roberto Mayor, who had made great contributions related to the molecular mechanisms that underlie the development of the neural crest in *Xenopus laevis* (Mayor *et al.*, 1999). These young researchers determined that a balance in the activity of the transcription factors Slug and *msx1* controls apoptosis in certain areas of the neural folds, defines the precise boundaries of the territory of the neural crest, and accurately controls the number of cells among neural crest derivatives (Tribulo *et al.*, 2004). They also cloned characteristic transcription factors of the progenitors of the migratory multipotent neural crest, as well as several of its differentiated derivatives such as *sox10* and *paraxis*, a regulator of the development of paraxial mesoderm and the formation of somites (Honoré *et al.*, 2003; Carpio *et al.*, 2004). At present, in Sánchez's laboratory, researchers determined that *paraxis* participates in the cellular rearrangements that take place during *Xenopus* somitogenesis through the regulation of cell adhesion and the differentiation of somites (Sánchez and Sánchez, 2015). They continue studying the processes and genes involved in the formation and establishment of the anuran spine pattern, considering the structural divergence that this group presents with respect to other vertebrates (Sánchez and Sánchez 2013).

Manuel J. Aybar, during his postdoctoral stay in Roberto Mayor's Laboratory, studied the complex sequence of inductive events responsible for the generation of *Xenopus* neural crest. He determined that the Snail transcription factor is required for the specification and migration of neural crest cells and that the early activation of this gene defines the competent territory of the neural crest (Aybar *et al.*, 2003). Upon his return to the INSIBIO (UNT-CONICET), Aybar leads a new research group aimed in studying the role of different genes and cell signaling pathways (BMPs, *Ihh/gli*, Endothelins, Notch) in the molecular, cellular and morphogenetic regulation of the processes that take place during neural crest development in

vertebrates (Bonano *et al.*, 2008; Tribulo *et al.*, 2012; Agüero *et al.*, 2012; Vega Lopez *et al.*, 2015). In this group, Celeste Tribulo also assesses the molecular aspects of epidermal development in amphibians (Tribulo *et al.*, 2012).

## Developmental Biology throughout the country

### Buenos Aires

Until the mid-twentieth century, the teaching of embryology and histology at the Faculty of Medicine of the University of Buenos Aires (UBA), Buenos Aires city, was integrated into a single subject. The tenured professor of the chair of histology, Julio Lacoste, was a strong promoter of both subjects and, in 1922, he created the Institute of General Comparative Anatomy and Embryology, thus laying the foundations for the creation of the Embryology Museum.

Between 1922 and 1957, the Institute of General Comparative Anatomy and Embryology was run successively by Pedro Rojas, who returned to the country after having worked with the prestigious embryologist Albert Brachet, Manuel Varela, who created the Chemical Embryology section of the Institute, Jorge Porto and Eduardo De Robertis (1913-1988). De Robertis started teaching cytology, describing the subcellular ultrastructure not only from a morphological but also from a biochemical and genetic point of view, thus laying the foundations of Cellular Biology in Argentina.

In the 1960s, De Robertis invited Bradley M. Patten (1889-1971), Emeritus Professor of the University of Michigan, to spend a season at the Chair in his charge. During this period, Patten promoted the training of Roberto Narbaitz as an embryologist, who then began teaching embryology to medical students. Narbaitz focused his research on the analysis of sexual differentiation in birds and their hormonal control, analyzing sexual inversion from a morphological and functional point of view (Narbaitz and Sabatini 1963; Narbaitz and Kolodny 1964; Narbaitz and Teitelman 1965). Their findings confirmed the bisexuality and asymmetry of the embryonic gonads of birds (Rahil and Narbaitz 1972). Together with his disciple Rubén Adler (1940-2007), he studied the differentiation of germ cells in embryonic gonads and described submicroscopic aspects during the differentiation of fetal Leydig cells from rodents (Narbaitz and Adler 1966). Narbaitz made important contributions to the knowledge of the parathyroid gland and its role in  $Ca^{2+}$  homeostasis during chicken embryogenesis (Narbaitz and Gartke 1975).

Adler graduated from the Faculty of Medicine of the UBA in 1963. In 1968, he made a postdoctoral stay at the embryology school of the Hubrecht Institute of the University of Utrecht, one of whose most prominent members was the researcher Pieter Nieuwkoop (1917-1996), a pioneer in the induction analysis of the mesoderm and in the processes that lead to neural induction. There Adler started his research in a field that would become his scientific passion, developmental neurobiology (Adler, 1968; Adler *et al.*, 1976, 1977), establishing the basis for modern experimental analysis of development processes at the morphogenetic, cellular and genetic level. On his return to Argentina, Adler became Assistant Professor of Histology, Cytology and Embryology at the Faculty of Medicine of the UBA, where he wrote a book dealing with the teaching of embryology titled "Developmental biology and congenital malformations" and published in 1974. One of the main aims of the book was to bridge the gap between morphology of development, the predominant viewpoint taught at that time, and the biology and genetics of development, which expanded its influ-



ence in the world under the enthusiastic leadership of Conrad H. Waddington (1905-1975), Joseph Needham (1900-1995), Gregory Bateson (1904-1980) and Pieter Nieuwkoop (1917-1996), among others. From the beginning of his career, Adler focused his interest on ocular development with novel approaches to identify genetic factors and microenvironmental signals that regulate neuron survival. In 1977, because of political conditions in Argentina, Adler, Narbaitz and other researchers left the country. After obtaining a Guggenheim Fellowship, Adler held research scientist positions on the campus of the University of California, Berkeley and San Diego in the US. In 1982, he joined the Wilmer Eye Institute at Johns Hopkins University, where he spent the rest of his remarkable career. Adler's work revealed key molecular pathways that regulate the survival and differentiation of retinal cells both in normal development and in degenerative retinal disease, and played a key role in the discovery of the CNTF neurotrophic factor (Adler *et al.*, 1979; Wahlin *et al.*, 2000; Canto Soler *et al.*, 2008). His laboratory is currently under the direction of one of his successors, Valeria Canto Soler.

The current Institute of Cell Biology and Neurosciences (IBCN) of the UBA School of Medicine originated from the Institute of Comparative General Anatomy and Embryology. Vladimir Flores started his research in the IBCN Electronic Microscopy laboratory. There he earned his doctor's degree, spending most of his time in the study of nervous system regeneration under the direction of Amanda Pellegrino de Iraldi. Flores completed his postgraduate training at the University of Cambridge, working at Downing College under the direction of professors J. Treherne and N.J. Lane, and specializing in techniques applicable to the study of nervous system development. Upon his return to the country, he went back to the IBCN, where he studied the development of local circuits and synaptogenesis during the development of the dorsal midbrain of the chick embryo, the *tectum opticum*. In the IBCN, he created the Laboratory of Developmental Neurobiology which up to now is directed by his former student Gabriel E. Scicolone (Scicolone *et al.*, 1995). Later, at Favaloro University, Flores created an Interdisciplinary Group of Theoretical Biology devoted to the analysis of cell behavior dynamics involved in the development of the central nervous system (Rapacioli *et al.*, 2012; 2016). At present, Scicolone's research is focused on neuronal determination and differentiation of progenitor cells and on the acquisition of competence to respond to signals that guide axonal growth, using the retinotectal system of the chick embryo as an experimental model. He is investigating the molecular bases of the development of topographically ordered connections in the central nervous system. His team studies the role of the Eph tyrosine kinase receptor system and its ligands, ephrins, uPA/uPAR and neurotrophic factors in growth and axonal guidance (Ortalli *et al.*, 2012; Lino *et al.*, 2014).

At the IBCN, Andrés Carrasco (1946-2014) worked as a professor of physiology, cell biology and embryology at the UBA. In 1981, he made a postdoctoral stay in Edward De Robertis' laboratory at the University of Basel, Switzerland. He cloned and sequenced the first vertebrate homeobox gene involved in development known as *AC1/Hoxc 6* (Müller *et al.*, 1984). In 1984, Carrasco started working at the Institute of Cellular and Molecular Biology at the University of Indiana, where he carried out the first *in situ* hybridization in *Xenopus laevis* embryos. It was the first demonstration of the spatial distribution of a *Hox* gene, a low abundance mRNA

in amphibian embryos (Carrasco and Malacinski, 1987). Then he moved to the Department of Molecular Genetics at the University of Texas to work with antisense RNA. In 1990, when he returned from his postdoctoral stay, Carrasco settled in the Institute of Cellular Biology and Neurosciences of the Faculty of Medicine of the UBA. He continued investigating the role of *Hox* genes in the development of the nervous system of *Xenopus laevis*, initiating studies on gene function using molecular biology methods (Lopez and Carrasco, 1992; Paganelli *et al.*, 2001). Silvia López, a disciple of Carrasco, continues to investigate the signaling pathways with *Xenopus laevis*, especially focusing on the Notch pathway and other factors in the segregation of embryonic layers and their impact on the structures derived from them (Revinski *et al.*, 2010; Favaro and Lopez, 2018).

In 1968, Marta Fernández de Recondo, who developed her doctorate by studying *R. arenarum* oogenesis under the direction of Barbieri in Tucumán (1967), started teaching at UBA as Professor of Embryology at the Faculty of Natural and Exact Sciences-UBA. Together with Graciela Guerrero and María Cristina Maggese (1944-2014), formed a new Developmental Biology group (Maggese *et al.*, 1976; Cukier *et al.*, 1979). Maggese and Guerrero were trained in the study of the amphibian model in the Postgraduate Courses

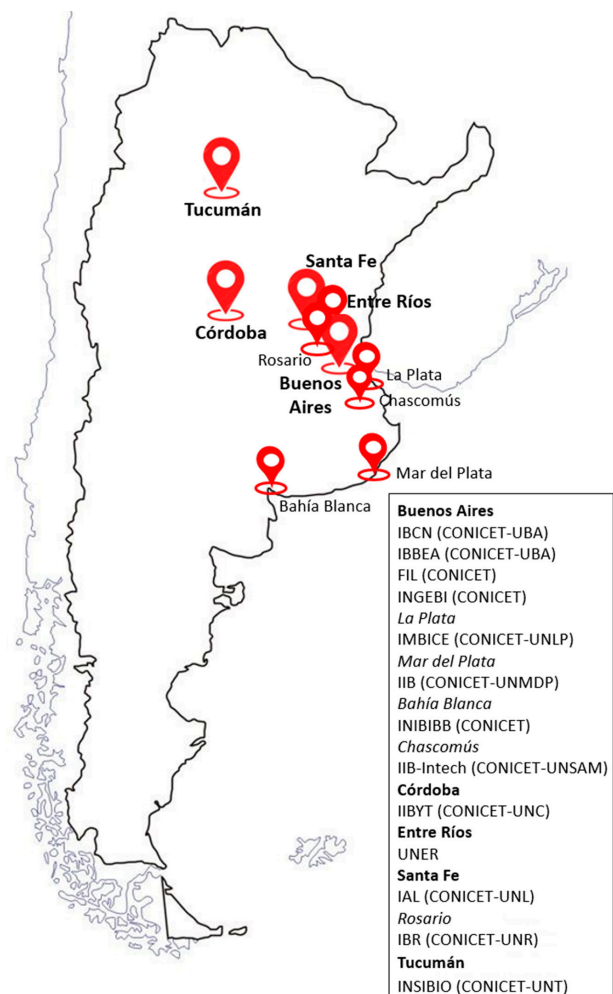


Fig. 7. Geographical distribution of some of the main Developmental Biology laboratories in Argentina.

run by Barbieri and also during several stays in his laboratory. For several years, together with other research teachers, Maggese and Guerrero taught the postgraduate course “Topics in Developmental Biology” at the UBA. At the same time, Guerrero started to study the implication of the germinal epithelium in the reversal of fish sex (Lo Nostro *et al.*, 2003). In the meantime, Maggese continued her studies on the hormonal control of the reproduction of native fish, training numerous researchers who analyzed various aspects of fish biology at the Institute of Biodiversity and Experimental and Applied Biology (IBBEA) (Vissio *et al.*, 2002; Pandolfi *et al.*, 2002; Ravaglia and Maggese 2003; Cánepa *et al.*, 2006; Genovese *et al.*, 2011). Her former students developed independent lines of research, giving rise to three autonomous working groups, one of them being the Laboratory of Neuroendocrinology of Growth and Reproduction, run by Paula Vissio. This group is dedicated to the study of morphological and adaptive changes during the early development of regulated teleosts at the neuroendocrine level. Vissio continued teaching Animal Embryology which, in 2019, changed its name to Biology of Growth and Reproduction.

Dante A. Paz did his doctoral thesis with Pisanó, analyzing the processes of differentiation of the heart area of *R. arenarum*. With his team, he continued working on the structuring of the olfactory system in anuran amphibians, during larval development and in adults, studying the signaling pathways involved in the perception of olfactory stimuli, neuronal projections and their connections with the central nervous system. He was also interested in the study of the regenerative processes of the olfactory epithelium, analyzing the participation of different growth factors and the participation of stem cells, the differentiation of olfactory neurons and of sustainable cells (glial cells) (Jungblut *et al.*, 2010; Frontera *et al.*, 2015).

At the Institute of Physiology, Molecular Biology and Neurosciences (IFIBYNE), Flavio Silva Junqueira de Souza is currently leading the Embryonic Development Neurobiology Laboratory, which is devoted to the study of the genes controlling the early patterning of the hypothalamus and the pineal gland in mammals and amphibians. In the same laboratory, María Cecilia Cirio investigates the role of cofactors of the transcriptional complex Ldb1-Lhx1 in the development of vertebrate kidneys (Espíritu *et al.*, 2018).

Alfredo Vitullo, made a post-doctoral stage at the Centre de Recherches de Jouy-en-Josas, Institut National de la Recherche Agronomique -INRA-, France, working on mechanisms of oocyte electroactivation and cloning of mammalian embryos. Subsequently, he served as a Guest Researcher at the Lerner Research Institute of the Cleveland Clinic Foundation, Cleveland, USA, in the molecular biology of T-box genes in breast cancer and its role in mouse embryological development. Nowadays he works at the Center for Biomedical, Biotechnological, Environmental and Diagnostic Studies (CEBBAD) of the Maimonides University, conducts research on the specification of germ cells in eutherian mammals (Proietto *et al.*, 2019).

At the Leloir Institute Foundation (FIL), Luis A. Quesada Allué began his scientific research with ecological studies in yellow clams and scallops, two mollusks found on Argentine shores, and then moved on to investigate the behavioral physiology of honeybees. His main scientific contributions are the biochemical and molecular studies in the development of insects concerning the mechanism of synthesis and deposition of cuticle proteins of insects, which were later extended to crustaceans, trypanosomatids and somatic embryos of plants (Quesada-Allue, 1981, 1982; Pérez *et al.*, 2002).

In recent years, his extensive work on the biology of insects become focused on two little explored aspects worldwide: the study of functional senescence (aging) and a new mechanism of regulation of neurotransmitters in insect brains (Pujol-Lereis *et al.*, 2016).

Since 1998, Pablo Wappner leads the Laboratory of Genetics and Molecular Physiology at the Leloir Institute using *Drosophila melanogaster* as a model system for the study of molecular and cellular mechanisms that mediate stress adaptation. Since its creation, the group has focused on the elucidation of the mechanisms that mediate hypoxia adaptation (Centanin *et al.*, 2005; Galagovsky *et al.*, 2014), developing transgenic lines to characterize physiologically and biochemically the elements that take part in the response to hypoxia. In recent years, the group has expanded its studies to elucidate hypoxia genes linked to the metabolic reprogramming that occurs under certain pathologies such as tumor development in cancer, a line of work that might lead to future therapeutic applications (Gándara and Wappner, 2018).

In recent years, young researchers who conducted postdoctoral studies and training in centers of great relevance in La Jolla, California, acquiring significant experience in new technologies, have conducted their research at the Leloir Institute. Among them is Maria Fernanda Ceriani, who, after a stay in the laboratory of Steve Kay at The Scripps Research Institute, made important contributions to the knowledge of the neural control mechanisms of the generation of circadian rhythms in *Drosophila* while directing the Behavioral Genetics laboratory (Franco *et al.*, 2018). Another team member is Marcelo J. Yanovsky, who also carried out postdoctoral studies in the laboratory of Steve A. Kay, and is currently focusing his research on the genetic plot that regulates the daily and seasonal rhythms of plant growth and development in response to the environment. His work is conducted in the laboratory of Comparative Genomics of Plant Development (Mora-Garcia *et al.*, 2017; Hernando *et al.*, 2019). Pablo D. Cerdán, who carried out his postdoctoral studies at the Plant Molecular and Cellular Biology Laboratory of the same Institute, has devoted his research to analyzing the gene cascade involved in *Arabidopsis thaliana*'s response to temperature and light, in the Plants Molecular Biology laboratory at the Leloir Institute (Jaskolowski *et al.*, 2019). At the FIL, Guillermo Lanuza, upon his return from the Laboratory of Molecular Neurobiology at the Salk Institute for Biological Studies, started to direct the Laboratory of Genetics and Neural Development. He is working on the ontogenic mechanisms that control the diversification of neuronal and glial types in the central nervous system in experimental models in mice and birds (Petraça *et al.*, 2016; Di Bella *et al.*, 2019).

At the Research Institute in Genetic Engineering and Molecular Biology “Dr. Héctor N. Torres” (INGEBI), Lucia F. Franchini is studying the genetic basis that underlies the evolution of the primate brain, especially in humans and in the inner ear of mammals in the Laboratory of Genetics and Molecular Evolution (Pisciottano *et al.*, 2019). Jorge Muschietti, in the Laboratory Molecular Mechanisms of Fertilization in Plants of INGEBI, is at present investigating the bases that regulate the development of pollen grain. He is also conducting research on the molecular mechanisms that control the specificity of pollination in plants (Sede *et al.*, 2018).

At the Biomedicine Research Institute of Buenos Aires (IBioBA), Luis Morelli is studying the principles that control the processing of information that cells receive from their environment and use it to make decisions, during morphogenesis and in adult tissues, using the zebrafish as an experimental model (Uriu *et al.*, 2017).



### La Plata

Nestor Carri, who at the beginning of his career had worked with Adler and Suburo (Suburo *et al.*, 1979), completed his PhD with Ted Ebendal in the Department of Developmental Biology, Uppsala University, Sweden, studying the influence of axon terminal fields of the *optic tectum* on the selective stimulation of the growth of neurites of the chick retina. Upon his return to Argentina in 1983, he created the Molecular Development Biology laboratory within the Multidisciplinary Institute of Cellular Biology (IMBICE-CONICET) of the city of La Plata in Buenos Aires, which was devoted to the study of the embryological mechanisms of nervous development. There he worked on trophic molecules and signaling cascades that activate the “gene tandem” of survival and neuroprotection of nerve and glial cells in primary cultures and established cell lines (Blanco *et al.*, 2001; Villegas *et al.*, 2006).

Upon his return from a postdoctoral stay with Herbert Jäckle at the Abteilung Molekulare Entwicklungsbiologie, Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany, Rolando Rivera-Pomar began working on gene regulation in the *Drosophila* embryo (Rivera-Pomar *et al.*, 1995). For some years now, Rivera-Pomar has been working on the comparative genomic study of insects. In the Laboratory of Genetics and Functional Genomics, Regional Center for Genomic Studies, UNLP, he investigates early development genes and their regulation with a focus on the segmentation process, genes related to resistance to insecticides and small peptides, and neuropeptides in different species of insects, some of them of medical and agricultural interest (Lavore *et al.*, 2015).

### Bahía Blanca

In 1963, Nicolás G. Bazán began to study developmental biology in Tucumán as a Barbieri fellow, being trained in the embryonic model of amphibians (Brauckmann and Bazán 1963; Barbieri and Bazán 1964). In 1970, on his return from a stay abroad (Harvard Medical School, Boston, MA, US, and a postdoctoral stay at the University of Toronto, Canada), he moved to the National University of the South (UNS), and organized the Biochemical Research Institute of Bahía Blanca (INIBIBB) and the Department of Biology. In addition to his managerial skills, his enthusiasm for biological studies led him to focus his research on the central nervous system, including the retina, and on early embryo development on vertebrate models, with a strong interest in lipid composition and metabolism (Bazán, 1971; Crupkin *et al.*, 1973; Avelano and Bazán, 1975). During his stay in the country until 1980, Bazán trained a significant group of developmental biologists interested in the lipid metabolism of amphibians, among them Ana M Pechen, Ida C. Bonini and Telma Alonso (Pechen *et al.*, 1974; Bonini de Romanelli *et al.*, 1981; Alonso *et al.*, 1982), who made important contributions in this area. Telma Alonso continued to explore Development Biochemistry using the *R. arenarum* toad as an experimental model. She focused on the biochemical mechanisms involved in oocyte maturation, fertilization and early embryogenesis (Alonso *et al.*, 1986; Buschiazzo *et al.*, 2008), establishing close collaboration with other related research groups, especially with members of the Biology Institute of Tucumán and in particular with Armando Pisanó. Pisanó taught postgraduate courses in Bahía Blanca and members of INIBIBB also participated in courses taught by him in Buenos Aires and in Neuquén. The INIBIBB Development Biology Laboratory was named after him as a tribute to his outstanding scientific career.

Luis E. Politi, one of Rubén Adler’s students (Politi and Adler,

1986; Politi *et al.*, 1989), on his return from the Wilmer Eye Institute in Johns Hopkins created in the INIBIBB the Developmental Neurobiology group, devoted to the search for cellular or molecular trophic factors involved in the survival and differentiation of retinal neurons. Politi was also interested in the use of stem cells during neurodegenerative diseases that cause blindness, such as retinitis pigmentosa or age-related macular degeneration (Garelli *et al.*, 2006; De Genaro *et al.*, 2013).

In recent years, the study of neuroendocrine control of *Drosophila* morphogenesis during pupariation was also addressed by Andres Garelli. His work sheds new light on the function and evolution of the relaxin receptors, revealing a novel neuroendocrine circuit responsive to growth aberrations mediated by *Drosophila* insulin-like peptide (Dil8) and leucine-rich repeat-containing G protein-coupled receptor 3 (Lgr3) (Garelli *et al.*, 2012; 2015).

### Chascomús

In 2013, Pablo H. Strobl Mazzulla and Juan I. Fernandino returned from their postdoctoral training abroad and created the Development Biology Laboratory at the Biotechnological Research Institute (IIB-Intech, CONICET-UNSAM). Their research is aimed at understanding the epigenetic basis that regulates the development and differentiation of cells in vertebrates, analysing the dynamics of the epigenetic-microRNA regulation network during delamination and condensation of neural crest cells in vertebrates using chicken as animal model. They also analyse the transgenerational epigenetic effect of folic acid deficiency during embryo development in vertebrates (Alata Jimenez *et al.*, 2018; Sánchez-Vasquez *et al.*, 2019). Another line of research focuses on the study of the molecular control of gonadal development in an embryonic model in *Oryzias latipes* (medaka fish) and the molecular pathways mainly involved in the proliferation of primordial germ cells (Fernandino and Hattori, 2019; Castañeda Cortés, 2019; Castañeda Cortés and Fernandino, 2020).

### Mar del Plata

At the Institute for Biological Research (IIB-CONICET), National University of Mar del Plata, Gabriela C. Pagnussat is studying the molecular basis of gametophytic development and fertilization in plants using *Arabidopsis Thaliana* as a model. She also studies iron-dependent cell death in response to adverse environmental conditions (Distéfano *et al.*, 2017; Jaskolowski *et al.*, 2019).

### Córdoba

In the early 1980s, Roberto A. Rovasio, initially trained by Pisanó in the embryological area and by Benito Monis in cell biology, returned to the Institute of Cell Biology of the Faculty of Medical Sciences from the National University of Córdoba (UNC), after a stay at the Institut d’Embryologie Cellulaire et Moléculaire (CNRS et Collège de France). During his training in France, under the direction of Nicole M. Le Douarin and Jean Paul Thiery, Rovasio made pioneering contributions on the role of fibronectin extracellular glycoprotein as an essential factor in the migration of neural crest cells (Rovasio *et al.*, 1983), which marked the focus of his future research on birds.

In the late 1980s, Rovasio worked as a Professor in the Faculty of Exact, Physical and Natural Sciences of the UNC. In 2002, he created the Center for Cellular and Molecular Biology (CEBICEM, UNC), and in 2011 he joined the new Institute of Biological and

Technological Research (IIBYT, CONICET-UNC) with his group. In Argentina, Rovasio oriented his studies toward the mechanisms involved in the dynamic behavior of avian embryo cells as well as their epigenetic disturbance. His research *in vitro* and *in vivo* showed that the cells of the cranial neural crest are oriented in relation to gradients of several molecules of their microenvironment. Rovasio's global contribution was the demonstration of a chemotactic mechanism in the emerging neural crest cells of different levels - brain and trunk - of the neural tube, induced by trophic factors and by the sonic hedgehog morphogen (Shh), proposing the convergence of molecular signals in the modulation of cellular directionality during embryo development (Zanin *et al.*, 2013; Tolosa *et al.*, 2016). On the other hand, he investigated the effects of the administration of ethanol on the proliferation, migration and distribution in the embryo of neural crest cells and its relationship with the Fetal Alcoholic Syndrome (Jaurena *et al.*, 2011). In his laboratory, Rovasio also conducted research on mammalian sperm-oriented motility (Fabro *et al.*, 2002).

Ana Macias, conducted her postdoctoral studies at the Morata G's laboratory at the Molecular Biology Center, Autonomous University of Madrid, Spain. There, she studied the functional hierarchy and phenotypic suppression among *Drosophila* homeotic genes (Macias and Morata, 1996). Since her return to Argentina, she studied the Genital disc growth in *Drosophila* in the Genetics laboratory of the Universidad Nacional de Córdoba. Her group analyzes the contribution of apoptosis to growth regulation and the participation of Jun-NH2-terminal kinase (JNK) pathway in the regulation of these events (Benitez *et al.*, 2010; Arias *et al.*, 2015; 2019).

### Entre Rios

Valeria Sigot, at the Laboratory of Microscopy Applied to Molecular and Cellular Studies of the National University of Entre Rios (UNER), has started a new group interested in studying the dynamic remodeling of intercellular contacts in embryogenesis of zebrafish.

### Santa Fe

In 1987, Marcelo Cabada, a former Barbieri student, started to work at the National University of Rosario (UNR), Rosario, Santa Fe, as Professor of Biology at the Faculty of Biochemical and Pharmaceutical Sciences. He created a developmental biology laboratory in this academic unit. His work continued to focus on the elucidation of the molecular mechanisms of recognition between gametes in amphibians and mammals. In 1999, together with other local scientists, created the Institute of Cellular and Molecular Biology of Rosario (IBR, CONICET-UNR). Together with his student Silvia E. Arranz, he isolated and biochemically characterized two highly glycosylated proteins from the jelly coats from *Rhinella arenarum* (previously known as *Bufo arenarum*) oviduct, the highly glycosylated protein (HGP) and the highly glycosylated low molecular mass protein (L-HGP). They demonstrated that HGP forms a stable fiber matrix around the oocyte and is the main structural component of the jelly coat, while L-HGP is present in the jelly coat and is released into the incubation medium, protecting the integrity of the acrosome until sperm contact the vitelline envelope (Arranz *et al.*, 1997; Krapf *et al.*, 2006). Cabada's team determined the glycoproteins composition of the vitelline envelope of *R. arenarum* and its participation in the spermatozoid-oocyte interaction during the early stages of fertilization (Scarpeci *et al.*, 2008; Barisone *et al.*, 2009). Arranz continued the studies on amphibian fertilization

(Morales *et al.*, 2012) and on the development of teleost fish, with particular interest in growth hormone response of *Odontesthes bonariensis* (Di Prinzio *et al.*, 2010; Botta *et al.*, 2016). Dario Krapf, another Cabada's disciple, leading a group at the IBR, focusses his efforts to understand the molecular events that underpin sperm capacitation. He has shown that PKA activity acts as a key player during this process. His studies aim to elucidate how a highly promiscuous kinase manages to activate different signaling cascades at different time points, and at different locations (Stival *et al.*, 2015; Stival *et al.*, 2018; Ritagliati *et al.*, 2018).

Nora Calcaterra began her studies in Developmental Biology by identifying and characterizing mRNA binding proteins differentially expressed during oogenesis and early developmental stages of *R. arenarum* embryos (Calcaterra *et al.*, 1999; Armas *et al.*, 2001). Later, Calcaterra incorporated *Danio rerio* (zebrafish) as a study model to analyze the molecular mechanisms controlling gene expression during the specification and differentiation of craniofacial structures. Calcaterra's lab is pioneer in Argentina in the use of zebrafish as animal model for studying developmental biology. Her research group analyzes the role of CNBP (cellular nucleic acid binding protein) in the folding/unfolding of non-canonical DNA structures (guanine quadruplexes) and its possible role in controlling the balance between proliferation and cell death of cranial neural crest cells (Armas *et al.*, 2004; Weiner *et al.*, 2007; Armas *et al.*, 2008; David *et al.*, 2019). They also study the participation of miRNAs in the differentiation of melanocytes as an experimental approach to clarify the molecular basis of some genetic diseases (Weiner *et al.*, 2019). The laboratory is also focused in deciphering the molecular bases of the Treacher Collins Syndrome (Rosas *et al.*, 2019), a neurocristopathy that arise from defects in the development of the cranial neural crest.

At the IBR, a group of scientists led by Javier Palatnik studies the biogenesis and the role of microRNAs in the development of *Arabidopsis Thaliana*. They analyze the participation of miR396 and miR319 in the complex regulatory plot of proliferative processes in the generation and growth of the different organs throughout the plant life cycle (Palatnik *et al.*, 2003; Rodriguez *et al.*, 2016; Ercoli *et al.*, 2018). The laboratory seeks to develop tools for practical use that can be applied to species of agronomic importance.

In the city of Santa Fe, in the Institute of Agrobiotechnology of Litoral (IAL, CONICET-UNL), Andrés Dekanty leads the branch of the Molecular Biology laboratory devoted to the study of the cellular and molecular mechanisms that control cell growth and proliferation during the development of *in vivo* organs and tissues, using *Drosophila melanogaster* as a model system (Sánchez *et al.*, 2019). The geographic distribution of the main Developmental Biology laboratories in Argentina is shown in Fig.7.

## Promotion of Developmental Biology in Argentina

Since 2013, and on a biannual basis, a "Workshop on Cellular and Development Biology" is held with great enthusiasm at the city of Chascomús. This workshop aims to generate a discussion space for researchers from the developmental biology field. Throughout the different editions (2013, 2014, 2016 and 2018) there was a growing participation of consolidated researchers with outstanding experience, as well as undergraduate students, doctoral and post-doctoral fellows from various research centers throughout



Argentina and abroad. This broad participation generated an active scientific discussion and favored genuine collaboration among the participants. The active scientific discussion was achieved through the implementation of a dynamic format of the Workshop activities where many young scientists from the country and abroad present various themes and experimental models of animals and plants.

## Final considerations

As can be seen through the evolution of Developmental Biology in Argentina, studies and the interest in solving biological development issues changed over the years. In the beginning, investigations were centered on the analysis of the large biological questions such as the reproduction, early development, gastrulation, the organogenesis that would lead them to understand the origin and formation of a new organism. Using the tools available during those first years, the biological processes were analyzed from morphological, descriptive and chemical and functional points of view, trying to answer the great questions of biology. The studies initiated in Tucumán and Buenos Aires pioneered works and caused the migration of researchers who settled in different parts of the country, constituting the bases for the growth of developmental biology in Argentina, despite the great ups and downs of the socio-political situations of the country. Since the 1970s, the great technological advances with the great expansion of cellular and molecular biology and genetics allowed investigators to study the molecules involved in the different processes and their gene regulation. These very exciting facts are sometimes so difficult to contextualize within developmental biology that it would seem that it is difficult to determine the boundaries between the various areas of knowledge. At present, thanks to the enthusiasm of many young researchers, developmental biology has made considerable progresses, albeit still rather smaller compared to what happens in the most advanced countries. Finally, mention should be made to the active participation of the different research groups in the LASDB, the Latin American Society of Development Biology.

Last, but not least, the authors would like to apologize for facts and colleagues whose names and relevant work have not been cited here due to space limitations.

## References

- ADLER, R. (1968). Submicroscopical study of chick embryo neural tube experimentally reopened "in vitro". *Z Zellforsch Mikrosk Anat.* 1968;86: 422-429.
- ADLER, R., LANDA, K.B., MANTHORPE, M., VARON, S. (1979). Cholinergic neurotrophic factors: intraocular distribution of trophic activity for ciliary neurons. *Science* 204: 1434-1436.
- ADLER, R., TEITELMAN, G., SUBURO, A.M. (1977). Cell interactions and the regulation of cholinergic enzymes during neural differentiation in vitro. *Dev Biol* 50: 48-57.
- ADLER, R., TEITELMAN, G., SUBURO, A.M. (1976). Cell interactions and the regulation of cholinergic enzymes during neural differentiation in vitro. *Dev Biol* 50: 48-57.
- AGÜERO, T.H., FERNÁNDEZ, J.P., LÓPEZ, G.A., TRÍBULO, C., AYBAR, M.J. (2012). Indian hedgehog signaling is required for proper formation, maintenance and migration of *Xenopus* neural crest. *Dev Biol* 364: 99-113.
- ALATA JIMENEZ, N., TORRES PÉREZ, S.A., SÁNCHEZ-VÁSQUEZ, E., FERNAN-DINO, J.I., STROBL-MAZZULLA, P.H. (2018). Folate deficiency prevents neural crest fate by disturbing the epigenetic Sox2 repression on the dorsal neural tube. *Dev Biol* 444: S193-S201
- ALONSO, T.S., BONINI DE ROMANELLI, I.C., BAZAN, N.G. (1982). Membrane lipids composition and metabolism during early embryonic development. Phospholipid subcellular distribution 32P labeling. *Biochim Biophys Acta* 688: 145-151.
- ALONSO, T.S., BONINI DE ROMANELLI, I.C., BAZAN, N.G. (1986). Changes in triglycerides, diglycerides and free fatty acids after fertilization in developing toad embryos. *Biochim Biophys Acta* 875: 465-447.
- ARIAS, C., FUSSERO, G., ZACHARONOK, M., MACÍAS, A. (2015) Dpp-expressing and non-expressing cells: two different populations of growing cells in *Drosophila*. *PLoS One* 10: e0121457.
- ARIAS, C., ZAPATA, A., LUDUEÑA-ALMEIDA, F., ZACHARONOK, M., MACÍAS, A. (2019). Genital disc growth in *Drosophila*. *Int J Dev Biol* 63: 17-27.
- ARMAS, P., AGÜERO, T.H., BORGOGNONE, M., AYBAR, M.J., CALCATERRA, N.B. (2008). Dissecting CNBP, a zinc-finger protein required for neural crest development, in its structural and functional domains. *J Mol Biol* 382: 1043-1056.
- ARMAS, P., CABADA, M.O., CALCATERRA, N.B. (2001) Primary structure and developmental expression of *Bufo arenarum* cellular nucleic acid-binding protein: changes in subcellular localization during early embryogenesis. *Dev Growth Differ* 43: 13-23.
- ARMAS, P., CACHERO, S., LOMBARDO, V.A., WEINER, A., ALLENDE, M.L., CALCATERRA, N.B. (2004). Zebrafish cellular nucleic acid-binding protein: gene structure and developmental behaviour. *Gene* 337: 151-161.
- ARRANZ, S.E., ALBERTALI, I.E., CABADA, M.O. (1997). *Bufo arenarum* egg jelly coat: purification and characterization of two highly glycosylated proteins. *Biochem J* 323: 307-312.
- AVELDANO, M.I., BAZAN, N.G. (1975). Rapid production of diacylglycerols enriched in arachidonate and stearate during early brain ischemia. *J Neurochem* 25: 919-920.
- AYBAR, M.J., FUENTES, A., SÁNCHEZ, S.S. (2000b). Inhibition of the synthesis of glycosphingolipid by a ceramide analogue (PPMP) in the gastrulation of *Bufo arenarum*. *Zygote* 8: 159-169.
- AYBAR, M.J., GENTA, S.B., SÁNCHEZ RIERA, A.N., SÁNCHEZ, S.S. (2000a). Participation of the GM1 ganglioside in the gastrulation of anuran amphibian *Bufo arenarum*. *J Exp Zool* 286: 457-472.
- AYBAR, M.J., NIETO, M.A., MAYOR, R. (2003). Snail precedes slug in the genetic cascade required for the specification and migration of the *Xenopus* neural crest. *Development* 130: 483-494.
- BARBIERI, F.D. (1954a). [Case of heterotypic division in somatic tissue of *Telmatobius schreiteri* tadpole]. *Sci Genet* 4: 272-274.
- BARBIERI, F.D. (1950). Observaciones sobre los cromosomas y espermatozoides en algunos batracios del género *Leptodactylus*. *Acta Zool. Lilloana* 9: 455-462.
- BARBIERI, F.D. (1960). Incorporation de l'adenine-C14 dans l'embryon de poulet. *J Embryo. Exp. Morph.* 174-181.
- BARBIERI, F.D. (1961). L'action de l'irradiation ultraviolette sur le metabolisme des acides nucléiques dans l'embryon de Poulet. *J Embryo. Exp. Morph* 9: 9-13.
- BARBIERI, F.D., (1976). Diffusible factors in anuran fertilization. *Acta Physiol. Latinoam* 26:1-9.
- BARBIERI, F.D., BAZAN, N.G. (1964). Observaciones preliminares sobre la molecula de glucogeno de embriones de anfibios en desarrollo por medio de la reacción de yodo. Segundo Simposio sobre Metabolismo de los hidratos de carbono, Tucumán, Argentina.
- BARBIERI, F.D., CABADA, M. (1969). The role of the diffusible factor released by the egg jelly in fertilization of the toad egg. *Experientia* 25: 1312-1313.
- BARBIERI, F.D., DEL PINO, E.J., ARMAYOR, M.R. (1977). Properties and isolation of the diffusible factor involved in *Bufo arenarum* fertilization. *Acta Physiol Lat Am* 27: 293-302.
- BARBIERI, F.D., FERNANDEZ, M. (1962). Biosintesis del acido desoxirribonucleico en huevos en desarrollo de *Bufo arenarum*. *Arch. Bioq. Qca. Farm* 10: 67-76.
- BARBIERI, F.D., LEGNAME, A. (1957). Acción de agentes inhibidores y activadores de las oxidaciones sobre el desarrollo de *Bufo arenarum*. *Arch Bioq, Qca y Fcia* 8: 211-226.
- BARBIERI, F.D., SALOMON, H. (1963). Some experimental studies on glycogen and lactic acid in the developing eggs of *Bufo arenarum*. *Acta Embryol Morph. Exper* 6: 304-310.
- BARBIERI, F.D., SÁNCHEZ, S.S., DEL PINO, E.J. (1980). Changes in lectin-mediated agglutinability during primary embryonic induction in the amphibian embryo. *J Embryol Exp Morphol* 57: 95-106.
- BARBIERI, F.D., VALDEZ TOLEDO DE MORENO C.L. (1966). Fluoride and respiration in amphibian eggs. *Acta Embryol Morphol Exp* 9: 77-82.

- BARBIERI, F.D., VILLECCO, E.I. (1966). A fertilizin-like substance in the egg jelly of the toad *Bufo arenarum*. *Arch. Zool. Ital* 51: 227-237.
- BARISONE, G.A., KRAPP, D., CORREA-FIZ, F., ARRANZ, S.E., CABADA, M.O. (2007). Glycoproteins of the vitelline envelope of Amphibian oocyte: biological and molecular characterization of ZPC component (gp41) in *Bufo arenarum*. *Mol Reprod Dev* 74: 629-640.
- BAZAN, N.G. (1971). Modifications in the free fatty acids of developing rat brain. *Acta Physiol LatinoAmer* 21: 15-20.
- BELECKY-ADAMS, T.L., SCHEURER, D., ADLER, R. (1999) Activin family members in the developing chick retina expression patterns, protein distribution, and *in vitro* effects. *Dev Biol* 210: 107-123.
- BENITEZ, S., SOSA, C., TOMASINI, N., MACÍAS, A. (2010). Both JNK and apoptosis pathways regulate growth and terminalia rotation during *Drosophila* genital disc development. *Int J Dev Biol* 54: 643-653.
- BLANCO, V., LOPEZ, CAMELO, J., CARRI, N.G. (2001). Growth inhibition, morphological differentiation and stimulation of survival in neuronal cell type (Neuro-2a) treated with trophic molecules. *Cell Biol Int* 25: 909-917.
- BONANO, M., TRÍBULO, C., DE CALISTO, J., MARCHANT, L., SÁNCHEZ, S.S., MAYOR, R., AYBAR, M.J. (2008). A new role for the Endothelin-1/Endothelin-A receptor signaling during early neural crest specification. *Dev Biol* 323: 114-129.
- BONINI DE ROMANELLI, I.C., ALONSO, T.S., BAZAN, N.G. : Phosphatidic acid, phosphatidylinositol, phosphatidylserine and cardiolipin in the course of early embryonic development. Fatty acid composition and content in whole toad embryos and in mitochondrial fractions. *Biochim Biophys Acta* 664: 561-571.
- BOTTA, P.E., SIMÓ, I., SCIARA, A.A., ARRANZ, S.E. (2016). Growth hormone receptors in the atherinid *Odontesthes bonariensis*: characterization and expression profile after fasting-refeeding and growth hormone administration. *J Fish Biol* 88: 1870-1885.
- BOUCAUT, J.C., DARRIBERE, T., LI, S.D., BOULEKBACHE, H., YAMADA, K.M., THIERY, J.P. (1985). Evidence for the role of fibronectin in amphibian gastrulation. *J Embryol Exp Morphol.* 89 Suppl: 211-227.
- BRACHET, J. (1934) Etude du métabolisme de l'oeuf de grenouille (*Rana fusca*) au cours du développement. 1. La respiration et la glycolyse, de la segmentation à l'éclosion. *Arch. de Biol.*, 45: 611-727.
- BRAUCKMANN, E.S., BARBIERI, F.D. (1957) Acción del glutatión reducido sobre la morfogénesis del tubo neural en el embrión de pollo. *Arch Bioq, Qca y Fcia* 8:227-230.
- BRAUCKMANN, E.S., BAZAN, N.G. (1963). Analisis histoquímico de las proteínas sulfhidrílicas en el huevo de *Bufo Arenarum* en desarrollo. *Arch Bioq, Qca y Fcia* 11:43-54
- BRIGGS, R., KING, T.J. (1952). Transplantation of Living Nuclei From Blastula Cells into Enucleated Frogs' Eggs. *Proc Natl Acad Sci USA.* 38: 455-463.
- BUSCHIAZZO, J., BONINI, I.C., ALONSO, T.S. (2008). Inhibition of *Bufo arenarum* oocyte maturation induced by cholesterol depletion by methyl-beta-cyclodextrin. Role of low-density caveolae-like membranes. *Biochim Biophys Acta.* 2008;1778: 1398-406.
- CABADA, M.O. (1975). Sperm concentration and fertilization rate in *Bufo arenarum*(Amphibia: Anura). *J Exp Biol* 62: 481-486.
- CABADA, M.O., DARNBROUGH C., FORD, P.J., TURNER, P.C. (1977). Differential accumulation of two size classes of poly(A) associated with messenger RNA during oogenesis in *Xenopus laevis*. *Dev Biol* 57: 427-439.
- CÁNEPA, M.M., PANDOLFI, M., MAGGESE, M.C., VISSIO, P.G. (2006). Involvement of somatolactin in background adaptation of the cichlid fish *Cichlasoma dimerus*. *J Exp Zool A Comp Exp Biol* 305: 410-419.
- CANTO-SOLER, M.V., HUANG, H., ROMERO, M.S., ADLER, R. (2008). Transcription factors CTCF and Pax6 are segregated to different cell types during retinal cell differentiation. *Dev Dyn* 237: 758-767.
- CARPIO, R., HONORÉ, S.M., ARAYA, C., MAYOR, R. (2004). *Xenopus* paraxis homologue shows novel domains of expression. *Dev Dyn* 231: 609-613.
- CARRASCO, A.E., MALACINSKI, G.M. (1987). Localization of *Xenopus* homoeobox gene transcripts during embryogenesis and in the adult nervous system. *Dev Biol* 121: 69-81.
- CASTAÑEDA CORTÉS, D.C., PADILLA, L.F.A., LANGLOIS, V.S., SOMOZA, G.M., FERNANDINO, J.I. (2019). The central nervous system acts as a transducer of stress-induced masculinization through corticotropin-releasing hormone B. *Development.* 146 pii: dev172866.
- CASTAÑEDA-CORTÉS, D.C. and FERNANDINO, J.I. (2020). Stress and sex determination in fish: from brain to gonads. *Int. J. Dev. Biol.* 65: 207-214.
- CEI, J. (1953). *Biología general*. Ed. Violetto. Tucumán.
- CENTANIN, L., RATCLIFFE, P.J., WAPPNER, P. (2005). Reversion of lethality and growth defects in Fatiga oxygen-sensor mutant flies by loss of hypoxia-inducible factor-alpha/Sima. *EMBO Rep* 6: 1070-1075.
- COHEN, A.I (1955) Anaerobiosis in the *Rana pipiens* Embryo. *J. Embryol. exp. Morph.* 3 : 77-85.
- CRUPKIN, M., BARASSI, C.A., BAZAN, N.G. (1973). Incorporation of <sup>32</sup>P during early amphibian embryo-genesis. *Comp Biochem Physiol* 45:523-528.
- CUKIER, M., GUERRERO, G.A., MAGGESE, M.C. (1979). Parthenogenesis in *Coptopteryx viridis*, *Giglio tos* (1915) (*Dyctioptera, mantidae*). *Biol Bull* 157: 445-452.
- DAVID, A.P., PIPPIER, A., PASCUTTI, F., BINOLFI, A., WEINER, A.M.J., CHALLIER, E., HECKEL, S., CALSOU, P., GOMEZ, D., CALCATERRA, N.B., ARMAS, P. (2019). CNBP controls transcription by unfolding DNA G-quadruplex structures. *Nucleic Acids Res* 47: 7901-7913.
- DE FELICI, M., SIRACUSA, G. (2000). The rise of embryology in Italy: from the Renaissance to the early 20th century. *Int. J. Dev. Biol.* 44: 515-521.
- DE GENARO, P., SIMÓN, M.V., ROTSTEIN, N.P., POLITI, L.E. (2013). Retinoic acid promotes apoptosis and differentiation in photoreceptors by activating the P38 MAP kinase pathway. *Invest Ophthalmol Vis Sci* 54: 3143-3156.
- DE ROBERTIS E.M. (2009). Spemann's organizer and the self-regulation of embryonic fields. *Mech Dev.* 126(11-12): 925–941.
- DEL PINO, E.J., CABADA, M.O. (1987). Lectin binding sites in the vitelline envelope of *Bufo arenarum* oocytes: role in fertilization. *Gamete Res* 17: 333-342.
- DELARUE, M., SANCHEZ, S.S., JOHNSON, K.E., DARRIBÈRE T., BOUCAUT J.C. (1992). A fate map of superficial and deep circumblastoporal cells in the early gastrula of *Pleurodeles waltl*. *Development* 114:135-146.
- DI BELLA, D.J., CARCAGNO, A.L., BARTOLOMEU, M.L., PARDI, M.B., LÖHR, H., SIEGEL, N., HAMMERSCHMIDT, M., MARÍN-BURGIN, A., LANUZA, G.M. (2019). Ascl1 Balances Neuronal versus Ependymal Fate in the Spinal Cord Central Canal. *Cell Rep* 28: 2264-2274.e3.
- DI PRINZIO, C.M., BOTTA, P.E., BARRIGA, E.H., RÍOS, E.A., REYES, A.E., ARRANZ, S.E. (2010). Growth hormone receptors in zebrafish (*Danio rerio*): adult and embryonic expression patterns. *Gene Expr Patterns* 10: 214-225.
- DIAZ FONTDEVILA, M.F., BLOJ, B., CABADA, M.O. (1988). Effect of cholesterol and phosphatidylcholine of different chain length on acrosome breakdown and fertilizing capacity of amphibian spermatozoa. *Gamete Res* 21: 59-70.
- DISTÉFANO, A.M., MARTIN, M.V., CÓRDOBA, J.P., BELLIDO, A.M., D'IPPOLITO, S., COLMAN, S.L., SOTO, D., ROLDÁN, J.A., BARTOLI, C.G., ZABALETA, E.J., FIOL, D.F., STOCKWELL, B.R., DIXON, S.J., PAGNUSSAT, G.C. (2017). Heat stress induces ferroptosis-like cell death in plants. *J Cell Biol* 216: 463-476.
- ERCOLI, M.F., FERELA, A., DEBERNARDI, J.M., PERRONE, A.P., RODRIGUEZ, R.E., PALATNIK, J.F. (2018). GIF Transcriptional Coregulators Control Root Meristem Homeostasis. *Plant Cell* 30: 347-359.
- ESPIRITU, E.B., CRUNK, A.E., BAIS, A., HOCHBAUM, D., CERVINO, A.S., PHUA, Y.L., BUTTERWORTH, M.B., GOTO, T., HO, J., HUKRIEDE, N.A., CIRIO, M.C. (2018). The Lhx1-Ldb1 complex interacts with Furry to regulate microRNA expression during pronephric kidney development. *Sci Rep* 8: 16029.
- FABRO, G., ROVASIO, R.A., CIVALERO, S., FRENKEL, A., CAPLAN, S.R., EISENBACH, M., GIOJALAS, L.C. (2002). Chemotaxis of capacitated rabbit spermatozoa to follicular fluid revealed by a novel directionality-based assay. *Biol Reprod* 67: 1565-1571.
- FANTINI, B. (2000a). The "Stazione Zoologica Anton Dohrn" and the History of Embryology. *Int. J. Dev. Biol* 44: 523-535.
- FANTINI B. (2000b). Molecularizing embryology: Alberto Monroy and the origins of Developmental Biology in Italy. *Int. J. Dev. Biol.* 44: 537-553.
- FAVAROLO, M.B., LÓPEZ, S.L. (2018). Notch signaling in the division of germ layers in bilaterian embryos. *Mech Dev* 154:122-144.
- FERNANDINO, J.I., HATTORI, R.S. (2019). Sex determination in Neotropical fish: Implications ranging from aquaculture technology to ecological assessment. *Gen Comp Endocrinol* 273: 172-183.
- FRANCO, D.L., FRENKEL, L., CERIANI, M.F. (2018). The Underlying Genetics of *Drosophila* Circadian Behaviors. *Physiology* (Bethesda) 33: 50-62.
- FRONTERA, J.L., CERVINO, A.S., JUNGBLUT, L.D., PAZ, D.A. (2015). Brain-derived



- neurotrophic factor (BDNF) expression in normal and regenerating olfactory epithelium of *Xenopus laevis*. *Ann Anat* 198:41-48.
- GALAGOVSKY, D., KATZ, M.J., ACEVEDO, J.M., SORIANELLO, E., GLAVIC, A., WAPPNER, P. (2014). The *Drosophila* insulin-degrading enzyme restricts growth by modulating the PI3K pathway in a cell-autonomous manner. *Mol Biol Cell* 25: 916-924.
- GÁNDARA, L., WAPPNER, P. (2018). Metabo-Devo: A metabolic perspective of development. *Mech Dev* 154:12-23.
- GARCÍA, E.V., MICELI, D.C., RIZO, G., VALDECANTOS, P.A., BARRERA, A.D. (2015). Effect of early addition of bone morphogenetic protein 5 (BMP5) to embryo culture medium on *in vitro* development and expression of developmentally important genes in bovine preimplantation embryos. *Theriogenol.* 84: 589-599.
- GARELLI, A., ROTSTEIN, N.P., POLITI, L.E. (2006). Docosahexaenoic acid promotes photoreceptor differentiation without altering Crx expression. *Invest. Ophthalmol. & Vis. Sc* 47: 3017-3027.
- GARELLI, A., HEREDIA, F., CASIMIRO, A.P., MACEDO, A., NUNES, C., GARCEZ, M., DIAS, A.R.M., VOLONTE, Y.A., UHLMANN, T., CAPARROS, E., KOYAMA, T., GONTIJO, A.M. (2015). *Dilp8* requires the neuronal relaxin receptor *Lgr3* to couple growth to developmental timing. *Nat Commun* 6:8732
- GARELLI, A., GONTIJO, A.M., MIGUELA, V., CAPARROS, E., DOMINGUEZ, M. (2012). Imaginal discs secrete insulin-like peptide 8 to mediate plasticity of growth and maturation. *Science* 336: 579-582.
- GENOVESE, G., DA CUÑA, R., TOWLE, D.W., MAGGESE, M.C., LO NOSTRO, F. (2011). Early expression of zona pellucida proteins under octylphenol exposure in *Cichlasoma dimerus* (Perciformes, Cichlidae). *Aquat Toxicol* 101: 175-185.
- GENTA, S.B., AYBAR, M.J., PERALTA, M.A., SÁNCHEZ, S.S. (1997). Evidence for the presence and participation of 85-75 KDa extracellular matrix components in cell interactions of *Bufo arenarum* gastrulation. *J Exp Zool* 277: 181-197.
- GIUSTO, N.M., BAZAN, N.G. (1973). High increment of triglycerols with ether linkages in the retina during anoxia. *Biochem Biophys Res Comm* 55:515-521
- GOULD, S.J. (1997). Ontogeny and phylogeny. Cambridge, Mass: Belknap Press of Harvard University Press.
- GRAMAJO-BÜHLER, M.C., ZELARAYÁN, L., SÁNCHEZ-TORANZO, G. (2016) Involvement of Protein cAMP-dependent Kinase, Phospholipase A2 and Phospholipase C in Sperm Acrosome Reaction of *Chinchilla lanigera*. *Reprod Domest Anim.* Feb;51: 150-7.
- GROEBEN, C., DE SIO, F.(2006). Nobel laureates at the Stazione Zoologica Anton Dohrn: phenomenology and paths to discovery in neuroscience. *J Hist Neurosci* 15: 376-95.
- HERNANDO, C.E., GARCÍA HOURQUET, M., DE LEONE, M.J., CARENO, D., ISERTE, J., MORA GARCIA, S., YANOVSKY, M.J. (2019). A Role for Pre-mRNA-PROCESSING PROTEIN 40C in the Control of Growth, Development, and Stress Tolerance in *Arabidopsis Thaliana*. *Front Plant Sci* 10:1019.
- HONORÉ, S.M., AYBAR, M.J., MAYOR, R. (2003). Sox10 is required for the early development of the prospective neural crest in *Xenopus* embryos. *Dev Biol* 260: 79-96.
- HOPWOOD, N. (2015). Haeckel's Embryos: Images, Evolution, and Fraud. Chicago: University of Chicago Press.
- JASKOŁOWSKI, A., IÑIGO, S., ARELLANO, S.M., ARIAS, L.A., FIOŁ, D.F., SEDE, A.R., OLDRA, M.B., LORENZI, H., MUSCHIETTI, J.P., PAGNUSSAT, G.C., CERDÁN, P.D. (2019). The MED30 subunit of mediator complex is essential for early plant development and promotes flowering in *Arabidopsis Thaliana*. *Development.* 146 (10). pii: dev175224.
- JAURENA, M.B., CARRI, N.G., BATTIATO, N.L., ROVASIO, R.A. (2011). Trophic and proliferative perturbations of *in vivo/in vitro* cephalic neural crest cells after ethanol exposure are prevented by neurotrophin 3. *Neurotoxicol Teratol* 33: 422-430
- JUNGBLUT, L.D., POZZI, A.G., PAZ, D.A. (2010). Larval development and metamorphosis of the olfactory and vomeronasal organs in the toad *Rhinella* (*Bufo*) *arenarum* (Hensel, 1867). *Acta Zoologica* 92: 305-315.
- KOLLIKER, A. (1882). Embryologie ou Traite complet du developpement de l'homme et des amniaux superieurs. Reinwald. Paris.
- KRAPF, D., VIDAL, M., ARRANZ, S.E., CABADA, M.O. (2006). Characterization and biological properties of L-HGP, a glycoprotein from the amphibian oviduct with acrosome-stabilizing effects. *Biol Cell* 98: 403-413.
- LAVORE, A., PASCUAL, A., SALINAS, F.M., ESPONDA-BEHRENS, N., MARTINEZ-BARNETCHE, J., RODRIGUEZ, M., RIVERA-POMAR, R. (2015). Comparative analysis of zygotic developmental genes in *Rhodnius prolixus* genome shows conserved features on the tracheal developmental pathway. *Insect Biochem Mol Biol* 64:32-43.
- LEGNAME, C.R., BARBIERI, F.D. (1968) Effect of medium on transplantability of nuclei of *Bufo arenarum* embryonic cells. *Experientia* 24: 842.
- LEGNAME, R., BARBIERI, F.D. (1967) Nuclear transplantation in *Bufo arenarum* eggs. *Arch Biol (Liege)* 78: 623-31.
- LINO, N., FIORE, L., RAPACIOLI, M., TERUEL, L., FLORES, V., SCICOLONE, G., SÁNCHEZ, V. (2014). uPA-uPAR molecular complex is involved in cell signaling during neuronal migration and neurogenesis. *Dev Dyn* 243: 676-689.
- LO NOSTRO, F., GRIER, H., ANDREONE, L., GUERRERO, G.A. (2003). Involvement of the gonadal germinal epithelium during sex reversal and seasonal testicular cycling in the protogynous swamp eel, *Synbranchus marmoratus* Bloch 1795 (Teleostei, Synbranchidae). *J Morphol* 257: 107-126.
- LÓPEZ, S.L., CARRASCO, A.E. (1992). Retinoic acid induces changes in the localization of homeobox proteins in the antero-posterior axis of *Xenopus laevis* embryos. *Mech Dev* 36: 153-164.
- LUQUE, M.E., CRESPO, P.M., MÓNACO, M.E., AYBAR, M.J., DANIOTTI, J.L., SÁNCHEZ, S.S. (2008). Cloning and functional characterization of two key enzymes of glycosphingolipid biosynthesis in the amphibian *Xenopus laevis*. *Dev Dyn* 237: 112-123.
- MACÍAS, A., MORATA, G. (1996) Functional hierarchy and phenotypic suppression among *Drosophila* homeotic genes: the labial and empty spiracles genes. *EMBO J* 15: 334-43.
- MAGGESE, M.C., SPAIZMANN, R.C., DE RECONDO, M.E. (1976). Concentration and seasonal variations of purine bases, nucleotides and nucleotide-sugars in liver of *Bufo arenarum*. Comparison with early embryogenesis. *Acta Physiol Lat Am* 26: 472-480.
- MANES M.E., ELINSON R.P., BARBIERI F.D. (1978) Formation of the amphibian grey crescent: Effects of colchicine and cytochalasin B. *Wilehm Roux Arch Dev Biol* 185: 99-104.
- MANES, M.E., BARBIERI, F.D. (1976). Symmetrization in the amphibian egg by disrupted sperm cells. *Dev Biol* 53: 138-141.
- MANES, M.E., BARBIERI, F.D.(1977) On the possibility of sperm aster involvement in dorso-ventral polarization and pronuclear migration in the amphibian egg. *J Embryol Exp Morphol* 40:187-197.
- MAYOR, R., YOUNG, R., VARGAS, A. (1999).Development of neural crest in *Xenopus*. *Curr Top Dev Biol* 43:85-113.
- MICELI, D.C., DEL PINO, E.J., BARBIERI, F.D., MARIANO, M.I., RAISMAN, J.S. (1977). The vitelline envelope-to-fertilization envelope transformation in the toad *Bufo arenarum*. *Dev Biol* 59: 101-110.
- MONROY, A. (1947). Further observations on the fine structure cortical layer of unfertilized and fertilized sea urchin eggs. *J. Cell. Comp. Physiol* 30: 105-109.
- MONROY, A. (1950). A preliminary electrophoretic analysis of proteins and protein fractions in sea urchin eggs and their changes on fertilization. *Exp. Cell Res* 1: 92-104.
- MORA-GARCÍA, S., DE LEONE, M.J., YANOVSKY, M. (2017). Time to grow: circadian regulation of growth and metabolism in photosynthetic organisms. *Curr Opin Plant Biol* 35:84-90.
- MORALES, E.S., KRAPF, D., BOTTA, P.E., CABADA, M.O., ARRANZ, S.E. (2012)  $\beta$ -hexosaminidase from *Xenopus laevis* eggs and oocytes: from gene to immunochemical characterization. *J Cell Biochem* 113: 3709-3720.
- MÜLLER, M.M., CARRASCO, A.E., DE ROBERTIS, E.M. (1984). A homeo-box-containing gene expressed during oogenesis in *Xenopus*. *Cell* 39: 157-162.
- NARBAITZ, R., ADLER, R. (1966). Germ cell differentiation in embryonic gonads cultured in heterosexual parabiotic pairs. *Experientia* 22: 677-678.
- NARBAITZ, R., GARTKE, K. (1975). Fine structure of chick embryonic parathyroid glands cultured on media with different concentrations of calcium. *Rev Can Biol* 34: 91-100.
- NARBAITZ, R., KOŁODNY, L. (1964). Delta-5-3-beta-hydroxysteroid dehydrogenase in differentiating chick gonads. *Z Zellforsch Mikrosk Anat* 63:612-617.
- NARBAITZ, R., SABATINI, M.T. (1963). Histochemical demonstration of cholesterol in embryonic chick gonads differentiated "in vitro". *Z Zellforsch Mikrosk Anat* 61:52-53.
- NARBAITZ, R., TEITELMAN, G. (1965). A Histochemical study of sex inversion produced by estradiol in chick embryos. *J Embryol Exp Morphol* 13:45-50
- OPPENHEIMER, J.M. (1970). Hans Driesch and the theory and practice of embryonic transplantation. *Bull Hist Med* 44: 378-382.

- ORTALLI, A.L., FIORE, L., DI NAPOLI, J., RAPACIOLI, M., SALIERNO, M., ETCHENIQUE, R., FLORES, V., SANCHEZ, V., CARRI, N.G., SCICOLONE, G. (2012). EphA3 expressed in the chicken tectum stimulates nasal retinal ganglion cell axon growth and is required for retinotectal topographic map formation. *PLoS One* 7: e38566.
- PAGANELLI, A.R., OCAÑA, O.H., PRAT, M.I., FRANCO, P.G., LÓPEZ, S.L., MORELLI, L., ADAMO, A.M., RICCOMAGNO, M.M., MATSUBARA, E., SHOJI, M., AFFRANCHINO, J.L., CASTAÑO, E.M., CARRASCO, A.E. (2001). The Alzheimer-related gene presenilin-1 facilitates sonic hedgehog expression in *Xenopus* primary neurogenesis. *Mech Dev.* 107(1-2):119-131.
- PALATNIK, J.F., ALLEN, E., WU, X., SCHOMMER, C., SCHWAB, R., CARRINGTON, J.C., WEIGEL, D. (2003). Control of leaf morphogenesis by microRNAs. *Nature* 425: 257-263.
- PANDOLFI, M., PARHAR, I.S., RAVAGLIA, M.A., MEIJIDE, F.J., MAGGESE, M.C., PAZ, D.A. (2002). Ontogeny and distribution of gonadotropin-releasing hormone (GnRH) neuronal systems in the brain of the cichlid fish *Cichlasoma dimerus*. *Anat Embryol (Berl)* 205: 271-281.
- PECHEN, A.M., BAZAN, N.G. (1974). Membrane 32P-phospholipid labeling in early developing toad embryos. *Exp Cell Res* 88: 432-435.
- PERALTA, M.A., AYBAR, M.J., SÁNCHEZ, S.S. (1995). Alteration of morphogenetic cell interactions by 1-beta-D-arabinofuranosylcytosine in *Bufo arenarum* embryo. *Biocell* 19: 159-173.
- PÉREZ, M., WAPPNER, P., QUESADA-ALLUÉ, L.A. (2002). Catecholamine-beta-alanyl ligase in the medfly *Ceratitis capitata*. *Insect Biochem Mol Biol* 32: 617-625.
- PERRY, R., SABATINI, D., WIESEL, T. (2000). La biología molecular en la Argentina - *Ciencia Hoy* 10:57.
- PETRACCA, Y.L., SARTORETTI, M.M., DI BELLA, D.J., MARIN-BURGIN, A., CARGAGNO, A.L., SCHINDER, A.F., LANUZA, G.M. (2016). The late and dual origin of cerebrospinal fluid-contacting neurons in the mouse spinal cord. *Development* 143: 880-891.
- PISANO, A. (1939). Primi risultati sulla potenza dei blastomeri dell' uovo di Axolotl in condizioni di espianto. *La Ricerca Scientifica* 12: 1-5.
- PISANO, A. (1942). Espiantos de territorios presuntivos caudales de neurulas de Rana esculenta. *Ricerche di Morfologia* 1: 1-63.
- PISANO, A. (1975). Fragmentos de mis impresiones en la Antártida. *Informaciones del CONICET* 105: 1-10.
- PISANO, A. and BARBIERI, F.D. (1978). Anatomía comparada de los vertebrados. 1ª Edición, EUDEBA.
- PISCIOTTANO, F., CINALLI, A.R., STOPIELLO, J.M., CASTAGNA, V.C., ELGOYHEN, A.B., RUBINSTEIN, M., GÓMEZ-CASATI, M.E., FRANCHINI, L.F. (2019). Inner Ear Genes Underwent Positive Selection and Adaptation in the Mammalian Lineage. *Mol Biol Evol* 36: 1653-1670.
- POLITI, L.E., ADLER, R. (1986). Generation of enriched populations of cultured photoreceptor cells. *Invest Ophthalmol Vis Sci* 27: 656-665.
- POLITI, L.E., LEE, L., WIGGERT, B., CHADER, G., ADLER, R. (1989). Synthesis and secretion of interphotoreceptor retinoid-binding protein (IRBP) by isolated normal and rd mouse retinal photoreceptor neurons in culture. *J Cell Physiol* 141: 682-690.
- PROIETTO, S., YANKELEVICH, L., VILLARREAL, F.M., INSERRA, P.I.F., CHARIF, S.E., SCHMIDT, A.R., CORTASA, S.A., CORSO, M.C., DI GIORGIO, N.P., LUX-LANTOS, V., VITULLO, A.D., HALPERIN, J., DORFMAN, V.B. (2019). Pituitary estrogen receptor alpha is involved in luteinizing hormone pulsatility at mid-gestation in the South American plains vizcacha, *Lagostomus maximus* (Rodentia, Caviomorpha). *Gen Comp Endocrinol* 273: 40-51.
- PUJOL-LEREIS, L.M., FAGALI, N.S., RABOSI, A., CATALÁ, Á., QUESADA-ALLUÉ, L.A. (2016). Chill-coma recovery time, age and sex determine lipid profiles in *Ceratitis capitata* tissues. *J Insect Physiol* 87: 53-62.
- QUESADA-ALLUE LA. (1981). Early dolichyl sugar synthesis during differentiation of non-growing brine-shrimp (*Artemia salina*) embryos. *Biochem J* 198: 429-432.
- QUESADA-ALLUE LA. (1982). The inhibition of insect chitin synthesis by tunicamycin. *Biochem Biophys Res Commun* 105: 312-319.
- RAHIL, K.S. NARBAITZ, R. (1972). *Evolution of the lining bodies in the embryonic chick gonad* *Development* 28: 133-140
- RAPACIOLI, M., BOTELHO, J., CERDA, G., DUARTE, S., ELLIOT, M., PALMA, V., FLORES, V. (2012). Sonic hedgehog (Shh)/Gli modulates the spatial organization of neuroepithelial cell proliferation in the developing chick optic tectum. *BMC Neurosci* 13: 117.
- RAPACIOLI, M., PALMA, V., FLORES, V. (2016). Corrigendum: Morphogenetic and Histogenetic Roles of the Temporal-Spatial Organization of Cell Proliferation in the Vertebrate Corticogenesis as Revealed by Inter-specific Analyses of the Optic Tectum Cortex Development. *Front Cell Neurosci* 10:112.
- RAVAGLIA, M.A., MAGGESE, M.C. (2003). Ovarian follicle ultrastructure in the teleost *Synbranchus marmoratus* (Bloch, 1795), with special reference to the vitelline envelope development. *Tissue Cell* 35: 9-17.
- REVINSKI, D.R., PAGANELLI, A.R., CARRASCO, A.E., LÓPEZ, S.L. (2010). Delta-Notch signaling is involved in the segregation of the three germ layers in *Xenopus laevis*. *Dev Biol* 339: 477-492.
- RITAGLIATI, C., LUQUE, G.M., STIVAL, C., BARO GRAF, C., BUFFONE, M.G., KRAPP, D. (2018). Lysine acetylation modulates mouse sperm capacitation. *Sci Rep* 8: 13334.
- RIVERA-POMAR, R., LU, X., PERRIMON, N., TAUBERT, H., JÄCKLE, H. (1995). Activation of posterior gap gene expression in the *Drosophila* blastoderm. *Nature* 376: 253-256.
- RODRIGUEZ, R.E., SCHOMMER, C., PALATNIK, J.F. (2016). Control of cell proliferation by microRNAs in plants. *Curr Opin Plant Biol* 34:68-76.
- ROLDÁN-OLARTE, M., GARCÍA, D.C., JIMÉNEZ-DÍAZ, M., VALDECANTOS, P.A., MICELI, D.C. (2012). *In vivo* and *in vitro* expression of the plasminogen activators and urokinase type plasminogen activator receptor (u-PAR) in the pig oviduct. *Anim Reprod Sci* 136: 90-99.
- ROSAS, M.G., LORENZATTI, A., PORCEL DE PERALTA, M.S., CALCATERRA, N.B., COUX, G. (2019). Proteasomal inhibition attenuates craniofacial malformations in a zebrafish model of Treacher Collins Syndrome. *Biochem Pharmacol* 163:362-370.
- ROVASIO, R.A., DELOUVEE, A., YAMADA, K.M., TIMPL, R., THIERY, J.P. (1983). Neural crest cell migration: requirements for exogenous fibronectin and high cell density. *J Cell Biol* 96: 462-473.
- SALOMON DE LEGNAME, H. (1969). Biochemical studies on the energetics of *Bufo arenarum* segmenting eggs. *Arch Biol (Liege)* 80: 471-490.
- SALOMON DE LEGNAME, H., SANCHEZ RIERA, A.N., SANCHEZ, S.S. (1971). Pathways of glucose breakdown during *Bufo arenarum* ontogenesis. *Acta Embryol Exp (Palermo)* 3: 187-194.
- SALOMÓN DE LEGNAME, H., SÁNCHEZ RIERA, A.N., SÁNCHEZ, S.S. (1975). Source of precursors for nucleotide biosynthesis in *Bufo arenarum* segmenting eggs. *Acta Embryol Exp (Palermo)* 2: 123-136.
- SALOMÓN DE LEGNAME, H., VALDEZ TOLEDO, C.L., LEGNAME, A.H. (1979). Role of thyroid hormone on metabolic regulation during *Bufo arenarum* embryogenesis. *Acta Embryol Exp (Palermo)* 1: 17-27.
- SÁNCHEZ RIERA, A.N., SÁNCHEZ, S.S., CABADA, M.O. (1988a). RNA metabolism in the follicle cells of *Bufo arenarum* oocytes. I. Biochemical studies. *Microsc Electron Biol Celular* 12: 149-61.
- SÁNCHEZ RIERA, A.N., SÁNCHEZ, S.S., CABADA, M.O. (1988b). RNA metabolism in the follicle cells of *Bufo arenarum* oocytes. II. Autoradiographic studies. *Microsc Electron Biol Celular* 12: 163-175.
- SÁNCHEZ TORANZO, G., BONILLA, F., GRAMAJO BÜHLER, M.C., BÜHLER, M.I. (2010). Participation of MAPK, PKA and PP2A in the regulation of MPF activity in *Bufo arenarum* oocytes. *Zygote* 19:181-189.
- SANCHEZ, J.A., MESQUITA, D., INGARAMO, M.C., ARIEL, F., MILÁN, M., DEKANTY, A. (2019). Eiger/TNF $\alpha$ -mediated Dlp8 and ROS production coordinate intra-organ growth in *Drosophila*. *PLoS Genet* 15: e1008133.
- SÁNCHEZ, R.S., SÁNCHEZ, S.S. (2013). Characterization of pax1, pax9, and uncx sclerotomal genes during *Xenopus laevis* embryogenesis. *Dev Dyn*: 572-579.
- SÁNCHEZ, R.S., SÁNCHEZ, S.S. (2015). Paraxis is required for somite morphogenesis and differentiation in *Xenopus laevis*. *Dev Dyn* 244: 973-987.
- SÁNCHEZ, S.S., BARBIERI, F.D. (1983). Developmental and biochemical alterations caused by tunicamycin in *Bufo arenarum* embryos. *Wilehm Roux Arch Dev Biol* 192: 37-41.
- SÁNCHEZ, S.S., BARBIERI, F.D. (1988). Extracellular materials and determination of neuroectoblast in amphibian gastrula. *Exp Cell Biol* 56: 60-66.
- SÁNCHEZ, S.S., CABADA, M.O., BARBIERI, F.D. (1983). Newly synthesized extracellular ribonucleic acids in the amphibian gastrula. *Cell Differ* 13: 149-157.
- SÁNCHEZ, S.S., SALINAS, P., BARBIERI, F.D. (1984). Distribution of fibronectin in the gastrulating *Bufo arenarum* embryo. *Micr Electr Biol Cel* 8:81-89.

- SÁNCHEZ-VÁSQUEZ, E., BRONNER, M.E., STROBL-MAZZULLA, P.H. (2019). Epigenetic inactivation of miR-203 as a key step in neural crest epithelial-to-mesenchymal transition. *Development* 146: dev171017.
- SCARPECI, S.L., SANCHEZ, M.L., CABADA, M.O. (2008). Cellular origin of the *Bufo arenarum* sperm receptor gp75, a ZP2 family member: its proteolysis after fertilization. *Biol Cell* 100: 219-230.
- SCICOLONE, G., PEREYRA-ALFONSO S., BRUSCO, A., PECCI SAAVEDRA, J., FLORES, V. (1995). Development of the laminated pattern of the chick tectum opticum. *Int J Dev Neurosci* 13: 845-858.
- SEDE, A.R., BORASSI, C., WENGIER, D.L., MECCHIA, M.A., ESTEVEZ, J.M., MUSCHIETTI, J.P. (2018). *Arabidopsis* pollen extensins LRX are required for cell wall integrity during pollen tube growth. *FEBS Lett* 592: 233-243.
- SPEMANN, H., MANGOLD, H. (1924) Über Induktion von Embryonalanlagen durch Implantation Artfremder Organisatoren. *Roux' Arch. Entw. Mech* 100:599-638.
- STIVAL, C., RITAGLIATI, C., XU X., GERVASI, M.G., LUQUE, G.M., BARÓ GRAF, C., DE LA VEGA-BELTRÁN, J.L., TORRES, N., DARSZON, A., KRAPP, D., BUFONE, M.G., VISCONTI, P.E., KRAPP, D. (2018). Disruption of protein kinase A localization induces acrosomal exocytosis in capacitated mouse sperm. *J Biol Chem* 293: 9435-9447.
- SUBURO, A., CARRI, N., ADLER, R. (1979). The environment of axonal migration in the developing chick retina: a scanning electron microscopic (SEM) study. *J Comp Neurol* 184: 519-535.
- TAGASHIRA, R. (2009). La investigación científica y tecnológica en la Universidad Nacional de Tucumán entre 1914 y 1951; su relación con el contexto social. Tesis de Maestría.
- TERRACINI, L. (1989). Una inmigración muy particular: 1938~ los universitarios italianos en la Argentina. *Anuario IEHS* IV: 335-369.
- TOLOSA, E.J., FERNÁNDEZ-ZAPICO, M.E., BATTIATO, N.L., ROVASIO, R.A. (2016). Sonic hedgehog is a chemotactic neural crest cell guide that is perturbed by ethanol exposure. *Eur J Cell Biol* 95: 136-152.
- TORRES, P., BARBIERI, F.D. (1987). Structural analysis of materials present between the notochord and the overlying neuroectoblast in the chick embryo. *Acta Embryol Morph. Exper* 8: 51-59.
- TRÍBULO, C., AYBAR, M.J., SÁNCHEZ, S.S., MAYOR, R. (2004). A balance between the anti-apoptotic activity of Slug and the apoptotic activity of msx1 is required for the proper development of the neural crest. *Dev Biol* 275: 325-342.
- TRÍBULO, C., BARRIONUEVO, G.M., AGÜERO, T.H., SÁNCHEZ, S.S., CALCATERRA, N.B., AYBAR, M.J. (2012).  $\Delta$ Np63 is regulated by BMP4 signaling and is required for early epidermal development in *Xenopus*. *Dev Dyn* 241: 257-269.
- URIU, K., BHAVNA, R., OATES, A.C., MORELLI, L.G. (2017). A framework for quantification and physical modeling of cell mixing applied to oscillator synchronization in vertebrate somitogenesis. *Biol Open*. 6: 1235-1244.
- VALDECANTOS, P.A., ARGANÁRAZ, M.E., ABATE, C.M., MICELI, D.C. (2004). RNA fingerprinting using RAP-PCR identifies an EBAF homologue mRNA differentially expressed in rat oviduct. *Biocell* 28: 287-297.
- VEGA-LÓPEZ, G.A., BONANO, M., TRÍBULO, C., FERNÁNDEZ, J.P., AGÜERO, T.H., AYBAR, M.J. (2015). Functional analysis of Hairy genes in *Xenopus* neural crest initial specification and cell migration. *Dev Dyn* 244: 988-1013.
- VILLEGAS, S.N., NJAINE, B., LINDEN, R., CARRI, N.G. (2006). Glial-derived neurotrophic factor (GDNF) prevents ethanol (EtOH) induced B92 glial cell death by both PI3K/AKT and MEK/ERK signaling pathways. *Brain Res Bull.* 71: 116-126.
- VISSIO, P.G., ANDREONE, L., PAZ, D.A., MAGGESE, M.C., SOMOZA, G.M., STRÜSSMANN, C.A. (2002). Relation between the reproductive status and somatolactin cell activity in the pituitary of pejerrey, *Odontesthes bonariensis* (Atheriniformes). *J Exp Zool* 293: 492-499.
- WAHLIN, K.J., CAMPOCHIARO, P.A., ZACK, D.J., ADLER, R. (2000). Neurotrophic factors cause activation of intracellular signaling pathways in Müller cells and other cells of the inner retina, but not photoreceptors. *Invest Ophthalmol Vis Sci* 41: 927-936.
- WARBURG, O. (1908). Beobachtungen über die Oxidations Prozesse von Seeigelei. *Z. Physiol. Chem* 57: 1-16.
- WARBURG, O. (1910). Über die Oxidationen in Lebenden Zellen nach versuchen am Seeigelei. *IChem* 66: 305-340.
- WEINER, A.M., ALLENDE, M.L., BECKER, T.S., CALCATERRA, N.B. (2007). CNBP mediates neural crest cell expansion by controlling cell proliferation and cell survival during rostral head development. *J Cell Biochem* 102: 1553-1570.
- WEINER, A.M.J., SCAMPOLI, N.L., STEEMAN, T.J., DOOLEY, C.M., BUSCH-NENTWICH, E.M., KELSH, R.N., CALCATERRA, N.B. (2019). Dicer1 is required for pigment cell and craniofacial development in zebrafish. *Biochim Biophys Acta Gene Regul Mech* 1862: 472-485.
- WILLINK, A. (1999). Biografías zoológicas lilloanas\* Biographical notes on zoologist from the Lillo. *Rev. Soc Entomol. Argent.* 58: 3-10.
- XIE, H.Q., ADLER, R. (2000). Green cone opsin and rhodopsin regulation by CNTF and staurosporine in cultured chick photoreceptors. *Invest Ophthalmol Vis Sci* 41: 4317-4323.
- ZAMPINI, R., ARGANÁRAZ, M.E., MICELI, D.C., APICHELA, S.A. (2014). Detection of the matrix metalloproteinases MMP-2 and MMP-9 and tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2 in llama (*Lama glama*) oviduct. *Reprod Domest Anim* 49: 492-498.
- ZANIN, J.P., BATTIATO, N.L., ROVASIO, R.A. (2013). Neurotrophic factor NT-3 displays a non-canonical cell guidance signaling function for cephalic neural crest cells. *Eur J Cell Biol* 92: 264-279.
- ZAPATA-MARTÍNEZ, J., MEDINA, M.F., GRAMAJO-BÜHLER, M.C., SÁNCHEZ-TORANZO, G. (2016) Participation of PLA2 and PLC in DhL-induced activation of *Rhinella arenarum* oocytes. *Zygote* 24: 495-501.
- ZELARAYÁN, L., OTERINO, J., BÜHLER, M.I. (1995). Spontaneous maturation in *Bufo arenarum* oocytes: Follicle wall involvement, respiratory activity and seasonal influences. *J Exp Zool* 272: 356-362.



**Further Related Reading, published previously in the *Int. J. Dev. Biol.***

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

M De Felici and G Siracusa  
Int. J. Dev. Biol. (2000) 44: 515-521  
<http://www.intjdevbiol.com/web/paper/11061413>

**The “Stazione Zoologica Anton Dohrn” and the history of embryology**

B Fantini  
Int. J. Dev. Biol. (2000) 44: 523-535  
<http://www.intjdevbiol.com/web/paper/11061414>

**Molecularizing embryology: Alberto Monroy and the origins of Developmental Biology in Italy**

B Fantini  
Int. J. Dev. Biol. (2000) 44: 537-553  
<http://www.intjdevbiol.com/web/paper/11061415>

**Valerio Monesi, a pioneer of modern Reproductive Biology**

G Siracusa and M Stefanini  
Int. J. Dev. Biol. (2000) 44: 555-557  
<http://www.intjdevbiol.com/web/paper/11061416>

**On the present situation of Developmental Biology in Italy**

G Cossu and E Boncinelli  
Int. J. Dev. Biol. (2000) 44: 559-561  
<http://www.intjdevbiol.com/web/paper/11061417>

**On the present situation of Developmental Biology in Italy**

G Cossu and E Boncinelli  
Int. J. Dev. Biol. (2000) 44: 559-561  
<http://www.intjdevbiol.com/web/paper/11061417>

**From a home-made laboratory to the Nobel Prize: an interview with Rita Levi Montalcini**

Giovanni Giudice  
Int. J. Dev. Biol. (2000) 44: 563-566  
<http://www.intjdevbiol.com/web/paper/11061418>

