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Classics revisited: Miguel Fernández on germ layer inversion and specific polyembryony in armadillos

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ABSTRACT

Background: Miguel Fernández was an Argentinian zoologist who published the first account of obligate polyembryony in armadillos. His contribution is here discussed in relation to his contemporaries, Newman and Patterson, and more recent work.
Findings: Fernandez worked on the mulita (*Dasypus hybridus*). He was able to get early stages before twinning occurred and show it was preceded by inversion of the germ layers. By the primitive streak stage there were separate embryonic shields and partition of the amnion. There was, however, a single exocoelom and all embryos were enclosed in a common set of membranes comprising chorion towards the attachment site in the uterine fundus and inverted yolk sac on the opposite face. He showed that monozygotic twinning did not occur in another armadillo, the peludo (*Chaetophractus villosus*).
Conclusions: Fernández's work represented a major breakthrough in understanding how twinning occurred in armadillos. His work and that of others is of intrinsic interest to zoologists and has a direct bearing on the origin of monozygotic twins and birth defects in humans.

Contents

1. Introduction

Armadillos of the genus *Dasypus* are unique among vertebrates in exhibiting obligate polyembryony, i.e. each blastocyst gives rise to four or more identical embryos [1–3]. This does not happen in other armadillos [4]. The German-Brazilian zoologist Hermann von Ihering is credited with the first experimental study of this phenomenon. He opened the uteri of two pregnant females of *D. hybridus* and found nine embryos of identical sex in both. Each embryo had a separate amnion, but all shared a common chorion. He suggested that the embryos were the product of a single fertilized egg and that division into several embryos occurred early in development [5,6]. Miguel Fernández set out to explore this further and was fortunate in having a ready supply of material from the meat markets of Buenos Aires [7]. His initial publication [8] was based on 60 pregnant uteri of *D. hybridus* (Southern Long-nosed Armadillo; local name mulita). Two of these contained an embryo

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that had not yet undergone division. Concomitantly, Newman and Patterson [9] were tackling the same problem in *D. novemcinctus* (Nine-banded Armadillo) whose range extends into the United States. They reached similar conclusions though their earliest specimen was at the primitive streak stage when there were already four embryos. As related by Garcia [7], Newman and Patterson were dependent on specimens caught in the wild and transported to the laboratory. Later Patterson started collecting in the field and this resulted in a supply of very early stages of *D. novemcinctus*, including unattached blastocysts [10]. Meanwhile Fernández expanded his collection to over 200 specimens and published a weighty tome on the embryology of *D. hybridus* [11].

2. Brief biography

Miguel Fernández (Fig. 1) was born in Essen 25 September 1882 to an Argentinian father and a German mother. He attended school in Uruguay and enrolled for studies in industrial chemistry at the Polytechnic Institute in Zurich (Albert Einstein's *alma mater*). Soon, however, he came under the influence of Arnold Lang and transferred to biological sciences at the University of Zurich. Lang was a renowned comparative anatomist and himself a pupil of Haeckel. Fernandez's doctoral thesis on the vascular system of tunicates [12] was completed during a brief sojourn at the University of Heidelberg. He returned to Lang in Zurich but then, while visiting family in Montevideo, was offered a position at the University of La Plata, Argentina. He was professor of zoology and comparative anatomy at La Plata from 1906 to 1926 and it was here he embarked upon his studies of the embryology of the mulita. According to his biographers [7,13], Fernández had to endure much criticism from his colleagues at La Plata not least because he published in German and in foreign journals, which was seen as unpatriotic (notwithstanding

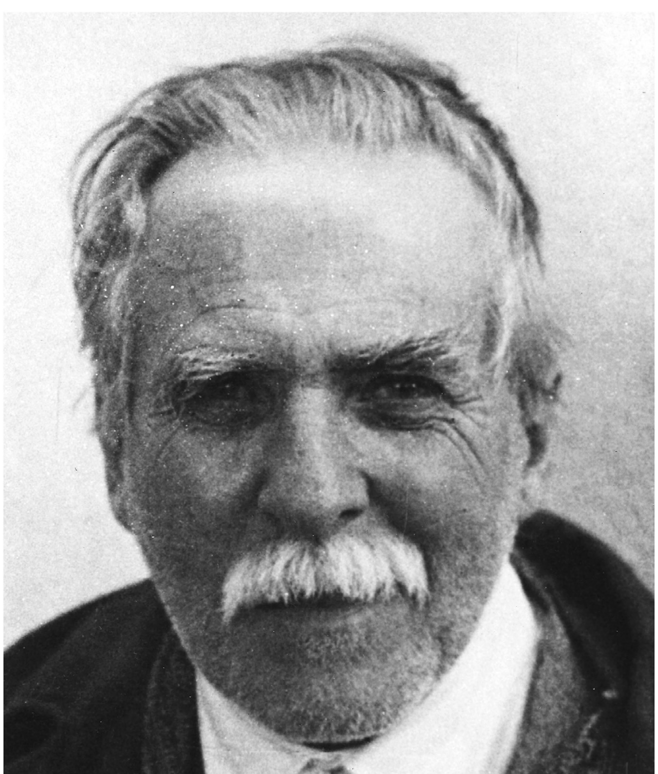

Fig. 1. Miguel Fernández (1882-1950) the discoverer of polyembryony in armadillos. Reproduced with permission from Solari et al. [14] © 2016 the authors.

his major work on the mulita had a 28-page summary in Spanish). In 1927 he transferred to the chair of zoology at the University of Córdoba, the senior academic institution in Argentina (founded in 1613). Though he was much appreciated as a mentor, Fernandez's own scientific output tailed off as most of his time at Córdoba was devoted to teaching. In his later years, Fernández is credited with establishing the study of genetics in Argentina [14]. Fernández was married to a fellow zoologist, Kati Fernández Marcinowski. He was a music lover and a keen photographer of wild birds. In 1943 he retired to a country home on the outskirts of Alta Gracia where he died 29 April 1950. A 1969 commemorative postage stamp bears his image. Further details can be found in his obituary [13], which has a complete list of his publications, as well as in more recent biographical sketches [7,15].

3. Germ layer inversion and early development of the mulita

In his 1909 paper Fernández [8] made two major observations. Firstly, that there was inversion of the germ layers as in mouse and guinea pig (see Ref. [16]). Secondly, that all the embryos in a litter were derived from a single blastocyst. He began by demonstrating that pregnant females had a single corpus luteum and confirming that all the embryos in a litter (7-12 in number) were of the same sex. Next he described two early embryos in which twinning had yet to occur (Fig. 2A-B). They were implanted in the fundus of the uterus and attached by a ring of trophoblast (the trophoblastic annulus) that he likened to the attachment cone (Träger) of rodents. In the middle of the Träger there was a cavity as also known from rodents (but see Discussion). The embryo protruded into the yolk sac cavity and it was covered by a layer of endoderm. However, the endoderm had not spread around the inside of the trophoblast on the outer wall of the cavity. Within the embryo was a cavity identified as the amnion. There was precocious differentiation of the mesoderm and appearance of an exocoelom. The embryonic ectoderm formed an epithelium that, throughout the embryo, was thicker at the base and thinner at the cervical end. This conformation was later confirmed for *D. novemcinctus* by Patterson [10] and several pertinent features can be seen in an early stage examined by Enders [2] (Fig. 2C).

The next embryos described by Fernández were at the primitive streak stage (Fig. 3A). The outer layer of trophoblast was no longer present (it is known to disintegrate [2]) so that inversion of the yolk was complete. There were now several separate embryonic shields. The exocoelom protruded into the amniotic cavity, which was compartmentalized in such a way that there was a portion associated with each embryo as well as a common amniotic cavity at the cervical end of the conceptus. This stage was important for understanding the arrangement at the 16-18 somite stage, when there had been further expansion of the exocoelom (Fig. 3B). Here each embryo appeared to be enclosed in its own amnion, yet there were channels connecting the several amnions to a common amniotic vesicle. Fernández was now able to refine Ihering's [5] interpretation that all embryos were within a common chorion. There was a stretch of chorion (trophoblast and extraembryonic mesoderm) spanning the trophoblastic annulus at the fundic end of the conceptus (see Discussion). However, the cervical end of the gestational sac was enclosed by the inverted yolk sac (Fig. 3B), which was supplied with blood vessels. In addition this stage saw the first appearance of an allantoic stalk (Bauchstiel) that Fernández compared to that described in the tarsier by Hubrecht [17]. At later stages the yolk sac vasculature fragmented and disappeared, whereas the umbilical vessels of the fetuses supplied the placenta. The cord also contained remnants of the urachus. There is but a cursory description of the placental villi in this paper.