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Spermatogenesis Following Early Ovariotomy in the Brown Leghorn Fowl.*

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In our earlier series of experiments on ovariotomy[1] operations were performed at a relatively late age, the majority of the birds ranging from 3 to 8 or 9 months. One of the extraordinary results revealed was the development of the rudimentary right gonad into an organ which is essentially a testis though, in our cases, always sterile. This was particularly baffling since during the course of our study 2 cases of spermatogenesis were recorded by Benoit[2] and 1 by Zawadowsky[3] in the ovariotomized fowl, from a relatively insignificant number of experiments. These birds were 4, 27, and 45 days of age respectively at the time of operation. Only 2 of our cases, one of 2, and one of 27 days, came within this age range.

Furthermore, according to the work of Brode[4] and others[5, 6] primordial germ cells are present in the medulla of both right and left gonads of the female chick in early stages. Brode has shown that these cells gradually decrease in numbers after hatching so that by approximately 3 weeks after they have practically all disappeared in the right gonad. Since it is highly probable from these observations that no primordial germ cells were present in the right rudimentary gonad at the time of ovariotomy in our earlier series it was considered advisable to supplement this otherwise extensive data by additional cases of early ovariotomy.

A large number of females were ovariotomized at an early age. Operations were performed every day from the first to the 30th day, and every other day from the 30th to the 50th day, after hatching. 175 birds operated under 30 days of age survived the operation; of these, 26 had been operated during the first 7 days.

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[1] Domm, L. V., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 28; *J. Exp. Zool.*, 1927, xlviii, 31.

[2] Benoit, Jacques, *C. R. Acad. Sc.*, 1923, T. 177, 1074.

[3] Zawadowsky, M., *Trans. Lab. Exp. Biol.*, Zoopark, Moscow, 1926, ii, 164.

[4] Brode, M. D., *J. Morph. and Physiol.*, 1928, i, 1.

[5] Swift, C. H., *Am. J. Anat.*, 1914, xv, 483; *Am. J. Anat.*, 1915, xviii, 441.

[6] Firket, Jean, *Arch. de Biol.*, 1914, xxix, 201; *Arch. de Biol.*, 1920, xxx, 393.

The birds were killed at regular intervals and the material carefully preserved. The study is not complete. Forty birds are still alive, and the gonads of those killed have not all been studied histologically. The experiments, as previously, were confined to the single comb light brown Leghorn secured from the same source immediately after hatching.

This supplementary series should give us a definite understanding of the histogenesis of this testis-like right gonad about which such varying views have been expressed. It was hoped that it would definitely reveal whether it had the capacity to develop spermatogenetic tissues following ovariotomy. We have now studied the histological preparations from 72 such testis-like right gonads of the second series and in 5 of these we find spermatogenesis.

Bird No. 1038. This bird was ovariotomized when 7 days old and killed 8 months after the operation. Following the operation it developed the plumage and head furnishings of the male. During the 2 months prior to its death the new plumage was intermediate in type. Post mortem examination revealed a relatively prominent right gonad measuring 2.0 cm. in length and 0.9 cm. in width. The entire gonad was serially sectioned. Active spermatogenesis appears in a few of the tubules on the 34th slide of the series and runs through to the 40th. These tubules are thus confined to a rather limited area of the gonad, the rest of which reveals a scattering of the characteristic sterile tubules previously found.

Bird No. 1083. This bird was operated when 8 days old and killed 11 months following the operation. By 6 months after the operation the bird was completely male plumaged, no other plumage was developed. The head furnishings developed very slowly and did not attain male proportions. Post mortem examination revealed a small right gonad measuring 1.8 cm. in length and 0.5 cm. in width. Only the anterior part of this gonad has been sectioned. It shows spermatogenetic tubules, many with fully differentiated spermatozoa, closely packed throughout the entire section.

Bird No. 1008. This bird was ovariotomized when 11 days old and killed 8 months following the operation. It developed the plumage and head furnishings of the male. During the first 4 months it developed juvenile male plumage which was followed by adult male for but a short period. By 5 months intermediate plumage began to appear, followed by female at 7 months. Female plumage continued to appear up to the time of killing. Post mortem examination revealed a prominent right gonad measuring 2.4 cm. in length and 1.0 cm. at its widest point. The anterior third of this gonad has been sectioned covering 30 slides. Spermatic tubules ap-

pear toward the end of the series, being limited to about 9 slides. They are rather closely packed and confined to a limited area dorsally immediately under the albuginea. The rest of the section reveals a scattering of sterile tubules interspersed with connective tissue.

Bird No. 1002. This bird was ovariotomized when 17 days old and killed 11½ months later. Following the operation it developed the plumage and head furnishings of the male. The juvenile plumage of the male was developed following the operation which was later followed by adult male. By 10 months intermediate plumage appeared and when the bird was killed the ingrowing back and saddle feathers were female. Post mortem examination revealed a prominent right gonad measuring 2.4 cm. in length and 0.9 cm. at its widest part. The anterior third of this gonad has been sectioned, covering 30 slides. Here spermatic tubules, many of which reveal mature sperm, appear in the 5th slide and run through the rest of the sectioned series. In general these tubules are loosely scattered through the entire section, though in places they occur in small groups. Some of the characteristic sterile tubules are also found.

Bird No. 923. This bird was ovariotomized when 38 days old and killed 12 months later. The juvenile plumage of the male was developed following the operation and this was later replaced by adult male. Seven and one-half months after, intermediate plumage was appearing, and from 9½ months after, until the bird was killed the new plumage was female. Head furnishings attained male proportions. The bird crowed. Post mortem examination revealed a prominent right gonad measuring 2.1 cm. in length and 0.9 cm. at its widest point. The entire gonad was serially sectioned, covering 115 slides. Here spermatic tubules, showing mature sperm, do not appear prior to the 64th slide of the series, where a few are found along the periphery. They are found in the next 3 slides and then apparently disappear in a few slides, then reappear, running through to slide 101, where some of them seem to end in the characteristic sterile tubule.

In other cases of this series, not here described, the right gonad developed ovotestis, also perfectly typical ovary, which definitely reveals that the changes following complete ovariotomy are very variable. Including the cases here described we have now demonstrated a range of possibilities for the right gonad that extends all the way from complete spermatogenesis following ovariotomy, through sterile testis and ovotestis, to complete and normal ovogenesis associated with a corresponding array of other sex characters such as

accessory organs of reproduction, head furnishings, behavior, plumage and spurs.

These results lead to the working hypothesis that primordial germ cells are necessary for gametogenesis, that when they become incorporated in the cords of the medulla they produce spermatogenesis and when in the cortical elements of the gonad they produce ovogenesis. This working hypothesis agrees with Witschi's[7] findings in Amphibia.

[7] Witschi, Emil, PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 729.