

Sex-Linked Feathering of Female Hybrid Chick Embryos Hormonally Conditioned

In some hybrids of fowl, the sex of the chicks can be recognized by a sex-linked difference in down colour. So, for instance, in the F1-generation of the cross Rhode Island Red or New Hampshire ♂ × Light Sussex ♀, the cockerels are white and the pullets reddish-brown. The brown colour of the females becomes visible on the 11th day of incubation as a yellow stripe on the back and neck. The stripe gradually broadens and darkens during the incubation period (Figure 1). As the difference in down colour is considered to be genetically determined and independent of the gonadal hormones, the hybrids have been used by many investigators, who studied the influence of oestrogenic or androgenic compounds on the development of the embryonic sex glands and ducts. When the latter were modified, the genetic sex of the embryo was diagnosed by the feather colour (VAN OORDT et al.¹, WOLFF et al.²). Likewise, in castration experiments, in which the gonads were totally removed, the sex was determined by the down colour (WOLFF et al.³); that is to say, a white embryo was diagnosed to be a male. However, while carrying out hemi- and subtotal castrations on hybrids of the cross New Hampshire ♂ × Light Sussex ♀, the author observed that the down colour of the females varied from brown to almost white, depending upon the amount of ovarian tissue that had remained. The subtotally castrated females could hardly be distinguished from the white males. Since these observations

open new insight into the characteristics of the sex-linked feathering in chicks, they are communicated here.

Materials and methods. On the fourth day of incubation either the left or right gonadal primordium, or both, were destroyed by local diathermic coagulation, as described previously⁴. As a rule, autopsy was performed on the 14th day of incubation, occasionally it was done on the 18th day. After inspection of the down colour, the abdominal organs were exposed and prefixed by intracardial perfusion with a 5% mercuric chloride solution, a colourless fluid which does not modify the feather colour. Then the remnants of the ovaries and the Müllerian ducts were drawn with the aid of a camera lucida. Photographs were taken of the embryo's back and occiput and of the sex organs. The gonads were fixed in Maximow's fluid, containing 2% osmic acid; the embryos were stored in alcohol 70%. The sex of the embryo was verified by histological examination of the gonadal remnants.

Results. The results are summarized in the Table. In the presence of large remnants of the left and right ovary, the colour of the female is brown as in the controls (Figure 1).

¹ G. J. VAN OORDT and G. L. RINKEL, Wilhelm Roux Arch. Entw. Mech. Org. 140, 59 (1940).

² ET. WOLFF, G. STRUDEL, and EM. WOLFF, Archs Anat. Histol. Embryol. 31, 241 (1948).

³ ET. WOLFF and EM. WOLFF, J. exp. Zool. 116, 59 (1951).

⁴ MARGOT M. GROENENDIJK-HUIJBERS, Acta morph. neerl.-scand. 7, 241 (1957).



Fig. 1. 14-day-old female control embryo; crown-rump length 5.7 cm. Feathers are brown, particularly on back, thighs, wings, neck and occiput.



Fig. 2. No. 6476. 14-day-old hemicastrated female; crown-rump length 5.8 cm. Feathers are pale brown on back, thighs, neck and occiput. Wings are white. Regression of the right oviduct is slightly retarded.

Influence of hemi- and subtotal castration on down colour of hybrid chick embryos (New Hampshire ♂ × Light Sussex ♀)

| | Embryos | | | | Ovarian remnant | Colour of down | | | | | |
|---------------------|-----------|----------|-------------|------------|-----------------|----------------|--------|-------|-------|------------|--------------|
| | Total No. | Dead No. | Alive ♂ No. | Down white | Alive ♀ No. | Large | Medium | Small | Brown | Pale brown | Almost white |
| Controls | 12 | — | 6 | 6 | 6 | 6 | | | 6 | | |
| Hemicastration | 15 | 7 | 4 | 4 | 4 | 1 | 3 | — | 1 | 2 | 1 |
| Subtotal castration | 57 | 50 | 4 | 4 | 3 | | 2 | 1 | | 2 | 1 |
| Total | 84 | 57 | 14 | 14 | 13 | 7 | 5 | 1 | 7 | 4 | 2 |

When only the right ovary or about two-thirds of the left ovary remained, the colour faded to a pale brown (Figure 2). When less than one-half of either the right or left ovary is present, the colour is almost white (Figure 3). The shortage of ovarian hormone is also manifested by the lack of regression of the right oviduct: when the colour is pale brown, the regression is retarded, when the colour is almost white the duct is fully preserved.

Conclusion and discussion. The experiments reported indicate that, although the brown feather colour of the female hybrid embryos of the cross New Hampshire ♂ ×

Light Sussex ♀ is genetically determined, the feather-pigmenting melanophores are unable to produce red pigment in the absence of the ovarian hormone, released by both the right and left ovary. The shade of the down is directly correlated with the amount of ovarian hormone produced. Below a certain threshold no red pigment can be formed. This means that *in a gonadless animal the white feather colour does not necessarily indicate the male sex*. This conclusion contradicts the results of previous investigations, which seemed to demonstrate that the embryonic melanophores are insensitive to hormones (reviewed by WILLIER⁵). These investigations, however, particularly concerned the Brown Leghorn breed. For the Rhode Island Red and the New Hampshire breeds, HAMILTON⁶ reported that melanophores of skin ectoderm of 6- to 7-day-old embryos, cultivated in vitro, did not produce red pigment unless gonadal hormones (either oestrogens or androgens) were added to the culture medium. The observations reported here fully agree with these findings. Further experiments are in progress to substantiate the preliminary conclusions⁷.

Zusammenfassung. Bei weiblichen Hühnerembryonen der Kreuzung New Hampshire ♂ × Light Sussex ♀ ist die rotbraune Farbe nach subtotaler Embryokastration verschwunden. Die embryonalen Melanophoren, welche die Daunen pigmentieren, können offenbar das rote Pigment nicht herstellen, wenn das ovarielle Hormon nicht vorhanden ist. Eine weisse Färbung der Daunen ist daher für die Feststellung des männlichen Geschlechts gonadenloser Tiere nicht entscheidend.

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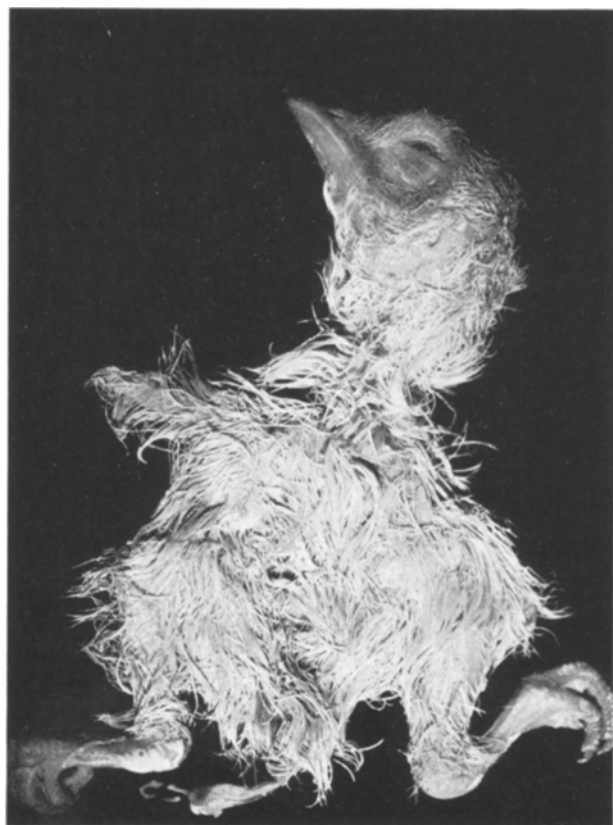


Fig. 3. No. 6460. 18-day-old subtotally castrated female; crown-rump length 6.0 cm. A few yellow feathers on tail, thighs and occiput. The rest of the feathers white. The right oviduct is completely preserved.

⁵ B. H. WILLIER, Archs Anat. microsc. Morph. exp. 39, 269 (1950).

⁶ H. L. HAMILTON, Anat. Rec. 78, 525 (1940).

⁷ It is a pleasure to thank Prof. Dr. W. K. HIRSCHFELD, Chairman of the Department of Poultry Breeding and Husbandry of the Utrecht University for his kind cooperation in supplying eggs from the cross New Hampshire ♂ × Light Sussex ♀, used in these experiments.