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Gonadal sex differentiation in the neonatal marsupial, *Monodelphis domestica*

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Summary

A quantitative and histological study of the gonads of newborn grey short-tailed opossums, *Monodelphis domestica*, is described. The pups were karyotyped, and comparisons were made within litters segregating for XX and XY sex chromosomes. A total of four litters including 25 pups were available. On the day of birth, developing testes were significantly larger than the ovaries of litter mates, and testes could be histologically distinguished by the formation of sex cords and a tunica albuginea. The data suggest that in this marsupial species gonadal differentiation may be initiated *in utero*.

Key words: marsupials, sex differentiation, gonads, testis development.

Introduction

In eutherian mammals the sexual differentiation of the gonads occurs prenatally and the newborn are anatomically distinguishable as male or female in consequence of the presence or absence of secretions by the fetal testis (Jost *et al.* 1973). By contrast, in the marsupial species, *Macropus eugenii*, the tammar wallaby, O *et al.* (1988) found neither histological nor quantitative differences between the gonads of newborns karyotyped as XX and XY. However, there was evidence of other sexual differences at birth, such as the presence of scrotal bulges in males and of mammary anlagen in females, and the authors proposed that the mechanism of differentiation of these structures must be hormone-independent and thus fundamentally different from that in eutherians.

In another species of marsupials, the Virginia opossum, *Didelphis virginiana*, McCrady (1938) found that the first histological signs of sexual differentiation can be detected at developmental stage 35, i.e. the time of birth, when the male gonad develops clearly defined testis cords, while the female gonad still appears indifferent. We now wish to report a quantitative and histological study in another didelphid species, the grey short-tailed opossum, *Monodelphis domestica*, which showed that XX and XY gonads could be distinguished by both criteria on the day of birth.

Materials and methods

Newborn grey short-tailed opossums were obtained from a breeding colony maintained at the Institute of Zoology. Oestrus was induced in mature females by the introduction of a male (Baggott *et al.* 1987) and matings were timed 5–8 days later by close-circuit video recording. It is known that ovulation and fertilization occur 24–30 h after the first mating and that the gestation length is 13.5 days (Baggott and Moore, 1990). The opossums were kept on a reverse light cycle with dusk at 9.00 h, and birth normally occurred in the early morning. Near to the expected time of birth, females were checked twice daily. The estimated minimum and maximum age of the litters examined was 2–18 h post-partum.

Newborns from timed matings were collected and weighed, and chromosome preparations were made from liver (Evans *et al.* 1972), as described previously (Moore and Thurstan, 1990). The bodies were trimmed, fixed in Bouin's solution and prepared for serial sectioning at 7 μm, followed by staining with haematoxylin and eosin.

Gonadal volumes were computed from section areas transposed *via* a Leitz camera lucida onto a digitizing tablet connected to an Opus microcomputer, and multiplying the section areas by the effective section thickness.

To test the statistical significance of the differences between body weights and gonadal volumes in XX and XY opossums, a weighted *t*-test was used. For each litter containing both sexes, the mean for each sex was calculated and the XX mean subtracted from the XY mean. The difference, d, thus obtained was assigned a weight, w, $=n_1n_2/n_1+n_2$, where n_1 and n_2 are the numbers in the two sex-chromosomal classes. The weighted mean difference,

$$\bar{d} = \frac{\Sigma dw}{\Sigma w},$$

where Σ=summation over all litters. The standard error, SE, of the weighted mean was calculated according to the formula

$$\sqrt{\left[\frac{1}{(N-1)\Sigma w}\left(\Sigma wd^2 - \frac{(\Sigma wd)^2}{\Sigma w}\right)\right]},$$

where N=number of litters. The weighted mean divided by its

standard error, with $N-1$ degrees of freedom, was referred to in Student's t table.

All measurements were carried out in the absence of knowledge of chromosomal sex, and the gonads of litter 734 were investigated in the absence of knowledge on quantitative studies.

Results

The chromosomes of a male opossum are shown in Fig. 1. The sex chromosome pair is the smallest, the Y being minute.

Quantitative results are shown in Table 1. It is evident that the difference in body weights between XX and XY opossums is not significant, but that there is a highly significant difference in gonadal volumes. On average, the volumes of developing testes exceed those of the ovaries of their litter mates by 46 %, while in the least developed litter, 734, this difference was 40 %.

The histology of the gonads is illustrated in Fig. 2. The gonad of pup 734.1 shows early signs of testicular differentiation by the presence of a tunica albuginea and the beginning of cord formation in the interior of the gonad (Fig. 2A,B), neither of which are present in the XX litter mate, 734.10 (Fig. 2C,D). Testicular differentiation is further advanced in pup 655.6 (Fig. 2E,F), whereas the gonad of a female litter mate 655.1 remains undifferentiated (Fig. 2G,H).

The gonads from all pups were correctly classified into testes and ovaries by detailed histological examination, in the absence of knowledge of their chromosome constitution.

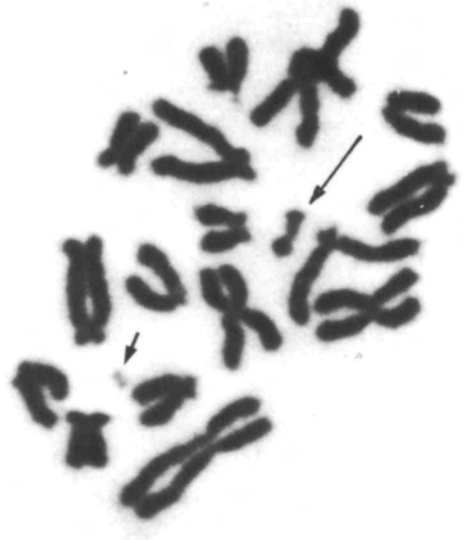

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Fig. 1. Chromosomes of a male opossum. The Y chromosome (short arrow) is minute and the X chromosome (long arrow) is small.

Table 1. *Body weights and gonadal volumes in four litters of short-tailed opossums on the first day of birth*

Litter no.	Individual no.	Sex chromosomes	Body weight (mg)	Mean gonadal volume (mm^3)
608	1	XY	133	0.01235
	3	XY	117	0.01735
	5	XY	123	0.0155
	2	XX	115	0.0097
	4	XX	121	0.01065
610	1	XY	111	0.01325
	2	XY	107	0.0116
	3	XX	73	0.0084
655	2	XY	140	0.01465
	3	XY	118	0.01345
	6	XY	136	0.01545
	1	XX	124	0.00855
	4	XX	132	0.01005
	5	XX	111	0.00965
	7	XX	127	0.01015
	8	XX	116	0.0101
	9	XX	119	0.0107
734	1	XY	96	0.00955
	2	XY	104	0.00915
	8	XY	89	0.00835
	9	XY	95	0.0085
	11	XY	103	0.01085
	12	XY	97	0.0113
	3	XX	94	0.0068
	10	XX	89	0.00695
Mean XY			115.5	0.01291
Mean XX			101.0	0.008845
Weighted difference $\bar{d}$ (XY-XX)			11.1824	0.004099
		S.E.	5.4921	0.0005076
		t_3	2.04	8.07
		P	n.s.	$0.002<P<0.005$

Discussion

This report describing definite signs of testicular differentiation in newborn pups belonging to the marsupial family *Didelphidae* supports the statement by McCrady (1938) that male gonads in another species of this family develop 'clearly defined' testis cords at the time of birth. Ullman (1989) wrote that in the bandicoot, *Isoodon macrourus*, a member of Peramelidae, sexual differentiation of the gonadal rudiment in prospective males begins on day 1 of pouch life, but she illustrates an indifferent gonad of a neonate. Our findings that even in the apparently youngest litter (734), the developing testes were not only histologically recognisable, albeit after detailed investigation of serial sections, but were also, on average, 40 % larger than the ovaries of the litter mates, suggest the likelihood that, in the short-tailed opossum, sexual differentiation begins in intrauterine life.

In view of their gestation time of 14 days, newborn opossums are of the same chronological age as rat fetuses aged 14–14.5 days, in which correct identification of gonadal sex by the presence or absence of sex cords and tunica albuginea is possible in fetuses from

day 14.5 onwards. The difference in size between XX and XY gonads reported by Lindh (1961) and by Mittwoch *et al.* (1969) is of a similar magnitude to that now found in neonatal opossums. The process of sex differentiation in the two species may therefore be chronologically similar, and this raises the question

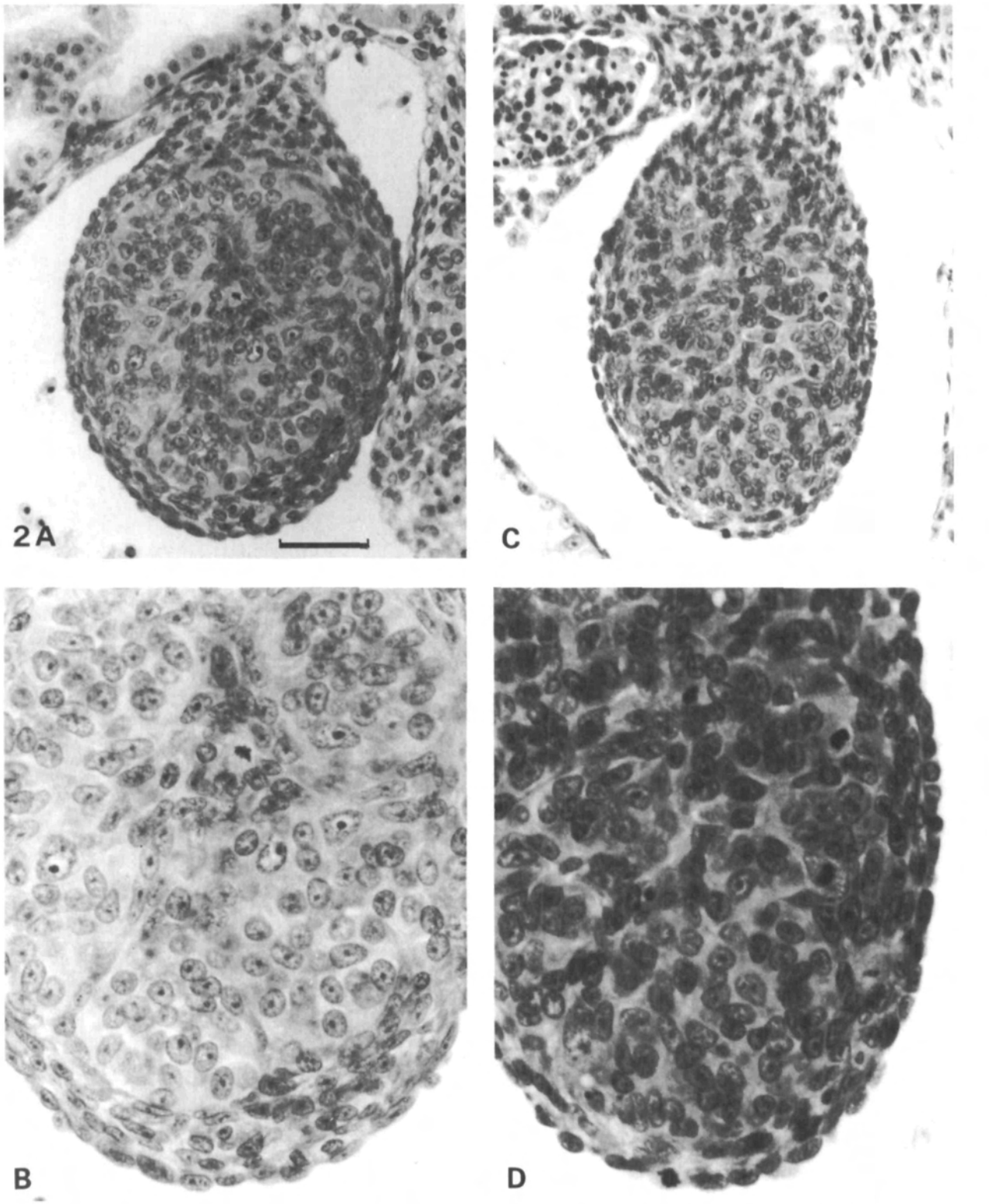

Fig. 2. Histology of transverse sections of XY and XX gonads on the day of birth. (A) Gonad of XY individual 734.1 (body wt.96 mg) showing beginning of cord formation and developing tunica albuginea. (B) The same at higher magnification. (C) Gonad of XX litter mate (734.10, body wt.89 mg), showing undifferentiated blastema and lack of tunica. (D) The same at higher magnification. Bar=46 μm in A, C; 25 μm in B, D. *Continued overleaf.*

whether the onset of steroidogenesis could also occur earlier in at least some species of marsupials than had hitherto been assumed.

The results obtained by Fadem and Tesoriero (1986) and by Moore and Thurstan (1990) of treating neonate short-tailed opossums with oestrogen are of particular

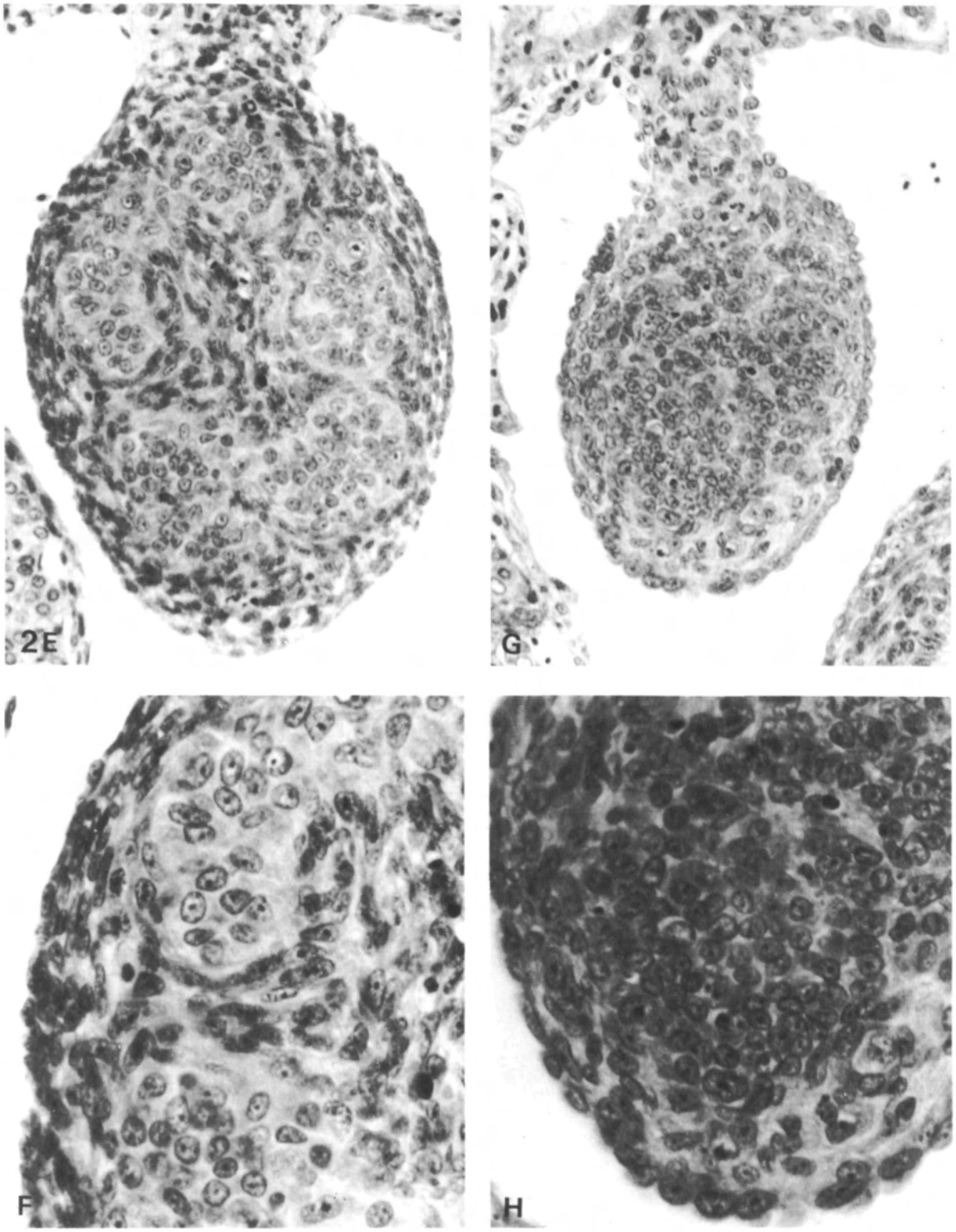

Fig. 2. (E) Gonad of XY individual 655.6 (body wt.136 mg), showing several layers of somatic cells separating sex cords and well developed tunica surrounding entire gonad. (F) The same at higher magnification. (G) Gonad of XX litter mate (655.1, body wt.124 mg), showing undifferentiated blastema surrounded by irregular layers of cells. (H) The same at higher magnification. Bar=46 μm in E, G; 25 μm in F, H.

interest in this respect. Oestrogen treatment had no detectable effect on females but, in males, testis development was severely disrupted leading to streak gonads in adults. Both internal and external genitalia were completely feminised, apart from the development of the scrotum, which appears not to be testosterone-dependent in marsupials. These findings, as well as those by Burns (1950, 1961), who reported ovarian development in male Virginia opossums treated with oestrogen neonatally, suggest that, even after histological differentiation has begun, the testis can still regress to become a streak gonad. In other words, testis determination is not a momentary decision but a process that must continue over a distinct period of time.

Histologically, the developing testes of newborns were distinguishable both by the formation of cords within the gonadal blastema, and the presence of a tunica albuginea, up to four or five cells in thickness, which forms a well-demarcated covering of the gonad. By contrast, the outer layer of developing ovaries consists of only one or at most two layers of cells. Although, following Jost *et al.* (1981), if it is often assumed that the formation of Sertoli cells is the first histologically distinguishable event in the process of gonadal differentiation, and that other processes of differentiation follow in its wake, the question arises whether formation of the tunica albuginea could be an independent event, also resulting from a high rate of proliferation of somatic cells of the gonadal ridge (Mittwoch, 1989).

Since gonadal sex differentiation in short-tailed opossums is already evident at birth, we shall be continuing our investigations backwards to the time of gonadal ridge formation in the embryo in order to find out at what stage the first signs of differentiation can be detected.

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