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The Marsupial Male: A Role Model for Sexual Development [and Discussion]

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The marsupial male: a role model for sexual development

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SUMMARY

Sexual differentiation in male marsupials has many similarities with that of eutherians. Marsupials have an XX-XY sex determining mechanism, and have a homologue of the testis-determining *SRY* gene on their Y-chromosome. However, the development pattern of *SRY* gene expression is different from the mouse in that it is expressed for a much longer period. *SRY* is expressed in a range of non-gonadal tissues in male pouch young and adults which is similar to the human pattern, and raises questions as to its particular role(s) in sexual differentiation. Similarly Müllerian inhibiting substance (MIS) is produced in the developing testis over a longer period than in the mouse. Since ovaries cultured with MIS or transplanted into male recipient pouch young develop tubular structures, MIS may induce Sertoli cell formation. Testosterone is produced by the neonatal testis, and this stimulates Wolffian duct development to form the vas deferens and epididymis. Virilization of urogenital sinus is also androgen-dependent. However, virilization of the prostate and phallus occurs more than three weeks after the onset of testosterone production, suggesting that the timing of this may be regulated by delayed activation of the androgen receptor pathway. Unlike in eutherians, differentiation of the scrotum and mammary glands is not dependent on testicular hormones, but is independently regulated by an X-linked genetic mechanism. Clearly marsupials provide a unique perspective to help us clarify the mechanisms underlying sexual development in all mammals.

1. INTRODUCTION

The pioneering studies of Professor Alfred Jost showed that the embryonic rabbit testis was essential for development of the male phenotype (Jost 1947). More recently, the cloning and characterization of several genes, including the testis-determining gene *SRY* (Sex determining Region of the Y chromosome, Sinclair *et al.* 1990), *MIS* (Mullerian Inhibitor Substance, Cate *et al.* 1986) and *AR* (Androgen Receptor, Chang *et al.* 1988) has led to a burgeoning interest in the pathways regulating mammalian sexual differentiation. Marsupials are valuable for investigation of the function of genes and hormones known to direct sexual development (Shaw *et al.* 1995). Marsupials provide an accessible model for the study of sexual differentiation, because most events occur post-natally whilst the young are attached to teats within their mothers' pouches.

Marsupial and eutherian mammals diverged from a common ancestor about 100 Ma, but retained many common mechanisms directing sex determination and differentiation. In marsupials the Y chromosome is testis-determining (Sharman *et al.* 1970) and contains a homologue of the eutherian *SRY* gene (Foster *et al.* 1992). The fetal testis produces MIS (Hutson *et al.* 1988) and androgens (Renfree *et al.* 1992) which direct subsequent male development. However, unlike eutherians, the scrotum, mammary primordia, gubernaculum and processus vaginalis form before the differentiation of a testis independently of the presence of gonadal hormones (O *et al.* 1988). These differences are informative because they provide clues as to the functions of the genes and hormones controlling phenotypic sex.

This paper examines the similarities and differences in male sexual differentiation between marsupial and eutherian mammals. We will discuss the morphology of the gonad and associated ducts, and germ cell migration in the tammar wallaby, to identify aspects of special interest for marsupial studies. The relatively long developmental time course for the marsupial gonad, with most differentiation occurring post-natally, provides an excellent advantage to correlate morphological changes with gene expression. Similarly, the long time lag between the production of androgens and their action on target tissues in marsupials offers a unique opportunity to investigate the mechanisms mediating steroid hormone function.

2. DEVELOPMENT OF GONAD AND DUCTS

The marsupial neonate is altricial, and most sexual differentiation occurs post-natally. While the general sequence of gonadal and internal genital tract development in marsupials is very similar to that of eutherians, the mesonephros remains the functional

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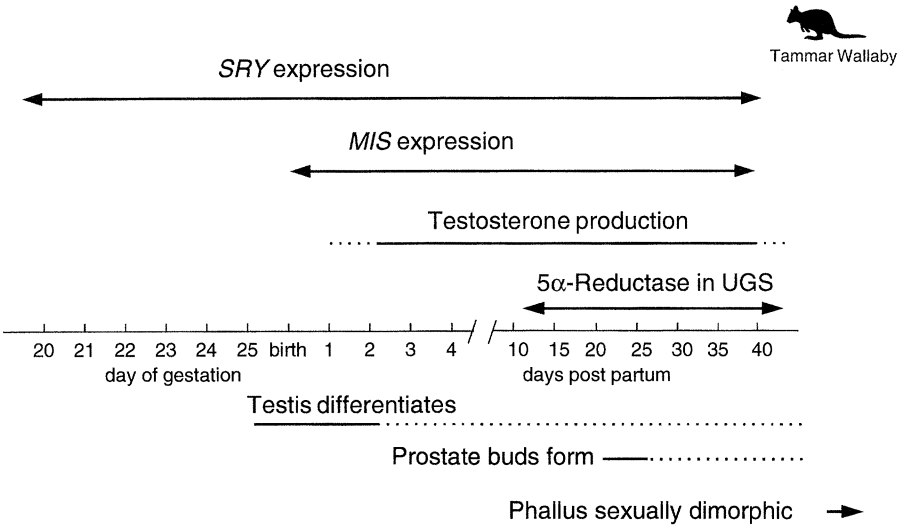

Figure 1. Summary of the key events and controlling factors in male sexual development of the tammar. Genes and hormones are shown above; morphological characters below.

kidney throughout the period of sexual differentiation, and this imposes major differences in the timing and development of the reproductive tract (figure 1). The marsupial urogenital system, like the gonad, develops from an indifferent stage at birth when both Wolffian and Müllerian ducts are present, to the phenotypically distinct male or female condition during early pouch life (figure 2). The Wolffian duct forms as the mesonephric duct, and is patent to the urogenital sinus about the time the indifferent genital ridge forms in the fetus (Renfree *et al.* 1995). In males the Wolffian duct develops into the epididymis and vas deferens. The Müllerian duct forms later in both males and females as an invagination on the cranio-lateral border of the mesonephros and in females develops into the Fallopian tubes, uterus and lateral vaginae.

The first sign of testis differentiation is the appearance of pre-Sertoli cells. Clearly developed seminiferous cords are obvious soon after birth in the tammar (Renfree *et al.* 1995) and the grey short-tailed opossum *Monodelphis domestica* (Moore & Thurstan 1990; Baker *et al.* 1990). The testis differentiates before the ovary in all six species of marsupial so far examined (reviewed in Renfree 1994). The testes of developing marsupials produce Müllerian inhibiting substance (MIS) (Hutson *et al.* 1988) and testosterone (George *et*

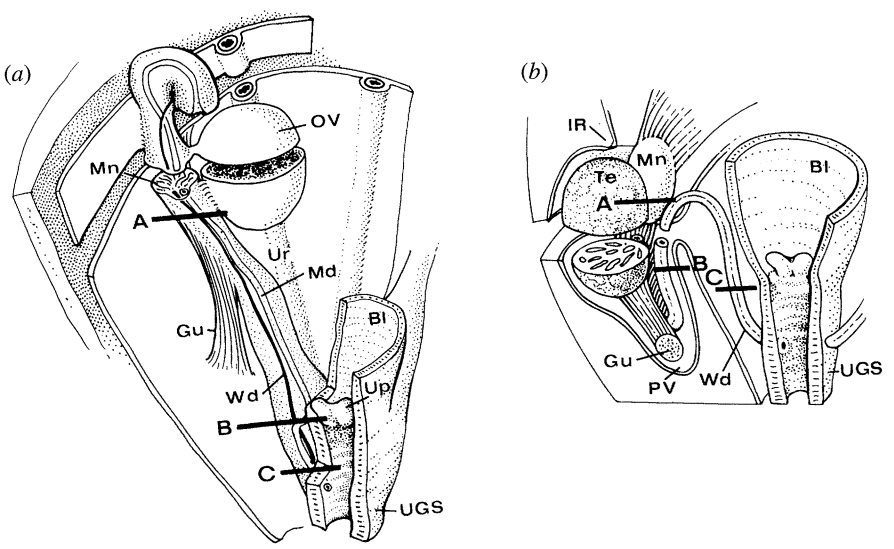

Figure 2. Reconstruction of the reproductive tract of a tammar female (*a*) and male (*b*) on day 25 of pouch life. (*a*) The Müllerian duct (Md) is well-developed throughout its length. The Wolffian duct (Wd) is regressing, particularly in its anterior regions, although it still connects with some remnant tubules of the mesonephros (Mn). The gubernaculum (Gu) terminates at the abdominal wall. The ureters (Ur) descend close to the dorsal abdominal wall before passing forward, between the Müllerian and Wolffian ducts, to enter the bladder (Bl) at its junction with the urogenital sinus as a distinct papillae (Up). (*b*) The testis (Te) has passed through the internal inguinal ring (IR). The Wolffian duct is well-developed. The Müllerian duct has regressed over most of its length, with small remnants present at the urogenital sinus (UGS) and near the remnant mesonephros. The gubernaculum passes along the processus vaginalis (PV) right into the scrotum. (From Shaw *et al.* 1988).

al. 1985; Renfree *et al.* 1992; Fadem & Harder 1992). MIS induces Müllerian duct regression (Hutson *et al.* 1988). Testosterone stimulates the Wolffian ducts to form the epididymis and vas deferens (Shaw *et al.* 1988). It is also necessary for the development of some male secondary sex characters such as the prostate, but there are some anomalies (see later section).

3. PRIMORDIAL GERM CELLS

Marsupials may be especially suitable for investigations of the origin and migration of primordial germ cells (PGCs) because the embryo develops on the surface of the vesicle, unencumbered by an egg cylinder as in the mouse. As yet, relatively few marsupials have been studied. In the bandicoots *Isoodon macrourus* and *Perameles nasuta* the PGCs are still migrating at birth (Ullmann 1978, 1981). In the tammar, migration occurs pre-natally and is essentially complete at birth (Alcorn 1975; Ullmann *et al.* 1995).

Marsupial PGCs are similar in appearance to those of other mammals (figure 3). They are large when compared with somatic cells, and have pale-staining cytoplasm containing characteristically spherical mitochondria. The cells are ovoid, sometimes pear-shaped and the cell membrane is usually difficult to see. The nuclear envelope is well defined. The nucleus appears vesicular, with finely beaded chromatin threads extending from the prominent nucleolus to the nuclear envelope, and may appear circular, subspherical or lobed in sections. At the ultrastructural level migrating PGCs may have pseudopod-like extensions (figure 3).

Primordial germ cells are positive for alkaline-phosphate activity in tammar wallaby embryos (Ullmann *et al.* 1995). At the earliest stages examined, in 10–15 somite embryos, 80–100 PGCs were found in the extraembryonic mesoderm, in the endoderm of the yolk sac, and in embryonic endoderm. The PGCs migrate to the gonad from the dorsal mesentery surrounding the hind gut adjacent to its entry into the cloaca. The majority of PGCs appear to migrate laterally to the posterior margins of the mesonephros, entering the gonadal ridges dorsally from the mesenchyme of the ventral mesonephric surface. The PGCs entering the posterior regions of the gonadal ridges then appear to migrate forward through the ridges. By the time of birth, around 12 500 PGCs have reached the gonads. This migration pathway, and the number of primordial germ cells observed in the tammar wallaby, agrees closely with that reported for eutherian mammals.

Migrating PGCs often occur in groups, rather than singly, suggesting that divisions are taking place rapidly during migration. Grouped, mitotic and mi-

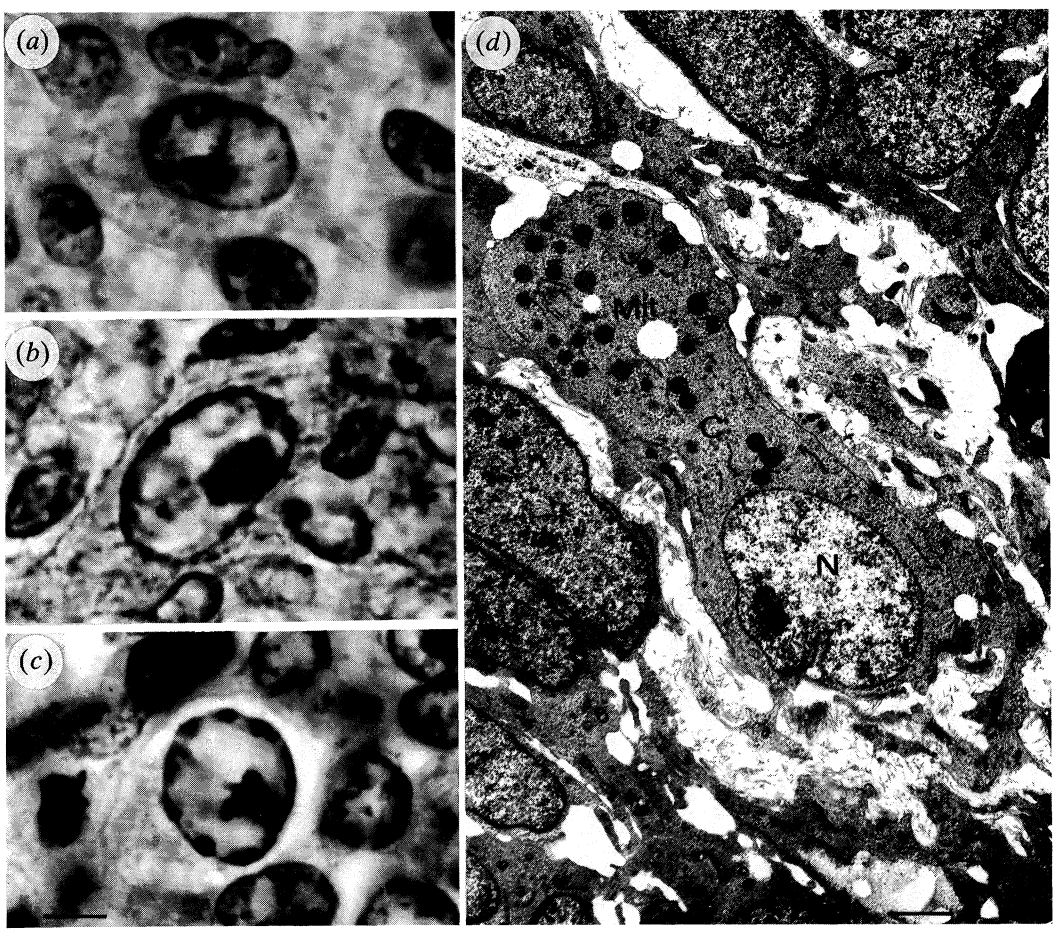

Figure 3. Germ cells from tammar wallaby fetuses: (*a*) d21 female, (*b*) d24 male, (*c*) d25 female (Scale bar = 5 μm), (*d*) Low power electron micrograph of a migrating PGC from a day-23 female fetus at the base of the developing gonadal ridge. Note the dumb-bell shape. Cytoplasmic polarization is notable and in particular the mitochondria are all distributed at the opposite pole from the nucleus. (Scale bar = 2.5 μm).

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grating PGCs also occur in the proximal region of the gonadal primordium in brushtail possums (Ullmann 1993). In the tammar, germ cells continue to multiply after birth in the female, and reach peak numbers in the gonad at about 50 days *post partum* (Alcorn & Robinson 1983).

4. MALE-DETERMINING GENES

(*a*) *SRY*

Marsupials, like eutherians, have an XX:XY sex determining mechanism (Hayman & Martin 1974). Development of a testis requires a Y chromosome (Sharman *et al.* 1970). Identification of a Y-specific homologue of the eutherian testis-determining gene *SRY* in species from two marsupial families (Foster *et al.* 1992) supports the hypothesis that *SRY* is the mammalian testis-determining factor. Fetal mouse *Sry* transcripts are found only in the somatic component of the male genital ridge between 10.5–12.5 days *post coitum* (Koopman *et al.* 1990) although there are reports of *Sry* transcripts detected in pre-implantation stages (Zwingman *et al.* 1993). The only *Sry* mRNA detected in the adult mouse is a circular, non-polyadenylated (and presumably inactive) transcript in the testis (Capel *et al.* 1993).

As the first indication of testis differentiation is the appearance of Sertoli cells and as they appear during this period of *Sry* expression, it is assumed that *Sry* directs the differentiation of Sertoli cells from the supporting cell lineage (Lovell-Badge 1993). In the tammar wallaby the time course of gonadal differentiation (figure 1) is much longer than in the mouse, making it possible to assess more critically the correlation of *SRY* expression with specific stages of testis differentiation. *SRY*, assessed by RT-PCR, is transcribed not only in the male tammar gonad throughout the period of testicular differentiation, but also at every stage examined for the male foetus from before genital ridge formation until at least 40 days after birth (Harry *et al.* 1995). *SRY* is also expressed in a variety of extra-gonadal male tissues (Harry *et al.* 1995). This is strikingly different to the transient and tissue-specific expression of SRY in the mouse.

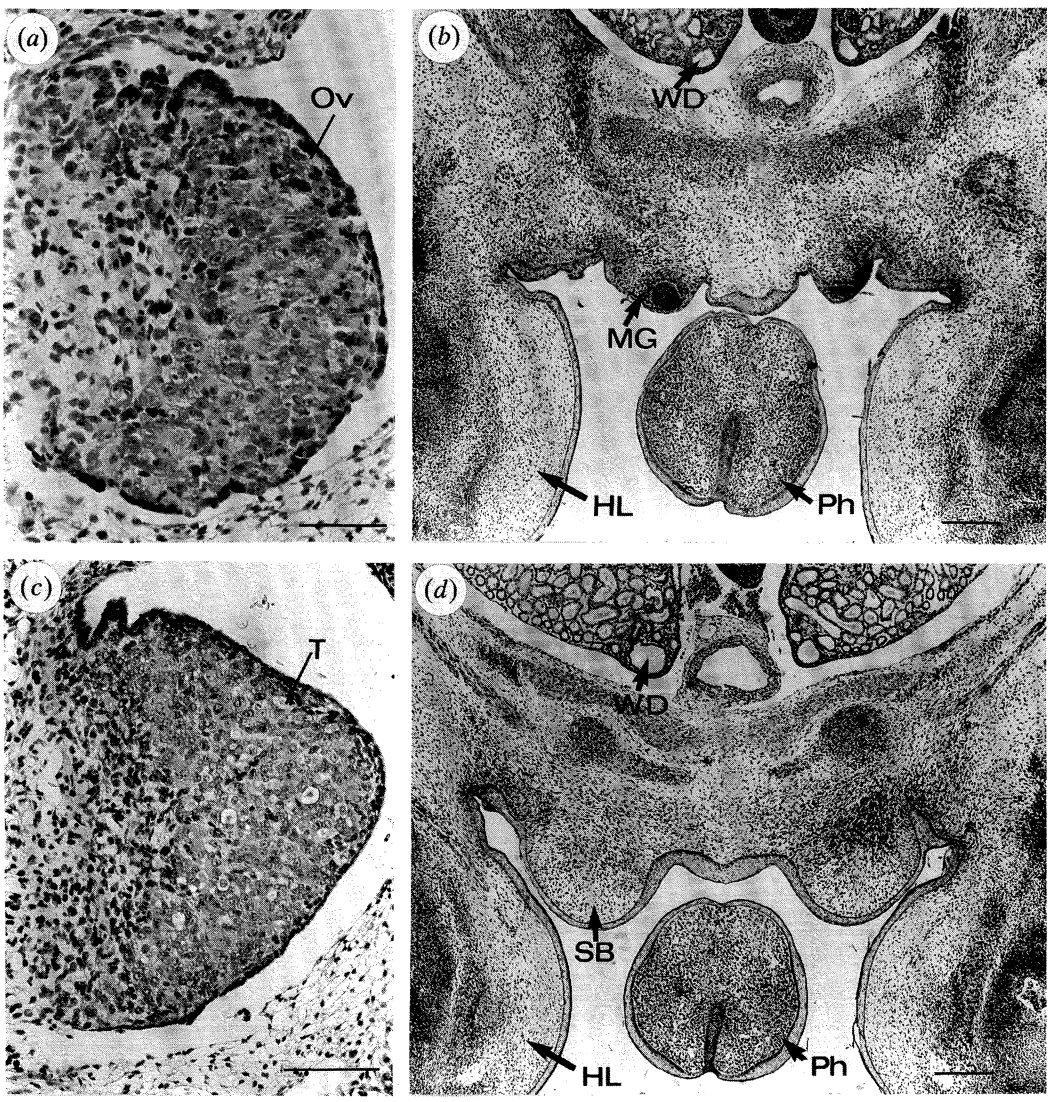

Figure 4. Histology of the ovary, testis, mammary gland and scrotal bulges in female (*a*, *b*) and male (*c*, *d*) day-25 tammar wallaby fetuses. (*a*) Ovary, (*b*) ventral abdominal wall (*c*) testis, (*d*) ventral abdominal wall. Ov, ovary; T, testis; MG, mammary gland; Ph, phallus; SB, scrotal bulges; HL, hindlimb. Scale bar = 100 μm.

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Because this Y-linked gene is transcribed in the male tammar genital ridge during the time that seminiferous tubules form, it is reasonable to assume that the marsupial *SRY* gene plays a role in testis determination, although there is no direct evidence so far. However, the long period of *SRY* transcription in a wide range of extra-gonadal tissues in tammars is intriguing. In the human, where mutational analysis has confirmed that the human *SRY* gene is testis-determining (Berta *et al.* 1990; Jager *et al.* 1990), *SRY* transcripts have been found in a range of tissues in late embryos and adults (Clepet *et al.* 1993). These observations suggest that *SRY* may have a more general function in addition to testis determination in marsupials and humans. This is difficult to reconcile with the normal female phenotype of human XY individuals with mutations in *SRY* but several explanations can be considered. One possibility is that *SRY* transcripts are not translated into functional gene products in extra-gonadal tissues, although functional SRY protein is produced at sufficient levels in the fetal testis. Alternatively, the general role of SRY may be complemented by the function of a related gene in females. A candidate is *SOX3*, which may be the ancestral gene from which *SRY* evolved because it maps to a conserved region of the mammalian X chromosome and shows strong similarity to the sequence encoding the DNA-binding domain of SRY (Foster & Graves 1994). A third possibility is that co-factors or activating proteins are required for the testis-determining function of SRY and that the production of such factors is restricted to the gonadal ridge.

(*b*) *MIS*

The *SRY* gene is believed to act as a transcription factor because part of its sequence encodes a DNA-binding domain (Sinclair *et al.* 1990). It has been speculated that SRY may interact with *MIS*, the first product known to be produced by Sertoli cells of the developing testis. SRY binds to sequences in the rat *MIS* promoter and is able to drive MIS production in a gonadal ridge cell line (Haqq *et al.* 1994). However, mutations in the SRY-binding region of the *MIS* promoter did not diminish the *in vitro* production of MIS (Haqq *et al.* 1994) and tissue *in situ* hybridization studies (Munsterberg & Lovell-Badge 1991) of mouse established that there is no overlap between *Sry* and *Mis* expression profiles. These observations suggest that *MIS* is not directly regulated by SRY, but that intervening transcription factors are required. By contrast, there is considerable overlap between *SRY* and *MIS* expression in the developing tammar testis. A bioassay using tammar testis showed that functional MIS is produced from day 2 *post partum* until at least day 80 (Hutson *et al.* 1988) (figure 5). *In situ* hybridization studies have shown that *MIS* is expressed exclusively by Sertoli cells of the differentiating testis from as early as the day of birth (Whitworth *et al.* 1994; Shaw *et al.* 1995). This is two days earlier than the equivalent stages of testis differentiation in the mouse, before the appearance of distinct seminiferous tubules (figure 1). There is no evidence of MIS production in the developing ovary.

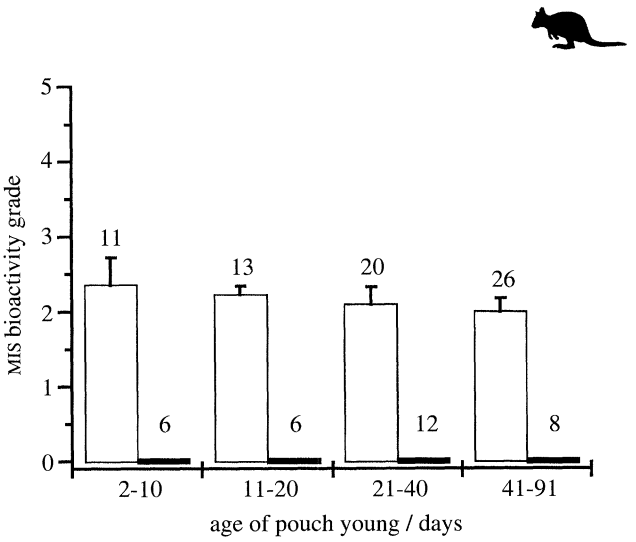

Figure 5. MIS bioactivity in developing tammar gonads between days 2 and 91 *post partum*. The numbers of gonad pools from male pouch young (open bars) and female pouch young (black bars). Data from Hutson *et al.* (1988).

Besides the role of MIS to induce Müllerian duct regression, MIS can promote testicular morphogenesis. As in the rat, where MIS induces the formation of seminiferous cord-like structures in fetal ovaries cultured *in vitro* (Vigier *et al.* 1987), neonatal tammar ovaries cultured with MIS develop seminiferous-like cords (Whitworth & Renfree 1994; Shaw *et al.* 1995). Similarly, ovaries from neonatal females transplanted under the skin of 10-day-old male pouch young develop cord-like structures. Although these data are consistent with the idea that MIS may induce testicular differentiation, transgenic male mice deficient in *MIS* still differentiate their testes (Behringer *et al.* 1994). Some of these MIS-deficient mice had descended testes and produced sperm, although they were not normal since all displayed Leydig cell hyperplasia.

(*c*) *The X factor*

There is substantial evidence that some sexually dimorphic features, such as the scrotum, the mammary primordia, the pouch, the gubernaculum and the processus vaginalis are hormone-independent. In the tammar, scrotal bulges only develop in genetic males, and primordia of the mammary glands only develop in genetic females. Both structures differentiate several days before birth and well before the gonadal primordia differentiate into testes or ovaries (Alcorn 1975; O *et al.* 1988; Renfree *et al.* 1995) and are unaffected by exogenous steroids (Shaw *et al.* 1988) (figure 4). Likewise, neonatal males of the marsupial mouse *Antechinus* (Bolton 1983), the American opossums (Renfree *et al.* 1990) and the brushtailed possum (Ullmann 1993) have scrotal bulges visible on or before the day of birth. Interestingly there is a dichotomy between male Australian and American marsupials in that mammary primordia are never present in the former, whereas a reduced number occur in fetuses and pouch young of the latter (Renfree *et al.* 1990; Robinson *et al.* 1991).

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Table 1. *Comparison of the phenotypes of XO and XXY mammals*

	eutherian	marsupial
Turner's syndrome XO		
gonads	streak ovaries	streak ovaries
internal phenotype	female	female
external phenotype	no scrotum	scrotum
Kleinfelter's syndrome XXY		
gonads	testes	testes (intra-abdominal)
internal phenotype	male	male
external phenotype	penis	penis
	scrotum	no scrotum pouch and mammary glands

In intersexual marsupials XXY male individuals have a pouch and mammary glands, but no scrotum, while XO individuals have an empty scrotum and no pouch or mammary glands (Cooper *et al.* 1977, 1990; Sharman *et al.* 1990) (table 1). These observations, together with the existence of bilateral mosaic tammars with a hemi-pouch and a hemi-scrotum suggest that the pouch and scrotum are developmental alternatives regulated by an X-chromosome switch (Cooper 1993). It has been suggested that this may be a dosage effect, where one X chromosome is activated and the other partly reactivated (Cooper *et al.* 1977; Renfree & Short 1988; Sharman *et al.* 1990). Alternatively, a hypothesis implicating parental imprinting has been proposed whereby the development of a scrotum is dependent on the presence of a maternal X and the pouch/mammary analgen upon the presence of a dominant paternal X chromosome (Cooper 1993). Identification of the gene system controlling this aspect of marsupial sexual differentiation may provide clues to the mechanisms regulating sexual dimorphisms preceding gonadal differentiation in eutherians.

We now know that in eutherian mammals there can be sexual dimorphisms before formation of the gonads. In a wide variety of species, male embryos tend to be larger and more developmentally advanced than females at a given stage after conception (mice: Tsunoda *et al.* 1985; cattle: Itoh & Goto 1986). These subtle developmental sex differences have been linked to both X- and Y-linked factors in different strains of mice (Burgoyne & Thornhill 1993).

5. ANDROGENS AND VIRILIZATION

Testosterone is the principal gonadal androgen produced during marsupial sexual differentiation (Renfree *et al.* 1992; Fadem & Harder 1992). In the tammar, gonadal testosterone levels are low in male and female gonads at birth but in males they rise between day 2 and day 10 with the formation of seminiferous tubules (Renfree *et al.* 1992) (figure 6). Testosterone levels remain high in the testis until after day 40, by which time sexual differentiation of the internal genitalia is essentially complete.

Androgens regulate differentiation of the Wolffian duct and urogenital sinus in a manner similar to that in eutherians. However, there are some intriguing differences in the timing of events. Prostatic buds are first visible in the urogenital sinus about day 25 *post partum*, despite the presence of high levels of gonadal

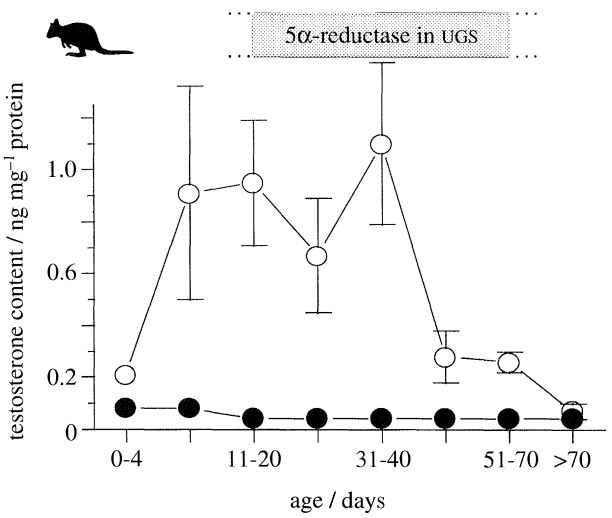

Figure 6. Testosterone content of the testis (○) and ovary (●) in the first 80 days of pouch life. The urogenital sinus and urogenital tubercle contain high levels of 5α-reductase activity at least as early as day 10, but the levels in the scrotum never increase over that of control tissues (data from Renfree *et al.* 1992).

testosterone during the preceding three weeks (figures 1 and 7). Daily treatment of female neonates with testosterone from birth to day 25 induces the formation of prostatic buds, but since these are similar to those of control males the prolonged treatment does not enhance development (Shaw *et al.* 1988). Treatment of male pouch young with flutamide, an androgen-receptor blocker, from day 20 to 45 dramatically reduced prostatic development compared to control untreated males (Butler *et al.* 1995). These experiments suggest that androgen is acting to induce prostate development in a narrow window between days 20 and 25 (figure 7).

The phallus is also androgen-dependent, but it becomes sexually dimorphic only after day 60, by which time testicular testosterone levels have declined (figure 6). Tyndale-Biscoe & Hinds (1989) removed testes from day-10 male pouch young and grafted these into similar-aged female pouch young. The castrated males did not develop a phallus whereas the female pouch young with testicular grafts developed a penis.

In eutherian mammals low levels of circulating testosterone are converted in the urogenital sinus and phallus to the more potent androgen 5α-dihydrotestosterone (DHT), and defects in the 5α-reductase gene interfere with normal male sexual differentiation.

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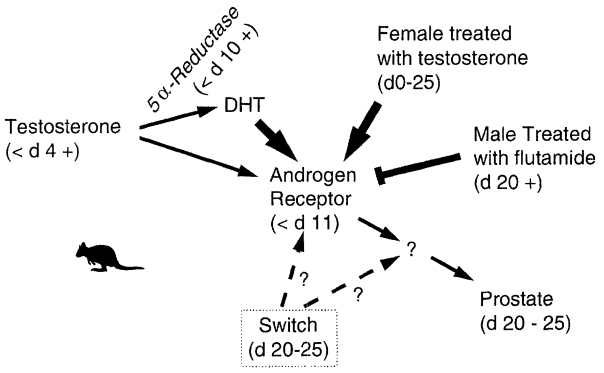

Figure 7. A model for virilization of the urogenital sinus in tammar pouch young. Testicular testosterone is converted to the more potent androgen DHT, which binds to the receptor and leads to prostate formation, possibly through intermediate steps. Large doses of exogenous steroid induce prostate formation in females, but do not advance the development compared to males. Blocking the androgen receptor from day 20 inhibits prostate formation suggesting that a switch that triggers prostate formation acts in the day 20–25 period, perhaps by direct or indirct activation of the androgen receptors.

However, in tammars the delay in virilization of the urogenital sinus and phallus cannot be due to lack of DHT, because the urogenital sinus and phallus both contain 5α-reductase in high levels by day 10–11 (Renfree *et al.* 1992) (figure 6).

Clearly low androgen levels cannot account for the delay in virilization of the prostate and phallus. It is possible that these events are controlled by expression or activation of the androgen receptor, but preliminary studies suggest that androgen receptor is present in the urogenital sinus from at least day 10 (Butler *et al.* 1995). Thus, events downstream of the androgen receptor may be the limiting factor (figure 7).

6. CONCLUSIONS AND PERSPECTIVES

Sexual differentiation in marsupial mammals is fundamentally similar to eutherians, reflecting their common evolutionary origins, but the differences that exist have made us question some of the paradigms of sex determination and differentiation in eutherians. The process of the marsupial urogenital duct development is exactly the same as the more familiar eutherian one, but there are significant differences in the control of the formation of four structures, namely the scrotum, mammary glands (and subsequently the pouch), the gubernaculum and processus vaginalis.

The scrotum of the marsupial, which arises from the abdominal cavity, is not homologous with the eutherian scrotum, which has its origins in the labioscrotal swellings. The scrotum is not androgen-dependent as in eutherians. Although the earliest marsupials are generally regarded as pouchless, as are many extant marsupials (Renfree 1993), the apparent origin of the pouch and scrotum from different primordia in the same morphogenetic field in marsupials (Robinson *et al.* 1991; Renfree 1994) may imply that their development is controlled by a single switch mechanism (Cooper 1993). Cytogenetic data (Sharman *et al.* 1970; Cooper *et al.* 1977, 1990) implicate the X chromosome in this switch, and hypotheses of gene dosage and parental imprinting have been proposed (Cooper 1993). The fact that there are marsupial genes which act before the gonad has formed is important in showing that there is more than one switch in male sexual determination.

The developmental profile of *SRY* expression has only been examined in two species to date, the mouse (Koopman *et al.* 1990) and the tammar (Harry *et al.* 1995), and the profiles are very different. It is interesting that widespread expression of the *SRY* transcript in tammars parallels the pattern reported for extra-gonadal tissues from the human fetus and adult (Clepet *et al.* 1993). Further characterization of the marsupial *SRY* gene and its product is likely to provide valuable information concerning the evolution and function of the *SRY* gene in all mammals. As the expression patterns of *SRY* and *MIS* overlap during the early stages of testicular differentiation, the tammar will be a useful system for examining the transcription profiles of genes encoding potential intervening factors. The late onset of virilization of the prostate and phallus in the face of apparently sufficient levels of testosterone, 5α-reductase and the androgen receptor is enigmatic, but suggests that a critical developmental switch operates between activation of the androgen receptor

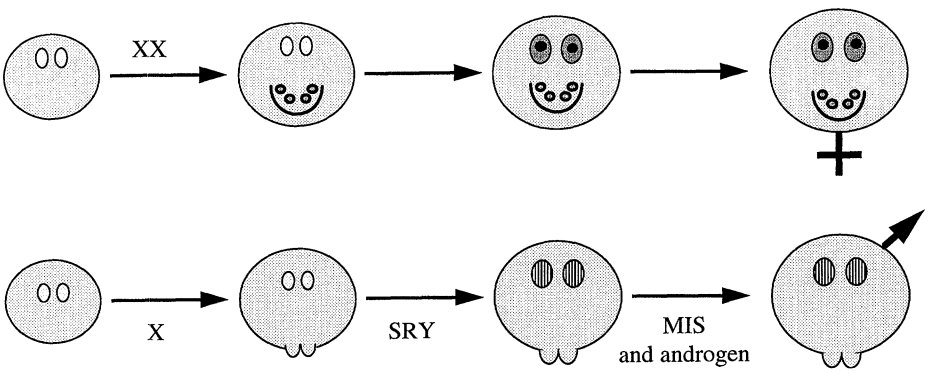

Figure 8. Sequential steps in sexual differentiation in tammars. Development in males and females is similar up to an early fetal stage. Then an X-linked factor induces mammary gland buds to form in XX females, whilst scrotal bulges develop in XY males. As the gonadal ridge develops, *SRY* acts in males to induce testicular differentiation about the time of birth. The testis rapidly commences production of MIS and testosterone, which drive subsequent male differentiation.

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and initiation of prostatic morphogenesis. Much remains to be learnt about the interactions of genes, hormones and receptors in the process of sexual differentiation, and it is clear that further study of marsupial mammals will continue to surprise.

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Discussion

N. Josso (*Ecole Normale Supérieure, Département de Biologie, 1 rue Maurice Arnoux–92120 Montrouge, France*). In relation to the detection of AMH by Western blot in homogenates (?) of mesonephroi, which antibodies were used and were the results of *in situ* hybridization also positive?

In relation to the late onset of phallic growth in marsupials, I would like to remind you that, in human fetuses also, the phallus grows essentially during the last trimester, at a time when testosterone levels in males are very low and may even overlap with values observed in females (Feldman & Smith 1975 *J. Pediatr.* **86**, 395).

M. B. Renfree. The antibodies used for the Western blots of the mesonephros and gonad were the mouse anti-bovine monoclonal antibody provided by Professor John Hutson, Royal Children's Hospital, Melbourne. Similar results were obtained from a rabbit anti-human recombinant MIS polyclonal antibody provided by Professor Pat Donahoe of Massachusetts General Hospital, Boston. Both antibodies recognized a 70 kDa band in testis, and male and female mesonephros, but not in ovary. The *in situ* hybridization also showed positive results in the mesonephros as well as in the gonad. The riboprobe for the *in situ* is generated from a 444 b.p. insert of rat MIS genomic DNA from the carboxy terminus as template, also kindly provided by Professor Donahoe.

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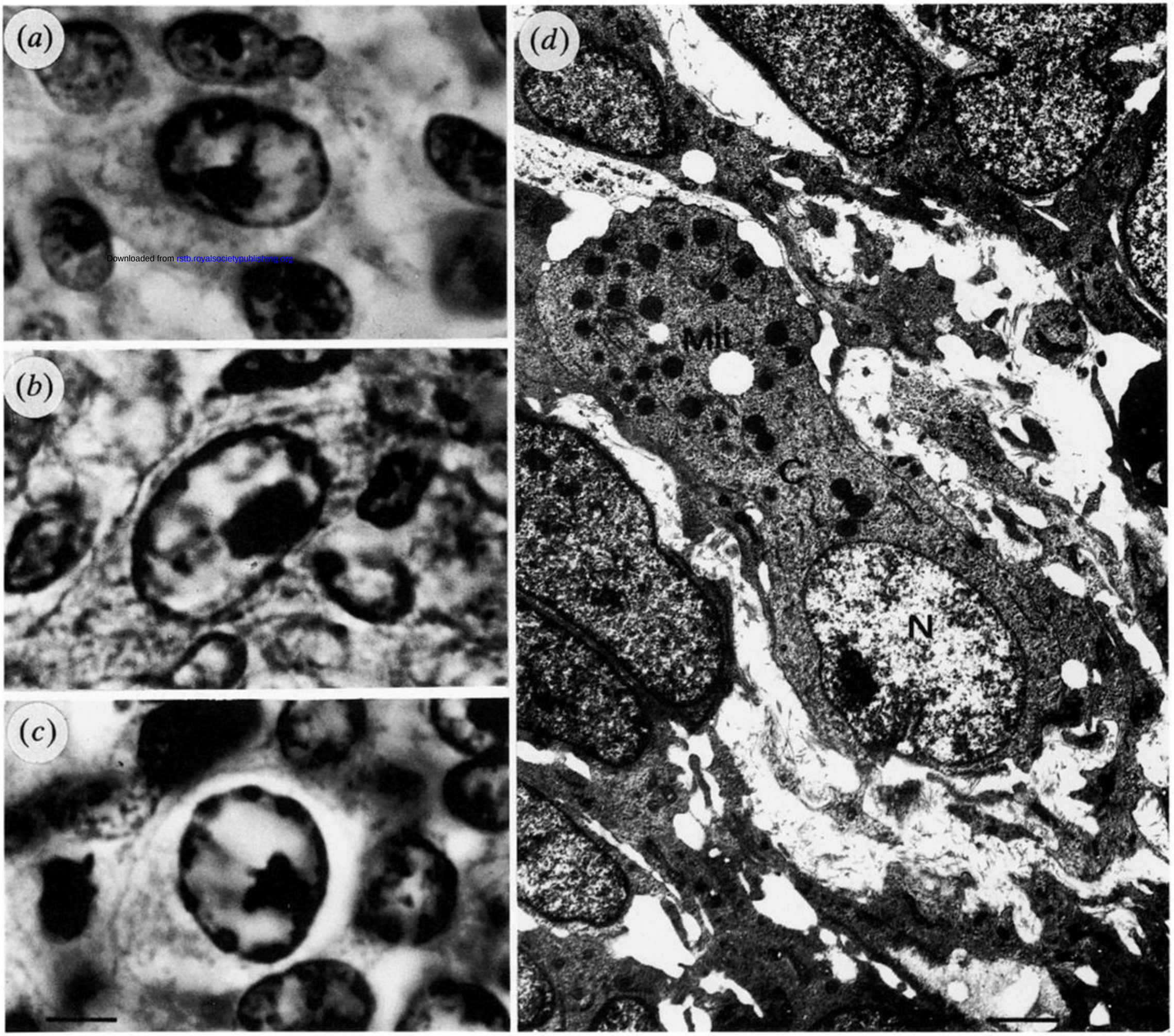
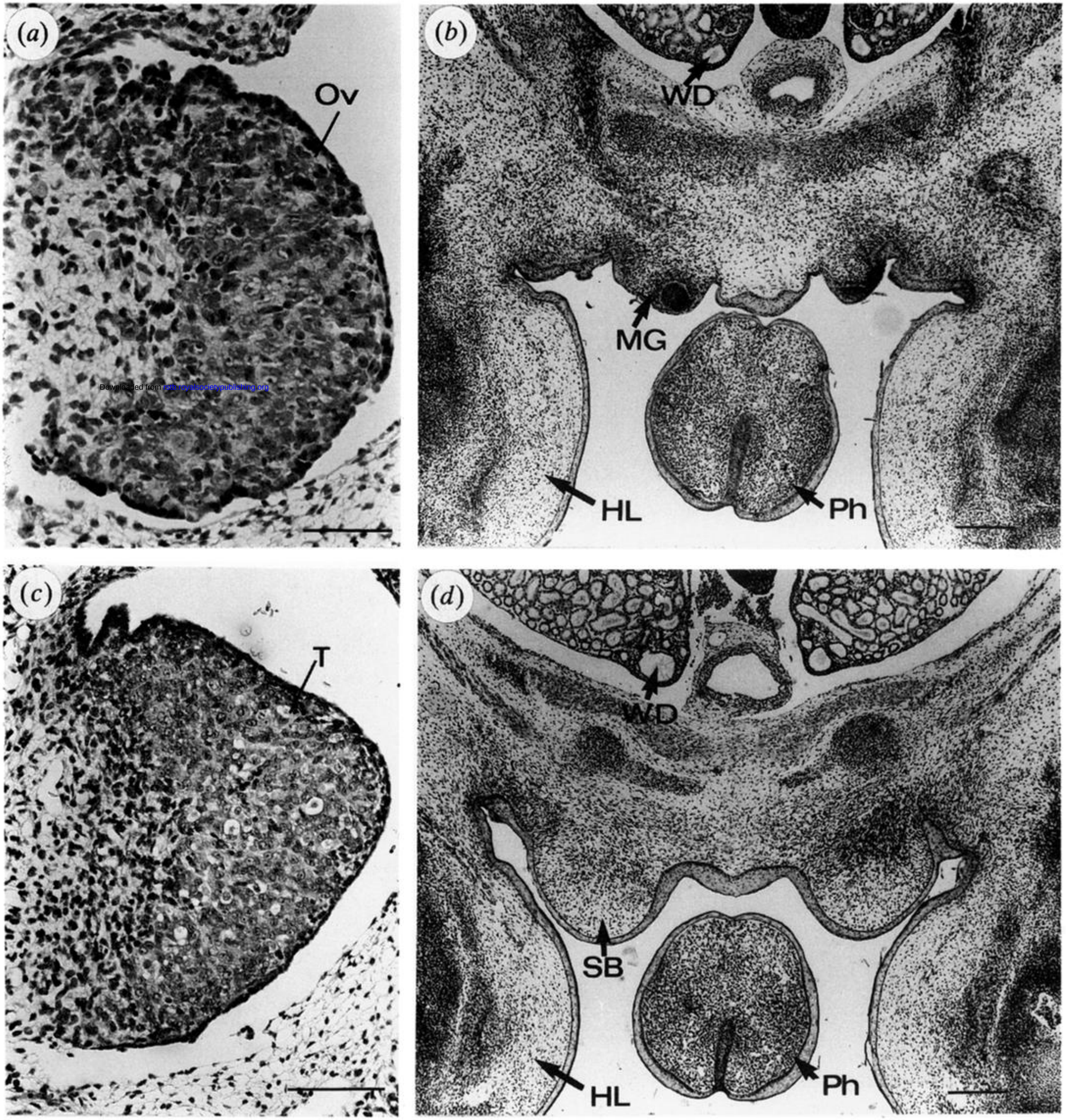


gure 3. Germ cells from tammar wallaby fetuses: (*a*) d21 female, (*b*) d24 male, (*c*) d25 female (Scale bar = 5 μm),) Low power electron micrograph of a migrating PGC from a day-23 female fetus at the base of the developing nadal ridge. Note the dumb-bell shape. Cytoplasmic polarization is notable and in particular the mitochondria e all distributed at the opposite pole from the nucleus. (Scale bar = 2.5 μm).



gure 4. Histology of the ovary, testis, mammary gland and scrotal bulges in female (*a*, *b*) and male (*c*, *d*) day-25 mmar wallaby fetuses. (*a*) Ovary, (*b*) ventral abdominal wall (*c*) testis, (*d*) ventral abdominal wall. Ov, ovary; T, stis; MG, mammary gland; Ph, phallus; SB, scrotal bulges; HL, hindlimb. Scale bar = 100 μm.