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Inducing sex reversal of the urogenital system of marsupials

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ABSTRACT

Marsupials differ from eutherian mammals in their reproductive strategy of delivering a highly altricial young after a short gestation. The young, with its undeveloped organ systems completes its development post-natally, usually within a pouch. The young is dependent on milk with a composition that varies through lactation to support its growth and changing needs as it matures over a lengthy period. Gonadal differentiation occurs after birth, providing a unique opportunity to examine the effects of hormonal manipulations on its sexual differentiation of the highly accessible young. In marsupials a difference in the migration of the urinary ducts around the genital ducts from eutherian mammals results in the unique tammar reproductive tract which has three vaginae and two cervices, and two distinctly separate uteri. In the tammar wallaby, a small member of the kangaroo family, we showed that virilisation of the Wolffian duct, prostate and phallus depends on an alternate androgen pathway, which has now been shown to be important for virilisation in humans. Through hormonal manipulations over differing time periods we have achieved sex reversal of both ovaries and testes, germ cells, genital ducts, prostate and phallus. Whilst we understand many of the mechanisms behind sexual differentiation there are still many lessons to be learned from understanding how sex reversal is achieved by using a model such as the tammar wallaby. This will help guide investigations into the major questions of how and why sex determination is achieved in other species. This review discusses the control and development of the marsupial urogenital system, largely drawn from our studies in the tammar wallaby and our ability to manipulate this system to induce sex reversal.

1. Introduction

Australia's marsupial mammals diverged from eutherian mammals over 160 million years, during which time they have evolved independently but in parallel with the eutherian mammals (Luo et al., 2011). The common ancestor probably had a fixed pelvic girdle (Qiang et al., 1999) so it is likely that after the divergence, eutherian mammals have evolved extended gestation and birth of relatively large young, whilst marsupials have retained an ancestral pattern of tiny, altricial young. As a result of these two evolutionary pathways, there are striking differences in reproduction between these two groups of mammal. The urogenital system of marsupials has many unique features: females have three vaginae, two completely separate uteri, and in males the scrotum is cranial to the penis. In many, but not all species, females have a pouch, and males of many, but not all species, have a bifid penis. Marsupials have a single urogenital opening (UGO) which is identical in appearance in both males and females. Within this opening are the openings of the gut and the genital ducts, each closed by its own tight sphincter, so the UGO is not a cloaca (Renfree, 1993; Renfree et al., 1992).

The differences in the development of the urogenital ducts in the embryo had profound effects on the anatomical arrangements of the male and female reproductive tracts. This review will describe how some of these unusual features come about during early development and how they may be altered experimentally to shed light on the controlling processes, drawing largely on our studies of the tammar wallaby, *Macropus eugenii*.

2. Reproductive anatomy

2.1. The indifferent urogenital tract

The origin of the marsupial tripartite vagina is the result of a simple developmental difference in the pathway of migration of the urinary ducts around the genital ducts. In all mammals, the urinary and genital ducts arise closely together. The Wolffian (mesonephric) ducts become the epididymis and vas, and the Müllerian (paramesonephric) ducts become the oviducts, uterus and cranial part of the vagina. These two parallel ducts are initially located on the lateral sides of the mesonephroi, whilst the metanephric blastema buds off from the dorsal side of the caudal Wolffian duct. In monotremes, the

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ureters open into the urogenital sinus opposite the bladder (Griffiths, 1978), but in both groups of therian mammals the ureters migrate from the dorsal position in the early fetus to make a direct ventral connection with the bladder (Fig. 1). In eutherians the ureters enter the bladder laterally so that it is possible for the Müllerian ducts to fuse into a single midline upper vagina and cervix, and uterine body (though in many species the upper uterus forms two uterine horns). In marsupials the ureters migrate medially to the bladder, between the Müllerian ducts. This effectively prevents fusion of the caudal section of the Müllerian ducts. As a result, female marsupials have a complex reproductive system, with paired lateral vaginae, two cervices and two completely independent uteri. There is a midline fusion of the upper vagina to form the anterior vaginal expansion, and a new midline connective tissue strand connects this to the urogenital sinus. This median vagina, lying between the lateral vaginae, becomes patent before the first birth and serves as the birth canal (Fig. 1). There are also differences between eutherians and marsupials in males, as a consequence of the differences in how the ureter reaches the bladder. In eutherians, after testicular descent, the vasa deferentia loop over the ureters, but in marsupials the vasa are lateral to the ureters, so in males it is eutherians that have the more complex duct arrangements.

2.2. Pouch and scrotum

The most notable characteristic of marsupials, that gives the group its name, is the pouch. However, not all marsupials have a pouch. No male marsupial has a pouch, and pouches are also

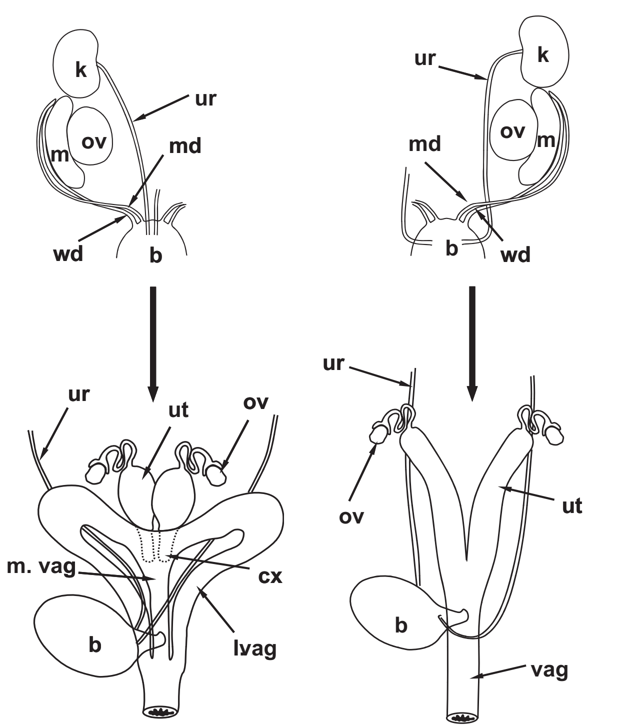

Fig. 1. Position of the genital and urinary ducts in the marsupial and eutherians. The position of the ureters dictates the development and structure of the female reproductive tract in the marsupial (left). The marsupial (as exemplified here by the tammar tract) has two lateral and a median vaginae with the ureters connecting to the bladder medially to the Mullerian and Wolffian ducts, thus preventing fusion of the two separated lateral vaginal canals. As a result, the marsupial tract also has two completely separate uteri and two cervical openings each protruding into the median vagina. In contrast, in the eutherian (right), the ureters migrate laterally in relation to the Mullerian and Wolffian duct. As a consequence the Mullerian ducts can fuse in the midline to form a single vagina and two uterine horns. b: bladder, cx: cervix, k: kidney, m. vag: median vaginae; Mullerian duct, m; Ov: ovary, m; mesonephros, ur: ureter ut: uterus, vag: vagina and wd: Wolffian ducts (Redrawn from Renfree, 1993).

absent in females of some didelphids, caenolestids (South American) and dasyurids (Australian). In addition, the scrotum is cranial to the penis in marsupials, and has a different mode of formation to that in eutherians. In eutherians the scrotum differentiates through fusion of the labio-scrotal folds caudal to the phallus in response to androgens. In marsupials the scrotum forms on the abdomen anterior to the phallus. The pouch and scrotum are probably not homologous to each other but arise from adjacent morphogenetic fields, and their differentiation depends on a gene or genes on the X-chromosome completely independent of gonadal hormones (O et al., 1988; Renfree et al., in press; Renfree and Short, 1988; Shaw et al., 1988). This means that pouch young can be sexed before gonadal differentiation by the presence of scrotal bulges (only males) or mammary primordia (only females) (O et al., 1988).

2.3. Gonads and germ cells

The testes and ovaries of both marsupial males and females are typically mammalian. The spermatozoa undergo maturation as they pass through the caput and corpus epididymis to the cauda epididymis where they are stored before release via the vas deferens. In females ovulated eggs pass into the oviduct where the egg is fertilised and reach the uterus in 24 h (Tyndale-Biscoe and Renfree, 1987). In the American marsupials, with the exception of one species, *Dromiciops*, the spermatozoa form into pairs in the epididymis by intimate association of the acrosomes. Sperm pairs separate in the oviduct and fertilisation is achieved by a single sperm (Tyndale-Biscoe and Renfree, 1987).

Testosterone, dihydrotestosterone, progesterone and oestradiol are key reproductive steroid hormones in monotremes, marsupials and eutherian mammals. In tammar wallabies, testicular testosterone content increases immediately after birth, and remains high until about day 45 post-partum (p.p.) and there is no measurable testosterone in females at this time (Renfree et al., 1992). Despite this, there is no difference in plasma testosterone or DHT between males and females during the period when androgen dependent sexual dimorphisms develop. The answer to this conundrum was our discovery of a new pathway of androgen biosynthesis (now known to operate in developing humans) involving 5α-reduction of progesterone or 17-hydroxy-progesterone with 5α-androstane-3α,17β-diol (adiol) being the key circulating androgen, which is converted in the target organs to the active androgen dihydrotestosterone (DHT) by 3α-hydroxysteroid dehydrogenase (Shaw et al., 2000; Wilson et al., 2003). Plasma adiol levels mirror the changes in testicular testosterone concentration during the period of sexual differentiation, and appears to fall at around the same time (Leihy et al., 2004; Wilson et al., 2003).Virilisation of the Wolffian ducts (WD), the urogenital sinus/prostate (UGS) are all driven by adiol (Wilson et al., 2002, 2003).

In the tammar wallaby the gonads arise as epithelial thickenings on the mediolateral aspect of the mesonephros by day 21 of the 26 day gestation (Fig. 2). However the somatic cell lineages of the testis and ovary do not undergo sexual differentiation until days 2 and 8 post-partum respectively (Renfree et al., 1996). Thus the male and female gonads appear to be identical on the day of birth (Fig. 2). One advantage of the marsupial model is that development of the gonad takes place over many days (as in humans) compared to the rapid and condensed development seen in mouse gonads. Most importantly, the development of the somatic cell lineages and germ cell entry into either meiotic or mitotic arrest occur after birth and are separated by weeks, whereas in the mouse these events occur concurrently (Harry et al., 1995; Renfree et al., 1996). The germ cells of the developing ovary begin to enter meiosis at 25 days p.p., at which time the somatic cells of the ovary are well defined (Alcorn and Robinson,