

MASCULINIZATION OF FEMALE MAMMALS: LESSONS FROM NATURE

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INTRODUCTION

Conventional understanding of mammalian sexual differentiation, first proposed by Alfred Jost in the late 1940's and early 1950's, requires the presence of androgens acting during a critical stage of fetal (or neonatal) life for normal development of the masculine penis and scrotum from embryonic primordia (Jost, 1953; Wilson *et al.*, 1981). Postnatally, a marked acceleration in growth of the penis is typically associated with increased secretion of androgen during puberty (at least in primates).

But, the preceding generalizations are based on the study of a relatively small number of species. There is a substantial cluster of mammal species in which all females display some masculinization of the external genitalia. In many ways, this natural masculinization of the female external genitalia is reminiscent of several human pathological conditions. The degree of virilization varies across species much as it does in several disorders of sexual development. For example, a cohort of girls born with congenital adrenal hyperplasia (CAH), owing to a deficiency in the steroidogenic enzyme 21-hydroxylase, may present with ambiguous genitalia that can vary from essentially feminine to almost completely masculine. Across species we witness the same degree of variability, from the isolated clitoromegaly of bonobos and spider monkeys (Wislocki, 1936), to the hypospadiac penile clitoris of various lemurs (Hill, 1958), to the extreme masculinization of female spotted hyenas (Matthews, 1939). This continuum of naturally masculinized female mammals may represent a set of useful animal models, whereby studies of females may provide insights into the ontogeny of male disorders of sexual differentiation.

The existence of such "masculine" clitoral development could be reconciled with the conventional theory, if: (a) androgens were found to circulate during fetal life with the requisite timing, and (b) interruption of androgenic activity during such critical periods resulted in a clitoris of reduced size, that was not traversed by a urethral canal. Moreover, since females in all of the preceding species, save for the spotted hyena, retain an external vaginal opening (not a scrotum), delicacy of timing for circulating androgens, or some odd distribution of androgen receptors may be required to account for selective "masculinization." To date, there is no direct evidence bearing on clitoral development in species characterized by natural masculinization. Although that can be attributed to gaps in the research literature, it is also possible that novel non-androgenic mechanisms are at work. The latter will not be found if they are not sought.

The present chapter is focused on two examples of natural masculinization in female mammals: (1) moles, which display a penile clitoris, but retain a distal vagina separate from the urethra, and (2) the spotted hyena, which is the only extant mammalian species with both a penile clitoris and a pseudoscrotum, but no distal vagina. Development of the female external genitalia in eutherian mammals has generally been considered a passive event, occurring by default in the absence of potent androgens, specifically 5 α -dihydrotestosterone (DHT). A corollary to this assumption is that any

masculinization of the female genitalia must require an alternate source of androgen. In each case, our research trail begins with a search for naturally circulating androgens in a female mammal. Our observations in moles and spotted hyenas raise important questions with regards to the conventional theory of sexual differentiation.

MOLES

Background

The layman rarely cares if the mole destroying his garden or lawn is male or female, and he probably couldn't tell if he did. Most, if not all species of mole belonging to the mammalian family Talpidae are difficult to sex, especially outside of the breeding season. In several of the species studied to date, females have a peniform clitoris, which is traversed by the urethra through most or all of its length. The vagina is also completely occluded prior to sexual maturity and during the nonbreeding season. In males, testes are intra-abdominal throughout the year, thus depriving individuals of another important clue for sexing moles. Scientific investigators frequently rely on internal inspection of the reproductive organs to definitively sex moles. Upon such inspections, some interesting findings have been described that add further to this unusual story.

The European mole (*Talpa europaea*) and several other related species in the genus *Talpa* are remarkable in that all females carry 'ovotestes', bipolar gonads that display discrete features of both ovaries and testes. This nomenclature has been considered by some to be inaccurate, as the 'ovotestes' do not exhibit any spermatogenic function, thus warranting the simple designation 'ovaries' (Beolchini *et al.* (2000). However, these ovaries are unusual and do contain a testis-like region with interstitial tissue and interspersed medullary cords (Jiménez *et al.*, 1993; Sanchez *et al.* 1996). Ovarian follicles are limited to a distinct pole in the gonad, while the ovarian interstitial gland (OIG) is found at the opposite pole. Additionally, the two poles vary seasonally in size and endocrine activity (Matthews, 1935; Deansesly, 1966; Whitworth *et al.*, 1999). The follicular pole enlarges at sexual maturity and at the beginning of subsequent breeding seasons, while the interstitial gland regresses; the arrangement reverses at the end of the breeding season. Testosterone, principally secreted from the OIG, increases in females outside of the breeding season to levels that are comparable to that of males (Whitworth *et al.*, 1999). Interestingly, this peculiar gonadal arrangement is present at birth (Gorman and Stone, 1990), and is found in pre- and post-pubertal females (Jiménez *et al.*, 1996). The timing of gonadal differentiation in *Talpa* has not been well studied, but Matthews (1935) noted differentiation of both regions of the female gonad *in utero*. Matthews also noted the abnormal development of epididymides adjacent to the OIG, which suggested to him that testosterone is secreted by the fetal ovary, and may provide a mechanism for masculinization of the female's external genitalia.

Comparative Studies

However, there is considerable variation in ovarian morphology among mole species not belonging to the genus *Talpa*. Moles of North America can also be very difficult to sex, owing to the same problems described above for the Old World moles. Mossman and Duke (1973) tabulated and described the ovarian morphology of many mammal species, including several from the family Talpidae, which includes both the Old and New World moles. Depending on the species, the latter may have bipolar ovaries very similar to those of the *Talpa* spp. (e.g. star-nosed moles, *Condylura cristata*),

conventional ovaries lacking an OIG (e.g. eastern moles, *Scalopus aquaticus*), or ovaries that are intermediate between the two extremes (e.g. shrew moles, *Neurotrichus gibbsii*). Given these findings, Rubenstein *et al.* (2004) recently set out to determine if the masculinization of female genitalia in several species of North American moles is consistently correlated with ovarian morphology, i.e. is a peniform clitoris ever found in species lacking an OIG. If so, an alternate source of androgen, or an alternate mechanism may be required to explain female masculinization in some species of mole. Additionally, Rubenstein *et al.* questioned whether the presence of ovotestes or a peniform clitoris is associated with a reduction in other indices of sexual dimorphism in moles, e.g. penile/clitoral length, anogenital distance, and body size.

In order to answer the questions above, Rubenstein *et al.* (2004) examined ovarian and clitoral anatomy in four species of North American moles, broad-footed, coast, star-nosed and shrew moles (*Scapanus latimanus*, *S. orarius*, *C. cristata*, and *N. gibbsii*, respectively). Specifically, they focused on the presence/absence of an OIG and the existence of a urethra that traverses the clitoris to its tip. This comparative approach set out to determine if a perfect cross-species correlation exists between the presence of an OIG and existence of a peniform clitoris. If so, these findings would be compatible with Matthews' suggestion that secretion of androgens by the ovaries is responsible for the masculinized clitoral anatomy of various moles.

Interestingly, Rubenstein *et al.* (2004) found that masculinization of the female gonad and external genitalia can occur in isolation of one another. All four species of mole were confirmed to have a peniform clitoris, but the ovaries of the broad-footed and coast moles (*Scapanus latimanus* and *S. orarius*) were unremarkable. Their ovaries showed no distinct polarity, as follicles were equally distributed throughout the cortex. Additionally, the *Scapanus* ovaries lacked a discrete interstitial gland, even when females were captured outside of the breeding season.

Moreover, the masculinization of the female external genitalia appears to be extensive, at least in the broad-footed mole, as the phallic length and anogenital distance (AGD) of females did not differ significantly from that of males. These findings are in contrast to those reported for the European mole, in which both phallic length and AGD are significantly larger in males. However, this sexual dimorphism in the external genitalia may simply reflect the strong sex difference in body size in European moles (Gorman and Stone, 1990). In fact, male European moles are so much larger than females that investigators can often sex individuals based on size alone. The same cannot be said about the broad-footed mole. Even though mean body size is sexually dimorphic in broad-footed moles, considerable overlap exists between the sexes, and the average male body length (165 ± 7 mm) is only about 4% larger than that of the average female (158 ± 7 mm) (Rubenstein *et al.*, 2004). That being said, the sexual dimorphism in the genitalia of *T. europaea* might well have an androgen-dependent component after all. Phallic length and AGD are known to be larger in males than females even at birth, however this difference disappeared when Godet (1946) treated pregnant European moles with testosterone propionate.

The mechanism behind the masculinization of the external genitalia in female moles remains a mystery. Does the presence of a peniform clitoris, traversed to its tip by the urethra, in species with and without so-called 'ovotestes' mean we need to look for an androgen-independent mechanism to explain the natural masculinization of female

moles? Research involving the spotted hyena suggests we might (see below). However, we need to continue to look for alternate sources of testosterone, be it from the maternal or fetal compartment. Neither the maternal or fetal ovary needs to express an 'ovotestis'-like phenotype to produce significant amounts of androgens, and theca-interstitial cells need not conglomerate into discrete interstitial glands to carry out this function. Clearly, additional research needs to be completed to further understand the 'intersexuality' of these elusive and interesting animals.

SPOTTED HYENAS

"Masculinization" of Females

Spotted hyenas are members of the family Hyaenidae, a lineage of carnivores that split from ancestral viverrids 25 - 30 million years ago. Presently, there are four extant species in the family: striped hyenas (*Hyaena hyaena*), brown hyenas (*Parahyaena brunnea*), spotted hyenas (*Crocuta crocuta*) and a small termite-eating outlier, the aardwolf (*Proteles cristatus*). Among the female hyenids, only spotted hyenas are characterized by exceptional masculinization of the external genitalia, and their internal urogenital anatomy follows the typical mammalian pattern.

Spotted hyenas are also the largest and most social of the hyenids, living in multi-male, multi-female "clans" and, when hunting in large groups, capable of killing zebra and cape buffalo. Within these groups, every adult female resident totally dominates every adult male immigrant, both at kills and in the course of other social interactions (Kruuk, 1972; Frank 1986a,b; Mills 1990). When viewed in combination with their "masculine" genitalia, this female behavioral dominance has provoked a search for naturally circulating androgens. Such androgens would have had to be present during prenatal life, in order to account for the genital masculinization which is complete at birth.

Androgens in Female and Male Spotted Hyenas

Testosterone and dihydrotestosterone appear to be the primary androgens circulating in adult male hyenas, while androstenedione, of ovarian origin, is the primary androgen circulating in female spotted hyenas (Glickman *et al.*, 1992; Lindeque *et al.*, 1986). Through a set of convergent observations on fetal steroid concentrations, and placental metabolism *in vivo* (Licht *et al.*, 1992), and detailed study of placental metabolism *in vitro* (Yalcinkaya *et al.*, 1993), it has become clear that androstenedione secreted by the maternal ovary is converted to estrogen and testosterone by the placenta and transferred to the developing fetus. This process has been observed as early as day 35 of the 110 d gestation, and may well begin at a still earlier date (Licht *et al.*, 1998). It is also the case, that formation of the fetal clitoris, with a completely enclosed central urogenital canal, has been observed in a 36 day fetus, prior to differentiation of the fetal ovaries (Licht *et al.*, 1998). This latter observation raised the possibility that formation of the clitoris of the female spotted hyena is, either driven via a non-traditional androgenic source (e.g., the placenta, or the fetal adrenal), or that it is formed by a non-androgenic mechanism. Experiments designed to test that possibility are described below.

Some Essential Genital Morphology in Relation to Behavior

As previously noted, the genital swellings of the female spotted hyena have fused to form a pseudoscrotum instead of an external vagina. The genital tubercle has hypertrophied, and is superficially similar to the penis of the male. From the first months of life, female and male hyenas display full erections during meeting ceremonies. In such "ceremonies," a pair of hyenas typically stand side-by-side, head-to-tail, and subject the ano-genital region of the partner to careful olfactory and (sometimes) gustatory inspection. Hyena etiquette requires that the subordinate hyena make the initial offering of the erect phallus for inspection by the dominant animal (East *et al.*, 1993).

There are clear and significant differences in phallic morphology, as is appropriate, given the very different tasks that these organs have evolved to fulfill (Neaves *et al.*, 1980; Frank *et al.*, 1990). The penis of the male is long and thin, with a small opening at the narrow tip of the glans. This urogenital meatus has little, if any, elasticity. But, the small meatus is adequate for urination and delivery of seminal fluid during mating. The angular shape of the glans facilitates entry into the prepuce and/or clitoral meatus during mating.

The shaft of the clitoris is shorter and thicker than the male penis and, at the tip of the blunt glans, the urogenital meatus is much larger and more elastic than that of the male (Glickman *et al.* 1992). The size and elasticity of the shaft and urogenital meatus permit receipt of the male penis during mating, and expansion of the organ during delivery of the fetus. However, at the time of the first birth, the meatus cannot stretch sufficiently to permit delivery of a 1.5 kg fetus and the urogenital meatus has to tear before the infant hyena can emerge (Frank and Glickman 1994). This is a reproductively costly arrangement, resulting in stillbirths as much as 60% of the time (Frank *et al.* 1995). Once torn, by the passage of the first fetus, subsequent births through the clitoris are uneventful.

Cunha *et al.* (2003) have identified a set of internal sex differences in phallic morphology that facilitate the diverse burdens placed on the clitoris and the penis. For example, the anterior placement of the clitoris on the abdomen, relative to the more usual location of an external vagina in close proximity to the anus, requires that the elongate male "flip" his semi-erect organ against the abdomen of the female as he "searches" for the opening of the clitoral prepuce. In both females and males cross-hatched collagen fibers provide more-than-usual firmness of structure. This would facilitate the flipping of the male penis, and may assist in stabilizing the clitoris for receipt of the male. In addition, the male urethra is centrally located within the shaft of the penis, surrounded by erectile tissue and a thick tunica, promoting firm erectile capability. In the female, the urogenital canal is located more ventrally within the shaft of the clitoris, and is not surrounded by an unyielding tunica, permitting appropriate expansion during mating and parturition (see Neaves *et al.* 1981).

Normal Postnatal Development

As observed above, the essential sexually differentiated features of the external genitalia are present at birth (Cunha *et al.*, 2003). Precise measurements are impossible in infant hyenas until 2 - 3 months of age, due to fusion of the prepuce and the glans. But, several features of postnatal development should be noted. First, sex differences in the size and elasticity of the urogenital meatus are obvious within the first six months of life.

Second, we have been unable to find any evidence of a pubertal growth spurt. In fact, 80% - 90% of genital growth occurs within the first year of life, well before puberty (Glickman *et al.*, 1998). The latter is typically achieved at roughly 18 - 24 months of age in males, and 30 - 36 months of age in females (Kruuk, 1972; Glickman *et al.*, 1992).

Effects of Pre-Pubertal Gonadectomy on Growth of the External Genitalia

We have observed the effects of pre-pubertal gonadectomy on growth of the external genitalia, employing a comprehensive set of measures. Castration, or ovariectomy, in spotted hyenas reduced circulating testosterone concentrations to barely measurable levels, and led to a reduction of approximately 90% in plasma concentrations of androstenedione (Glickman *et al.*, 1992). However, removal of the testes or ovaries at 4 - 7 months of age had little effect on erect (i.e., "stretched") length of the adult penis or clitoris (Glickman *et al.*, 1992), or on the density of "spines" found on the glans of both females and males (Glickman *et al.*, 1998). Such "spines" are found in many mammalian species, and are usually highly sensitive to the reduction in circulating androgens that typically follows castration (Beach and Levinson, 1950; Sachs *et al.*, 1984). In addition, although mating behavior was absent, full erectile function was displayed during meeting ceremonies, in a male hyena more than 10 years after pre-pubertal castration. The apparently normal growth, and maintenance of spine densities, observed in these pre-pubertally castrated/ovariectomized hyenas, once again raised the possibility that some non-androgenic mechanism might be at work.

To be sure, there were clear effects of prepubertal ovariectomy on clitoral morphology. The glans was noticeably thinner in ovariectomized subjects, and the urogenital meatus of the female lost its elasticity, responding much as the meatus of the male when gently distended. These effects were largely reversed by systemic estrogen administration (Glickman *et al.*, 1998).

A final note. Given the exceptionally early growth of the external genitalia of the spotted hyena, it may be the case that gonadectomy at 4 - 7 months of age was already too late. We have some morphological data available from two male hyenas, one castrated at 30 days of age, and the other at 37 days of age. There may be effects on stretched length of the adult penis, as well as effects on shaft diameter. We need more data, and these initial observations suggest that still earlier gonadectomies are warranted. Also, considering the effects of estrogens on diameter of the glans in ovariectomized females, and noting the effects of an aromatase inhibitor on penile development *in utero* (as described below), it is clear that further experiments on pre- and post-natal effects of estrogens are required. Although there is little circulating estrogen in male hyenas, the possibility of local conversion in target tissues needs to be explored.

Effects of Prenatal Androgen Blockade

When one encounters a female mammal that is so thoroughly masculinized as the spotted hyena, you have to wonder if the process by which masculinization has occurred can be reversed. The scientific literature is replete with papers describing the effects of prenatal androgen treatment on female anatomy, physiology, and behavior, however the spotted hyena requires the obverse. As such, exhaustive efforts have been made to treat pregnant spotted hyenas with a variety of drugs, which can be generally categorized as 'anti-androgens', with the goal of producing a female spotted hyena with the external

genitalia of a more typical female mammal. These experiments have been complicated by the fact that such treatments in spotted hyenas are regularly associated with poor pregnancy outcomes (stillbirths and early neonatal deaths). However, a handful of anti-androgen treated animals have survived to adulthood, and even the stillborn cubs have provided useful information.

Pregnant spotted hyenas were treated with several different regimens, yet not a single female has been born with a distal vagina, and the clitoris has always been prominent. These traits persisted in female offspring whether mothers were treated with an androgen receptor blocker alone (cyproterone or flutamide) or in combination with an inhibitor of 5 α -reductase (finasteride + flutamide). Even when the latter regimen was initiated well before the anticipated time of sexual differentiation (as early as 10 days post-mating), newborn females continued to display a large clitoris and the absence of an external vaginal opening.

This is not to say that there was no effect of the treatment on genital morphology. In fact, the effects of the prenatal anti-androgens are structurally and functionally significant. In anti-androgen treated males (AA-males) the penis became 'feminized', in that it took on many of the characteristics of the clitoris. The penis of AA-males is shorter and thicker than that of control males (C-males), with dimensions more akin to those seen in C-females. Similarly, the diameter and elasticity of the urethral meatus is enlarged in AA-males, again approximating that of C-females (Drea *et al.*, 1998). Finally, the morphology of the bulbocavernosus (BC) and ichiocavernosus muscle, and the number of spinal motoneurons (Onuf's nucleus) that innervate them are sexually dimorphic in spotted hyenas, as they are in other mammals. Both the number of motoneurons in Onuf's nucleus and the morphology of the BC muscle were 'feminized' in AA-males (Forger *et al.*, 1996).

Although the clitoris of AA-females is quite prominent, the 'feminine' characteristics are more exaggerated in this group. The clitoris is even broader and shorter than that of C-females, and the urogenital meatus is much more elastic (Drea *et al.*, 1998). From a functional point-of-view, these modest changes in phallic morphology have had robust effects. The greater meatal elasticity of AA-females seems to have negated some of the high costs associated with the delivery of a fetus through a long and tortuous birth canal. Indeed, the first cub born to a C-female has only a 33% chance of being born alive, while the rate of live births in our four AA-females has been 100%. Conversely, the changes seen in males may have rendered some of our AA-males functionally sterile (Drea *et al.*, 2002). In the most profoundly affected AA-males the penis appears to be too short to reach the ventrally located urogenital opening. The increased thickness of the erect glans in AA-males may also make insertion problematic, especially when courting virginal females.

Effects of Prenatal Aromatase Inhibition

After several years of experiments involving prenatal treatment with anti-androgens we have recently begun to expand our repertoire by manipulating maternal and fetal hormones in new ways. We started a pilot program using the potent aromatase inhibitor Letrozole (Femura®, Novartis) for the following reasons: 1. A deficiency in placental aromatase activity has been associated with female pseudohermaphroditism in humans (Conte *et al.*, 1994). However, Licht *et al.* (1992) and Yalcinkaya *et al.* (1993) suggested that aromatase activity in the spotted hyena placenta is relatively low as

compared to that of 17β -hydroxysteroid dehydrogenase. Thus, most of the androstenedione derived from the maternal ovary is preferentially converted to testosterone rather than estrogen as it crosses the placenta. We queried whether we would induce further masculinization of the female genitalia by blocking aromatase. 2. We were also curious to know if the intense neonatal aggression seen between littermates is influenced by prenatal androgens, and if so, are those effects mediated by local aromatization of androgens into estrogens in critical areas of the brain. As circulating maternal androgens and estrogens are at their highest levels during late gestation (Licht *et al.*, 1992), our investigations with aromatase inhibition started here.

Initially, we used the potent third generation aromatase inhibitor Letrozole during the last 30 d of a twin pregnancy (average length of gestation = 110 d). Both cubs were males and only one was born alive, so we were unable to determine if the genitalia of a female spotted hyena was further masculinized by this treatment. However, our routine measurements of the surviving cub's phallus revealed an interesting effect. While the overall shape and contour of the phallus was unmistakably male, the elasticity of the urethral meatus was significantly greater than untreated males. The absolute values of the stretched meatal diameter were intermediate between untreated males and AA-males (recall the latter are very similar to untreated females).

This preliminary finding piqued our curiosity such that we treated a second pregnancy for a longer duration, encompassing the last 60 d of gestation. This pregnancy resulted in the birth of a single female cub with very unusual genitalia. The glans clitoridis appeared blunted, but more importantly, the urethral meatus was displaced, with part of the opening located along the ventral surface of the glans (Fig. 1). For want of a better term, this appears to be a case of clitoral hypospadias.

SUMMARY

Although varying degrees of genital masculinization are a reasonably common phenomenon in the world of female mammals, the majority of such variation has not been investigated. In this chapter we have described research on the "masculinized" genitalia of moles and hyenas. Such research raises intriguing possibilities regarding the coordinated role that androgens, estrogens and peptide hormones (e.g., relaxin) might play, at different stages of sexual differentiation and development, in preparing genital tissues for their functional roles in reproduction. Such studies also suggest that non-androgenic mechanisms need to be considered.

Arnold (1996) and Carruth *et al.* (2002) have recently presented the argument for broadening our view of sexual differentiation of brain and behavior, emphasizing direct genetic effects. A similar view has been presented for the Tammar wallaby, where formation of a scrotum, or a pouch, is a direct consequence of the presence/absence of two X chromosomes (Pask and Renfree, 2001). Although our research on moles and hyenas has not yet yielded such definitive results, the research reviewed in this chapter calls attention to processes that could well operate in other mammals, including humans.

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REFERENCES

- Arnold AP** (1996) Genetically triggered sexual differentiation of brain and behavior. *Hormones and Behavior* **30**: 495-505
- Beach FA and Levinson** (1950) Effects of androgen on the glans penis and mating behavior of castrated rats. *Journal of Experimental Zoology* **114** 159-168
- Beolchini F, Rebecchi L, Capanna E, and Bertolani R** (2000) Female gonad of moles, genus *Talpa* (Insectivora, Mammalia): ovary or ovotestis?. *Journal of Experimental Zoology* **286** 745-754
- Carruth LL, Reisert I, and Arnold AP** (2002) Sex chromosome genes directly affect brain differentiation. *Nature Neuroscience* **5** 933-934
- Conte, FA, Grumbach MM, Ito F, Fisher CR, and Simpson ER** (1994) A syndrome of female pseudohermaphroditism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (P450arom). *Journal of Clinical Endocrinology and Metabolism* **78** 1287-1292
- Cunha GR, Wang Y, Place NJ, Lui W, Baskin L, and Glickman SE** (2003) The urogenital system of the female spotted hyena (*Crocuta crocuta*): A functional histological study. *Journal of Morphology* **256** 205-218
- Deanesly R** (1966) Observations on reproduction in the mole *Talpa europaea*. In *Comparative biology of reproduction in mammals* Vol 15 pp 387-402 Ed IW Rowlands. Academic Press, London
- Drea CM, Weldele ML, Forger NG, Coscia EM, Frank LG, Licht P and Glickman SE** (1998) Androgens and masculinization of genitalia in the spotted hyaena (*Crocuta crocuta*). 2. effects of prenatal anti-androgens. *Journal of Reproduction and Fertility* **113** 117-127
- Drea CM, Place NJ, Weldele ML, Coscia EM, Licht P and Glickman SE.** (2002). Exposure to naturally circulating androgens in foetal life incurs direct reproductive costs in female spotted hyaenas, but is prerequisite for male mating. *Proceedings of the Royal Society, London B* **269** 1981-1987.
- East ML, Hofer H and Wickler, W** (1993) The erect "penis" is a flag of submission in a female-dominated society: greetings in Serengeti spotted hyaenas. *Behavioral Ecology and Sociobiology* **33** 335-370
- Forger NG, Frank LG, Breedlove SM, and Glickman SE** (1996) Sexual dimorphism of perineal muscles and motoneurons in spotted hyenas. *The Journal of Comparative Neurology* **375** 333-343
- Frank LG** (1986a) Social organisation of the spotted hyaena (*Crocuta crocuta*). I. Demography *Animal Behaviour* **34** 1500-1509
- Frank LG** (1986b) Social organisation of the spotted hyaena (*Crocuta crocuta*). II. Dominance and reproduction *Animal Behaviour* **34** 1510-1527
- Frank LG and Glickman SE** (1994) Giving birth through a penile clitoris: Parturition and dystocia in the spotted hyaena (*Crocuta crocuta*). *Journal of Zoology, London* **234** 659-665

- Frank LG, Glickman SE and Powch I** (1990) Sexual dimorphism in the spotted hyaena (*Crocuta crocuta*). *Journal of Zoology, London* **221** 308-313
- Frank LG, Weldele ML, and Glickman** (1995) Masculinization costs in hyaenas. *Nature* **377** 584-585
- Glickman SE, Frank LG, Pavgi S, and Licht P** (1992) Hormonal correlates of 'masculinization' in female spotted hyaenas (*Crocuta crocuta*). I. Infancy to sexual maturity. *Journal of Reproduction and Fertility* **95** 451-462
- Glickman SE, Coscia EM, Frank LG, Licht P, Weldele ML and Drea CM** (1998) Androgens and masculinization of genitalia in the spotted hyaena (*Crocuta crocuta*). 3. Effects of juvenile gonadectomy. *Journal of Reproduction and Fertility* **113** 129-135
- Gorman ML and Stone RD** (1990) *The natural history of moles*. Comstock Publishing Associates, Ithaca
- Godet R** (1946) Biologie experimentale - modifications de l'organogenèse des voies urogénitales des embryons de taupes (*Talpa europaea* L), par action du propionate de testostérone. *Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences* **222** 1526-1527
- Hill WCO** (1958) External genitalia. *Primatologia* **3** 630-704
- Jiménez R, Burgos M, Sanchez A, Sinclair AH, Alacron FJ, Marin JJ, Ortega E and Diaz de la Guardia R** (1993) Fertile females of the mole *Talpa occidentalis* are phenotypic intersexes with ovotestes. *Development* **118** 1303-1311
- Jiménez R, Alacron FJ, Sanchez A, Burgos M and Diaz de la Guardia R** (1996) Ovotestis variability in young and adult females of the mole *Talpa occidentalis* (Insectivora, Mammalia). *Journal of Experimental Zoology* **274** 130-137
- Jost A** (1953) Problems of fetal endocrinology: the gonadal and hypophyseal hormones. *Recent Progress in Hormone Research* **8** 379-418
- Kruuk H** (1972) *The spotted hyena*. University of Chicago Press, Chicago
- Licht P, Frank LG, Pavgi S, Yalcinkaya TM, Siiteri PK and Glickman SE** (1992) Hormonal correlates of 'masculinization' in female spotted hyaena (*Crocuta crocuta*). 2. Maternal and fetal steroids. *Journal of Reproduction and Fertility* **95** 463-474
- Licht P, Hayes T, Tsai P, Cunha G, Kim H, Golbus M, Hayward S, Martin MC, Jaffe RB and Glickman SE** (1998) Androgens and masculinization of genitalia in the spotted hyaena (*Crocuta crocuta*). 1. Urogenital morphology and placental androgen production during fetal life. *Journal of Reproduction and Fertility* **113** 105-116
- Lindeque M, Skinner JD, and Millar RP** (1986) Adrenal and gonadal contribution to circulating androgens in spotted hyaenas (*Crocuta crocuta*) as revealed by LHRH, hCG, and ACTH stimulation. *Journal of Reproduction and Fertility* **78** 211-217
- Matthews LH** (1935) The oestrous cycle and intersexuality in the female mole (*Talpa europaea* Linn.). *Proceedings of the Zoological Society, London Series 2* **1935** 347-383
- Matthews LH** (1939) Reproduction of the spotted hyaena (*Crocuta crocuta* Erxleben). *Philosophical Transactions of the Royal Society, London Ser B* **230** 1-78
- Mills MGL** (1990) *Kalahari hyaenas: Comparative behavioural ecology of two species*. Unwin-Hyman, London

- Mossman HW and Duke KL** (1973) *Comparative morphology of the mammalian ovary*. University of Wisconsin Press, Madison.
- Neaves WB, Griffin JE and Wilson JD** (1980) Sexual dimorphism of the phallus in spotted hyaena (*Crocuta crocuta*). *Journal of Reproduction and Fertility* **59** 509-513
- Pask A and Renfree MB** (2001) Sex determining genes and sexual differentiation in a marsupial *Journal of Experimental Zoology* **290** 586-596
- Rubenstein N, Cunha G, Wang YZ, Campbell K, Conley AJ, Catania KC, Glickman SE and Place NJ.** (2004) Variation in ovarian morphology in four species of New World moles with a peniform clitoris. **Reproduction** (*in press*)
- Sachs BD, Glater GB, and O'Hanlon JK** (1984) Morphology of the erect glans penis in rats under various gonadal conditions *Anatomical Record* **210** 45-52
- Sanchez A, Bullejos M, Burgos M, Hera C, Stomatopoulos C, Diaz de la Guardia R and Jimenez R** (1996) Females of four mole species of the genus *Talpa* (Insectivora, Mammalia) are true hermaphrodites with ovotestes. *Molecular Reproduction and Development* **44** 289-294
- Whitworth DJ, Licht P, Racey PA and Glickman SE** (1999) Testis-like steroidogenesis in the ovotestis of the European mole, *Talpa europaea*. *Biology of Reproduction* **60** 413-418
- Wilson JD, George FW and Griffin JE** (1981) The hormonal control of sexual development. *Science* **211** 1278-1284
- Wislocki GB** (1936) The external genitalia of the simian primates. *Human Biology* **8** 309-347
- Yalcinkaya TM, Siiteri PK, Vigne J-L, Licht P, Pavgi S, Frank LG and Glickman SE** (1993) A mechanism for virilization of female spotted hyenas *in utero*. *Science* **260** 1929-1931

FIGURE LEGEND

Figure 1. Ventral view of the distal clitoris in (A) 9 month old female spotted hyena, treated with an aromatase inhibitor (Letrozole) during the last 60 days of gestation, and (B) 3 month old untreated female. In the Letrozole-treated female, a hypospadias-like condition is seen, with part of the urethral meatus opening along the ventral surface of the glans (arrows). In untreated hyenas the meatus does not extend onto the ventral surface of the glans.

