The boar testis: the most versatile steroid producing organ known

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A review of the remarkable production of steroids by the testes of the boar is presented, with the principal aims of highlighting the achievements of the Leydig cells and, at the same time, pointing to the considerable deficiencies in our understanding of its biological relevance. The onset of gonadal steroidogenesis at an early stage of sex differentiation and the pattern of pre- and postnatal secretion of steroids are outlined. This is followed by a list of steroids identified in extracts of the boar testis, with emphasis on those that can reasonably be assumed to be secretory products of the Leydig cells. For example, the high concentrations of 16-unsaturated C_{19} and sulphoconjugated compounds are noted. Next, an impressive list of steroids found in venous blood from the boar testis is given; among them are the 16-unsaturated steroids, the oestrogens and dehydroepiandrosterone, all mainly in the form of sulphates. However, the list also includes some less likely members, such as 11-OH and 19-OH androgens as well as 5α-reduced steroids. Lastly, the high concentrations of steroids reported in testicular lymph, especially sulphates, are mentioned. Although roles for testosterone are uncontested, and even for the pheromone-like C_{19} steroids, there is little that can be said with assurance about the other compounds listed. Some speculations are made on their possible contributions to the reproductive physiology of the boar. This is done to provoke interest and, perhaps, even action towards reaching a more complete understanding of the biological significance of the steroidogenic powers of porcine Leydig cells.

Introduction

The boar is a remarkable animal in terms of reproductive attributes. A list of them would include the large size of the accessory sex glands and testes, and the impressive volume of the ejaculate delivered at mating or collection of semen. It is, however, the structural characteristics of a highly developed interstitial tissue in the testes of the boar that lead to a consideration of steroid production. Among the domestic animals, only the stallion shows a comparable abundance of Leydig cells in the interstitium (Fawcett *et al.*, 1973), suggesting a high capacity for steroid secretion. The striking difference between these two males lies not in the quantitative performance but rather in the surprising variety of steroid compounds that comprise the

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natural output of the boar testes. The following is an account of this variety to justify the title chosen for the present review.

Before addressing the justification of the title a few preliminary comments are in order. Firstly, non-mammalian vertebrate species are excluded from consideration. Then, a possible counterclaim for the adrenal gland being the most versatile steroidogenic organ needs to be acknowledged. It should be pointed out, however, that the list of steroids to be given for the boar testis is largely restricted to those compounds that have been reported as secretory products; it does not include some of the steroids seen in extracts of testicular tissues. Extracts contain steroids, such as pregnenolone and progesterone, which are precursors or intermediary compounds in steroidogenesis but are not released from the testes in quantitatively significant amounts. In contrast, a competing list of steroid compounds for the adrenal glands would have to be derived from those in extracts of tissues as well as from blood, and would need to be based on data from several species. Nevertheless, a comparison of steroid production by the boar testes and the adrenal glands is intriguing in many respects, as will be become evident below.

Also as preface, it should be emphasised that the physiological significance of the production of the great variety of steroids by the boar testis remains unknown. With the obvious exception of testosterone, and perhaps the delta-16 unsaturated steroids, there is little that can be cited as clear evidence for a role for the large number of other compounds. Several of these steroids are secreted from the testes in appreciable amounts and are present in high concentrations in peripheral blood; and, for this reason, it remains a challenge to reproductive endocrinologists to address this void in our understanding of the reproductive physiology of the boar. Some degree of overlap or fail-safe approach can hardly provide an answer to the biological significance of such a plethora of steroid products from the testes. Therefore, a principal aim in providing a list of testicular steroids for the boar is to issue a challenge. Acceptance would hopefully lead to uncovering specific roles for some of the compounds, for example in epididymal regulation.

Ontogenesis of gonadal steroidogenesis

Steroid hormones appear at an early stage in mammalian sex differentiation, although no specific role in gonadal differentiation has been demonstrated for mammalian species. From the classical studies of Jost (for review see Jost, 1970) it is clear that androgen secretion by the male gonads is of prime importance in the development of the male reproductive tract. The first suggested evidence of an endocrine role for the foetal gonads was reported just over one hundred years ago (Bouin and Ancel, 1903). It was followed almost immediately by a description of the development of the Leydig cells in the foetal pig testes (Allen, 1904; Whitehead, 1904). Incidentally, the term 'hormone' was introduced into the literature in the following year (Starling, 1905). After the discovery of the chemical nature of the steroid hormones in the 1930s, there have been many attempts, mainly in small laboratory animals, to determine the time of onset and the factors regulating steroidogenesis in the foetal gonads. Recent studies in mice have shown that pituitary hormones are not required for foetal development of Leydig cell activity and sexual differentiation (O'Shaughnessy *et al.*, 1998; Pakarinen *et al.*, 2002). Thus the conclusion reached was that either some paracrine factors or constitutive components were involved in the onset of steroidogenesis. Our earlier work had already demonstrated a large measure of autonomy in the initiation of functional development (steroid secretion) of foetal Leydig cells in the pig. Clearly, the principal advantage in working with the pig is the longer time-span for developmental events than is the case for the common laboratory animal

species. A brief review of our studies on steroidogenesis and gonadal differentiation has been reported (Raeside, 1985). Whatever the factors might be in the initial stimulation of steroid production by the Leydig cells, it is fascinating to learn from recent work that their progenitor cells, in the rat and human testis, are vascular smooth muscle cells and pericytes (Davidoff *et al.*, 2004).

Pattern of pre- and postnatal testicular steroid secretion

A high degree of differentiation of the interstitial (Leydig) cells in the pig can be seen during three phases of life. The first phase is a transient one in early foetal life during gonadal differentiation; the second is also transient in the perinatal period; and the third phase extends from early puberty onwards (Allen, 1904; Moon and Hardy, 1973; van Straaten and Wensing, 1978). A similar series occurs in the development of the human testes (Pelliniemi *et al.*, 1996; Ge *et al.*, 1996), but only two phases can be distinguished in rats and mice with much shorter time frames for development.

Testicular steroidogenesis in the pig follows the pattern shown for morphological development. It is characterised by a peak in testosterone secretion in early foetal life (Meusy-Dessolle, 1974; Raeside and Sigman, 1975; Stewart and Raeside, 1976; Colenbrander *et al.*, 1978; Ford *et al.*, 1980), elevated levels in the perinatal period (Segal and Raeside, 1975; Meusy-Dessolle, 1975; Booth, 1975; Colenbrander *et al.*, 1978; Schwarzenberger *et al.*, 1993), and a rise again with the approach of puberty (Gray *et al.*, 1971; Meusy-Dessolle, 1975; Colenbrander *et al.*, 1978; Lapwood and FlorCruz, 1978; Tan and Raeside, 1980). With few exceptions testosterone was the only hormone measured in these studies. In late foetal stages it has been possible to include assays of other androgens (androstenedione, dehydroepiandrosterone and 5-androsten-3ß,17ß-diol) in extracts of testes (Segal and Raeside, 1975). At all stages, however, testosterone was the androgen present in highest amounts. In primary cultures of immature Leydig cells from neonatal pigs these same androgens were secreted as unconjugated and sulphated steroids, and in increased amounts with gonadotrophic stimulation (Orava *et al.*, 1985). The presence of oestrone was noted in both free and conjugated forms; and this finding was supported by a report on the stimulation of aromatase activity in immature porcine Leydig cells (Raeside *et al.*, 1988). In further work on the ontogenesis of oestrogen secretion by the porcine foetal testes (Raeside *et al.*, 1993a), it was found that oestrogen secretion is a feature of steroidogenesis even in the early stages of development. With organ culture of foetal gonads, oestrogens appear in the media slightly later and in much lesser amounts than seen for testosterone during development.

Steroids present in the boar

The steroids found in biological material from the boar have been grouped according to source. For the most part the evidence for identity is definitive but for some it is based on reasonable inference from the information provided. Emphasis has been placed on secretion from the testis; although there remains the possibility that some compounds identified in extracts of testicular tissues are produced in the testis and serve in a paracrine manner to support activity in the seminiferous tubules. At the same time, it is recognised that some steroids might simply represent local metabolism to inactive products within the testis and, as such, would not be eligible for the list of biologically active steroids released from the boar testis. Many of the compounds in tissue extracts of the testes, such as pregnenolone and progesterone, are related to intermediary metabolism in the biosynthesis of steroids in the testis. Other steroids serve

both as secretory products from the testis and as intermediary compounds; a prime example is testosterone itself which is also a precursor for oestradiol formation in the testis.

Testis

a) Unconjugated steroids. A list of unconjugated (free) steroids found in extracts of boar testes is given in Table 1. For the C_{21} compounds, the presence of pregnenolone and progesterone in low amounts is a reflection of their role as intermediary compounds in steroidogenesis (Gower, 1972). The significance of the other C_{21} steroids remains uncertain; they might be intermediary compounds in the formation of the 16-unsaturated steroids, or represent a pathway to biologically inactive products within the testis. Although a testicular 20ß-hydroxysteroid dehydrogenase has been cloned from a pig testis cDNA library (Tanaka *et al.*, 1992), there are no reports of C_{21} 20ß-dihydro steroids in pig testes. The enzyme could be involved in the biosynthesis of the 16-unsaturated steroids postulated by Gower (1972), or lead to 20ß-OH compounds which may play a role in spermatogenesis in mammalian testes (Tanaka *et al.*, 1992). Its occurrence could thus be analogous to the presence of the enzyme responsible for forming a steroid implicated in the regulation of spermiation in salmonid fish (Ueda *et al.*, 1985). No 21-hydroxylase activity characteristic of the adrenal cortex has been reported for the boar testis although it has been demonstrated in testicular interstitial tumours in male mice (Dominguez *et al.*, 1960).

Table 1. Unconjugated steroids in boar testis tissue

Steroid	Reference
C_{21}	
Pregnenolone	Ruzicka and Prelog, 1943; Ruokonen and Vihko, 1974
Progesterone	Ruokonen and Vihko, 1974
3α-Hydroxy-5α-pregnan-20-one	Ruzicka and Prelog, 1943; Ruokonen and Vihko, 1974
3α-Hydroxy-5ß-pregnan-20-one	Ruzicka and Prelog, 1943; Ruokonen and Vihko, 1974
C_{19}	
Testosterone	Brady, 1951; Baulieu *et al.*, 1967; Gower, 1972; Ruokonen and Vihko, 1974; Booth, 1975
Androstenedione	Lindner, 1961; Baulieu *et al.*, 1967
Epiandrosterone	Ruokonen and Vihko, 1974
Dehydroepiandrosterone	Baulieu *et al.*, 1967; Ruokonen and Vihko, 1974; Raeside, unpublished
5-Androstene-3ß,17ß-diol	Booth, 1975; Raeside, unpublished
5α-Androst-16-en-3α-ol	Prelog and Ruzicka, 1944; Claus, 1970; Ruokonen and Vihko, 1974; Booth, 1975
5α-Androst-16-en-3ß-ol	Prelog and Ruzicka, 1944; Claus, 1970; Ruokonen and Vihko, 1984; Booth, 1975
5α-Androst-16-en-3-one	Claus, 1970; Gower, 1972; Booth, 1975
4,16-Androstadien-3-one	Gower, 1972; Booth, 1975
5,16-Androstadien-3ß-ol	Gower, 1972; Ruokonen and Vihko, 1974; Booth, 1975
C_{18}	
19-Nortestosterone	Ruokonen and Vihko, 1974
Oestradiol-17ß	Raeside, unpublished
Oestrone	Raeside, unpublished

The most outstanding feature of the C_{19} steroids found in extracts is the remarkably high concentrations of 16-unsaturated steroids that are formed from pregnenolone by the so-called andien-

ß pathway (Fig. 1). The most prominent by far is 5α-androst-16-en-3ß-ol (Ruokonen and Vihko, 1974). In the case of the C_{18} steroids, the presence of 19-nor-testosterone was recorded (Ruokonen and Vihko, 1974), along with large amounts of the two oestrogens, oestradiol-17ß and oestrone. This gave a profile similar to that for extracts of the stallion testes (Beall, 1940; Dumasia *et al.*, 1989) and different from that for the limited data for other species.

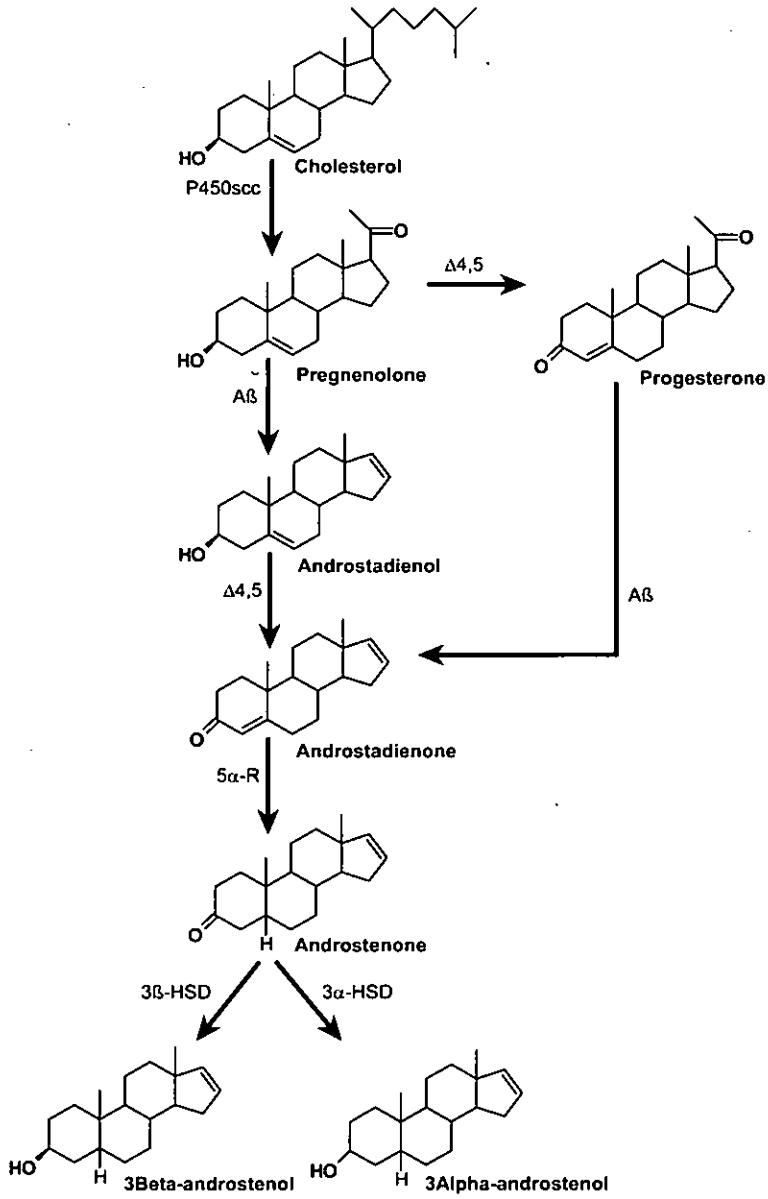

Fig. 1 The andien-ß pathway.
Abbreviations: $P450_{SCC}$, cytochrome P-450 side-chain cleavage; Aß, Andien-ß synthetase; Δ4,5, Δ4,5-isomerase/3ß-dehydrogenase; 5α-R, 5α-reductase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3ß-HSD,3ß-hydroxysteroid dehydrogenase

b) Steroids conjugated as monosulphates. Many of the steroids above appear also in the conjugate fraction of extracts of the boar testis (Table 2). Again, the C_{19} 16-unsaturated compounds are predominant. Also, steroids with the 3ß-OH configuration seem to be favoured in their abundance (Ruokonen and Vihko, 1974). Their source was clearly shown to be the Leydig cells (Raeside, 1983). It would appear that the high levels of the 3ß-sulphates in the extracts result from activity of neutral steroid sulphotransferases that are distinct from the oestrogen sulphotransferase present in the boar testis (Hobkirk *et al.*, 1989; Sinclair and Squires, 2005).

Table 2. Steroids conjugated as monosulphates in boar testis tissue

Steroid	Reference
C_{21}	
Pregnenolone	Ruokonen and Vihko, 1974
3α-Hydroxy-5α-pregnan-20-one	Ruokonen and Vihko, 1974
3ß-Hydroxy-5α-pregnan-20-one	Ruokonen and Vihko, 1974
3ß,17α-Dihydroxy-5ß-pregnan-20-one	Ruokonen and Vihko, 1974
C_{19}	
5α-Androst-16-en-3α-ol	Ruokonen and Vihko, 1974
5α-Androst-16-en-3ß-ol	Ruokonen and Vihko, 1974
5,16-Androstadien-3ß-ol	Ruokonen and Vihko, 1974
Androsterone	Ruokonen and Vihko, 1974
Epiandrosterone	Ruokonen and Vihko, 1974
Dehydroepiandrosterone	Ruokonen and Vihko, 1974
5α-Androstane-3α,17α-diol	Ruokonen and Vihko, 1974
5α-Androstane-3α,17ß-diol	Ruokonen and Vihko, 1974
5α-Androstane-3ß,17α-diol	Ruokonen and Vihko, 1974
5α-Androstane-3ß,17ß-diol	Ruokonen and Vihko, 1974
5-Androstene-3α,17ß-diol	Ruokonen and Vihko, 1974
5-Androstene-3ß,17α-diol	Ruokonen and Vihko, 1974
5-Androstene-3ß,17ß-diol	Raeside and Howells, 1971; Ruokonen and Vihko, 1974
C_{18}	
Oestradiol-17ß	Raeside, unpublished
Oestrone	Raeside, unpublished

Testicular vein blood

An impressive list of steroids found in venous blood from the boar testis is seen in Table 3. Since they are evidently secretory products from the testis, some physiological significance of their release from the testis might be assumed. It may be noted that testosterone is only one of the many steroids; and it is accompanied by several other androgens, albeit as biologically weaker forms. No clear explanation comes to mind as to why there is such apparent duplication. The fact that the amounts of testosterone leaving the testis are within the range seen for other domestic species (Lindner, 1961) does pose questions concerning the relevance of the other androgenic steroids. Many of these compounds are present in high concentrations as sulphoconjugates, and give rise to concentrations much higher than those for testosterone in the peripheral blood circulation (Schwarzenberger *et al.*, 1993). Their biological activity would then depend on the presence of a sulphatase in the target tissues; at which point the unconjugated forms liberated by the enzyme could act directly, or be converted to active products by local metabolism. In addition, a speculation about a direct action as a steroid sulphate is supported by evidence for the biological action of dehydroepiandrosterone sulphate in brain (Baulieu, 1997),

and for the nongenomic action of oestrone sulphate on cultured epithelial cells of the guinea pig endometrium (Chaminadas *et al.*, 1989).

Table 3. Steroids in boar testicular vein blood

Steroid	Reference
C_{19}	
Testosterone	Lindner 1961; Baulieu *et al.*, 1967
Androstenedione	Lindner 1961; Baulieu *et al.*, 1967
Dehydroepiandrosterone	Baulieu *et al.*, 1967; Raeside *et al.*, unpublished
Dehydroepiandrosterone sulphate	Clark *et al.*, 1965; Baulieu *et al.*, 1967; Raeside and Howells, 1971
Epiandrosterone sulphate	Raeside *et al.*, 1992a
5-Androstene-3ß, 17ß-diol sulphate	Raeside and Howells, 1971
5α-Androstane-3ß, 17ß-diol sulphate	Raeside *et al.*, 1992a
11ß-Hydroxytestosterone	Raeside *et al.*, 1989a
11ß-Hydroxyandrostenedione	Raeside *et al.*, 1989a
19-Hydroxytestosterone	Raeside *et al.*, 1993b
19-Hydroxyandrostenedione	Raeside *et al.*, 1993b
5α-Androst-16-en-3α-ol sulphate	Gower *et al.*, 1970; *Saat *et al.*, 1972; **Gower 1972
5α-Androst-16-en-3ß-ol sulphate	Gower *et al.*, 1970; *Saat *et al.*, 1972; **Gower 1972
5α-Androst-16-en-3-one	Gower *et al.*, 1970; **Gower 1972
5,16-Androstadien-3ß-ol sulphate	Gower *et al.*, 1970; tentative identification
C_{18}	
19-Nortestosterone	Raeside *et al.*, 1989c
Oestradiol-17ß sulphate	Raeside, unpublished
Oestrone sulphate	Raeside, unpublished

*Evidence from testicular infusion of [17α-^{3}H]-pregnenolone and collected *in vivo*.
**Cited in a review by Gower (1972).

Apart from the classical role of testosterone as the 'male sex hormone', the physiological importance of the other steroids remains largely unresolved, with the exception of the 16-unsaturated compounds. These latter steroids act as reproductive pheromones in the pig, stimulating mating behaviour in the female (Reed *et al.*, 1974) and accelerating attainment of puberty in gilts (Brookes and Cole, 1970; Gower, 1972; Kirkwood *et al.*, 1981). An explanation for the prodigious production of the 16-unsaturated steroids by the testis may lie in their role as exocrine, rather than endocrine messengers (Booth, 1980). Presumably this would require greater quantities to generate a response. Three of these compounds are of particular note in testicular vein blood; they are 5α-androsten-3-one, in both the free and sulphate fractions, and the two 5α-androstenols (3α and 3ß) in the sulphate fraction. The interesting observation that 5α-androsten-3-one in blood leaving the testes appeared in the sulphoconjugated fraction was repeated with extracts of media in our studies with Leydig cells from mature boars (Sinclair *et al.*, 2005). After a hydrolysis step the steroid was identified definitively by GC-MS. A possible explanation for the apparent sulphoconjugation of 5α-androsten-3-one is through enolisation of the 3-keto group to give a 3-OH product as a substrate for the sulphotransferase enzyme (Fig. 2). Given that steroid enols are relatively unstable, the high level of sulphotransferase activity in the boar testis could potentially stabilise this form through conjugation. At the same time, a high level of enolase enzyme activity would presumably be involved but this has yet to be examined. Later analyses of the unconjugated and sulphoconjugated fractions of peripheral plasma revealed that about 70 % of 5α-androsten-3-one was present in the sulphoconjugated form (Sinclair and Squires, 2005), which further supports the hypothesis of enol formation. The significance of these findings for the transport of the steroid and its ability to accumulate in boar fat is now the subject of further study.

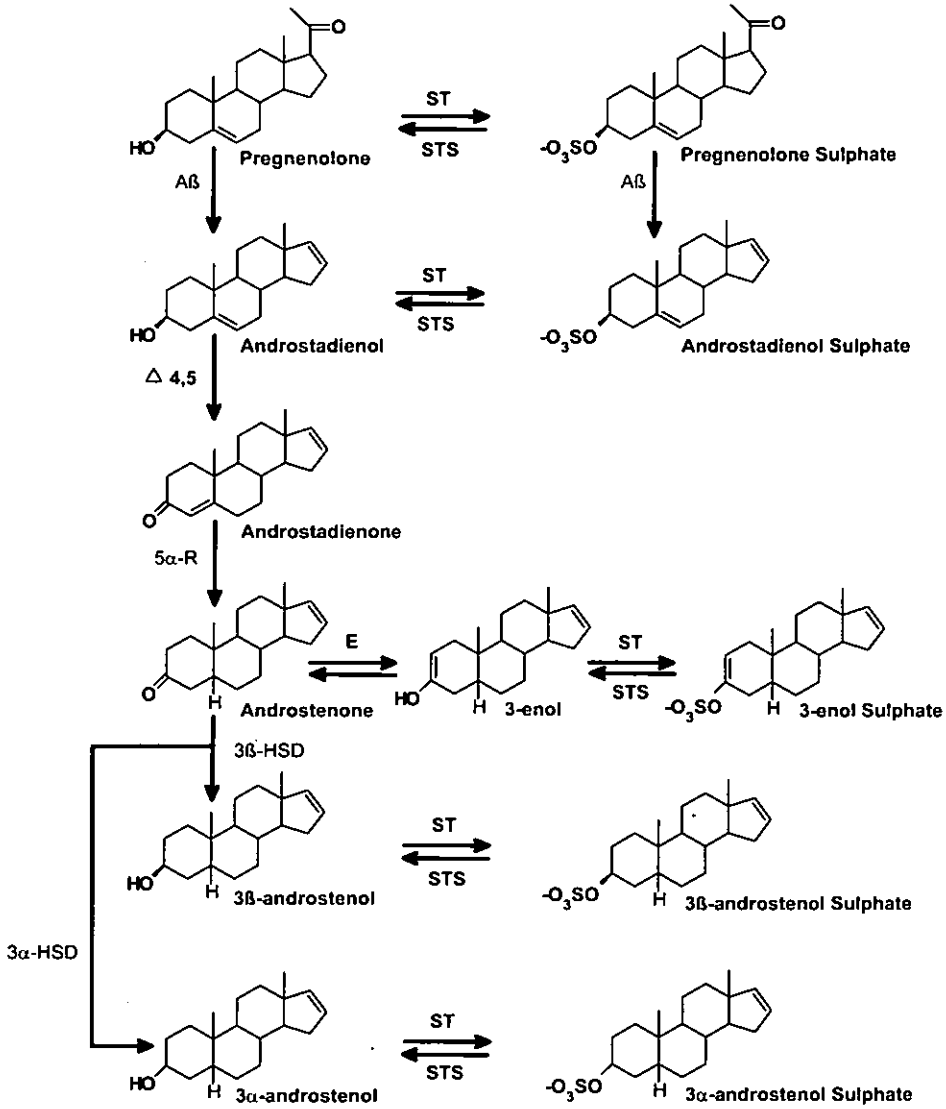

Fig. 2 Scheme illustrating the potential involvement of sulphoconjugation in the synthesis and metabolism of 16-unsaturated steroids.
Abbreviations: ST, sulphotransferase; STS, sulphatase; Aß, Andien-ß synthetase; Δ4,5, Δ4,5-isomerase/3ß-dehydrogenase; 5α-R, 5α-reductase; E, enolase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3ß-HSD,3ß-hydroxysteroid dehydrogenase

No firm evidence of biological roles for the other C_{19} steroids has been reported in the pig. At the same time, it may be noted that the physiological significance of the high concentrations of dehydroepiandrosterone sulphate in human peripheral blood, male and female, has defied an adequate explanation for over half a century. Comment on some of the remaining C_{19} compounds in Table 3 will be restricted, therefore, to a few observations on their actions in other

species, and mainly from *in vitro* studies. Both 5-androstene-3ß,17ß-diol and 5α-androstane-3ß,17ß-diol are weak oestrogens, as reflected in their binding to the oestrogen receptors (Kuiper *et al.*,1997). The latter compound exhibits relatively higher binding to the oestrogen receptor ß (ERß) than to ERα and, thus, may serve as a selective ligand to modulate the response to oestrogens in target tissues (Weihua *et al.*, 2002).

Although the adrenal glands in several species are known as a source of androgens with an oxygen function at C11, the mammalian testes do not normally form such steroids. Here, the boar is an exception. The secretion of 11ß-hydroxylated androgens from the boar testis and the demonstration of their formation by the Leydig cells have been recorded (Raeside *et al.*, 1989a, 1992b). Within the testis they might be converted into oestrogens, as has been shown to be possible with a purified bovine adrenal $P450_{11ß}$ preparation (Suhara *et al.*, 1986). In salmonid fish 11-keto-testosterone is the principal androgen from the testes (Idler *et al.*, 1960); but no studies have yet addressed their significance as testicular products secreted in the boar. The secretion from the boar testis of C_{19} steroids with a 19-OH group raises other interesting and similar questions. In the production of oestrogens from androgens the first intermediary step was shown to be 19-hydroxylation (Meyer, 1955). Both 19-OH testosterone and 19-OH androstenedione are steroids in the aromatisation pathway in boar Leydig cells where19-norandrogens and oestrogen production is remarkably high. Nevertheless, these steroids also occur as secretory products from the testis (Raeside *et al.*, 1993b). They have also been found in testicular tissue from the horse (Dumasia *et al.*, 1989), where oestrogens are present in large amounts (Beall, 1940). As for a physiological role, apart from their intermediary position in steroidogenesis, 19-OH androgens were claimed to form a new class of sodium retaining and hypertensinogenic steroids (Sekihara, 1982). Is it possible that they could participate in fluid regulation in the testis or epididymis in the same way that oestrogens regulate water reabsorption in the efferent ductules of the rodent testis (Hess, 2000)?

C_{18} steroids are the last group to be considered, and they include neutral steroids as well as the oestrogens. The presence of 19-nortestosterone once again provides an example of a compound that is both a good substrate for aromatisation to oestrogens by the Leydig cells (Raeside *et al.*, 1989b) and an end product in its own right since it has been identified as a secretory product in testicular vein blood (Raeside *et al.*, 1989c). An action as an amplifier of aldosterone has been recorded for 19-norandrostenedione in the rat (Sekihara *et al*, 1980). Production of a 19-norandrogen within the boar testis might invite speculation on a local action in water transport in the seminiferous tubules and beyond. No data are available on the concentrations of these steroids in testicular vein or peripheral blood. It should be noted that aldosterone is extremely efficient as a mineralocorticoid at levels much lower than those required for the actions of glucocorticosteroids. With that in mind, could small amounts of 19-norandrogens, leaving the testes, have some activity as hormones in the usual sense of the term?

Oestrogens make a major contribution, in quantitative terms, to the secretory pattern of steroids from the boar testes, as judged from the large quantities in the urine of the boar (Velle, 1958; Raeside, 1965) and on the steroid profile in peripheral blood (Schwarzenberger *et al.*, 1993). An even greater imbalance between testosterone and oestrogen is seen in the peripheral blood circulation of the stallion (Raeside, 1978). Little information is available on the physiological significance of such high levels of oestrogens in males of these two species. Oestrogens are generally thought to antagonise the action of testosterone on the accessory sex glands of the male (Mann, 1964). However, some synergistic effects of testosterone and oestrogens on the secretory activity of the accessory sex glands and on sexual behaviour of the boar have been demonstrated with hormone replacement in animals castrated as mature males (Joshi and Raeside, 1973). Yet another possibility for consideration has been raised by Claus *et al.*, (1987), who

reported the presence of oestrogens in the semen of the boar, with about half of the oestrogens bound to the sperm cells which appear to act as carriers. Implications for an effect on uterine contractions and the timing of ovulation in the sow were discussed. In a subsequent study we confirmed these findings but noted that the oestrogens were present largely as sulphates; and, interestingly, they remained with the sperm after multiple washes, as noted for stallion semen (Raeside and Christie, 1997).

Testicular lymph

An alternative and quantitatively significant route for transport of steroids from the testis is seen in the concentrations in lymph (Table 4). Setchell *et al.*, (1983) have shown that with higher concentrations, especially for the conjugated steroids, testicular lymph is an important route for secretion by the boar testis. Although lymph flow was about 7 % of plasma flow from a vein in the spermatic cord, about 80 % of the oestrone sulphate and dehydroepiandrosterone sulphate produced by the testis leaves the organ in the lymph. Thus, the implications for metabolism and establishment of peripheral blood concentrations of steroids need to be considered.

Table 4. Steroids in boar testicular lymph

Steroid	Reference
C_{19}	
Testosterone	Setchell *et al.*, 1983
Dehydroepiandrosterone	Setchell *et al.*, 1983
Dehydroepiandrosterone sulphate	Setchell *et al.*, 1983
C_{18}	
Oestradiol-17ß	Setchell *et al.*, 1983
Oestrone	Setchell *et al.*, 1983
Oestrone sulphate	Setchell *et al.*, 1983

Summary and conclusions

Information on comparative aspects of testicular steroidogenesis in mammalian species is extremely limited. In a review, Ewing and Brown (1977) were able to list data from only twenty species of mammals at the time. Most of the information was based on studies of biosynthesis from radioisotopes; and references for data on testicular concentrations were available for only six species, including the pig. No reference was made to steroid secretion from the testes; but it is evident from the present review that an impressive list can be compiled for steroids formed and released from the boar testis. Some are relatively rare, at least in quantitative terms, such as the 16-unsaturated steroids and the oestrogens. Both types of steroid seem to have a role to play in mating behaviour. The underlying significance of the preponderance of secretion as sulphoconjugated steroids remains largely unexplained. For some of the other steroids, such as 11-OH- and 19-OH-androgens, the speculation becomes even greater. Nevertheless, it is tempting to suggest a possible role for these compounds in a more paracrine manner in relation to the seminiferous tubules and upper regions of the reproductive tract of the boar. The claim postulated here, for a remarkably high level of versatility in steroidogenesis, seems a reasonable one to make for the boar testis. However, a major challenge remains, namely to provide some clear answers on the biological significance of this fascinating characteristic of high and varied steroid production by the testis in the pig. Meeting the challenge should surely lead to better

understanding of the reproductive physiology of the boar and to improvements in breeding performance.

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