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Pathways and genes involved in steroid hormone metabolism in male pigs: A review and update

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ABSTRACT

This paper reviews state-of-the-art knowledge on steroid biosynthesis pathways in the pig and provides an updated characterization of the porcine genes involved in these pathways with particular focus on androgens, estrogens, and 16-androstenes. At least 21 different enzymes appear to be involved in these pathways in porcine tissues together with at least five cofactors. Until now, data on several porcine genes were scarce or confusing. We characterized the complete genomic and transcript sequences of the single porcine *CYP11B* gene. We analyzed the porcine *AKR1* gene cluster and identified four *AKR1C*, one *AKR1C* like genes and one *AKR1E2* gene. We provide evidence that porcine *AKR1C* genes are not orthologous to human *AKR1C*. A new nomenclature is thus needed for this gene family in the pig. Thirty-two genes are now described: transcript (30 + 2 characterized in this study) and genomic (complete: 18 + 1 and partial: 12 + 1) sequences are identified. However, despite increasing knowledge on steroid metabolism in the pig, there is still no explanation of why porcine testes can produce androstenone and epiandrosterone, but not dihydrotestosterone (DHT), which is also a reduced steroid.

Abbreviations: aa, amino acid; S, sulphate; 5α-R, 5-α reductase; Cytb5, cytochrome b5; Cytb5-red, cytochrome b5 reductase; HSD, hydroxysteroid dehydrogenase; P450scc, cytochrome P450 side chain cleavage encoded by porcine *CYP11A1* gene; P450aro, P450 aromatase encoded by one of the three porcine *CYP19A* genes; P450c11, enzyme encoded by porcine *CYP11B* gene; P450c17, enzyme encoded by porcine *CYP17A1* gene; P450c21, 21 steroid hydroxylase enzyme encoded by porcine *CYP21* gene; StAR, steroidogenic acute regulatory encoded by porcine *STAR* gene; Pregnenolone, 5-pregnen-3β-ol-20-one; 17OH-pregnenolone, 17-hydroxy pregnenolone; Progesterone, 4-pregnen-3,20-dione; 17OH- progesterone, 17-hydroxy progesterone; 20-OH-progesterone, 20αprogesterone or 4-pregnen-20-α-ol-3-one; DOC, 11deoxycorticosterone or 21-hydroxyprogesterone (21-hydroxy-4-pregnene-3,20-dione); Δ4-AD, androstenedione or 4-Androstene-3,17-dione; Δ5-ADiol, androstenediol or 5-Androstene-3,17-diol; AD, androstanedione or 5α-androstan-3,17-dione; Androstanediol, 5α-androstan-3,17-diol; 11-OH-Δ4-AD, 11β hydroxy-androstenedione or 11β-hydroxyandrost-4-ene-3,17-dione; 19-OH-Δ4-AD, 19β hydroxy-androstenedione or 19β hydroxyandrost-4-ene-3,17-dione; DHEA, dehydroepiandrosterone or 3β-hydroxyandrost-5-en-17-one; EpiA, epiandrosterone or 3β-hydroxy-5α-androstan-17-one; Androstenone, Δ4-androstene -3-one; Androstadienone, Δ 4,16-androstadien-3-one; Androstadienol, Δ 4,16-androstadien-3-ol; Androsterone, 3α-hydroxy-5α-androstan-17-one; Adrenosterone, androst-4-ene-3,11,17-trione or 11-oxoandrostenedione; Testosterone, 17β-hydroxy-5alpha-androst-1-en-3-one; DHT, dihydrotestosterone or 17β-hydroxy-5α-androstan-3-one; 11-OH-Testo, 11β-hydroxy testosterone or 11β,17β-dihydroxy-4-androsten-3-one; 19-OH-Testo, 19β-hydroxy testosterone or 17β,19-dihydroxyandrost-4-en-3-one; 19-norTesto, 19-nortestosterone (or nandrolone) or 17β-hydroxyestra-4-en-3-one; 11-K-Testo, 11-ketotestosterone or 17-Hydroxyandrost-4-ene-3,11-dione; 11-K-DHT, 11-keto dihydrotestosterone; 11-OH-DHT, 11β-hydroxy dihydrotestosterone; Estrone, 3-hydroxyestra-1,3,5(10)-triene-17-one; Estradiol, 17β-estra-1,3,5(10)-triene-3,17-diol.

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1. Introduction

Even though it is commonly accepted and reported that the synthesis of androstenone is the major feature [1–3], testicular production of steroids has many other remarkable features in pigs [4]. The boar testis produces high amounts of unconjugated and conjugated estrogens as well as 16-unsaturated steroids [2,5,6]. Their production, as indicated by their plasma levels, increases regularly during sexual maturation in parallel to testosterone [7,8]. Active steroidogenesis is not limited to pubertal and mature animals. During the first month after birth, the peak in steroid production reaches similar levels to those observed in mature animals [9–11]. This pattern of steroids parallels that of the development of Leydig cells in the testes [11–13]. After some weeks of relatively low production, steroid production again increases to reach maximum values around puberty, i.e. between five and eight months of age. Circulating steroids with this typical pattern of production are testosterone, estrone, estradiol, epiandrosterone (3β androsterone = EpiA), DHEA (dehydroepiandrosterone), androstenone and 19-nortestosterone (19-norTesto).

Estradiol secreted by the testes plays an active role in the control of male sexual behavior [14]. Large quantities of estrogens are also excreted in boar semen and may influence female reproductive function by stimulating uterine contractions and the time of ovulation [14–16]. No circulating EpiA or 19-norTesto is observed in men. Very limited knowledge is available on the role of these two androgens in the physiology of the boar apart from the fact that these circulating compounds are produced by the pig testis [17,18]. Among 16-unsaturated steroids (Δ16-androstene steroids), androstenone (5 α-androst-16-en-3-one) is of particular importance in the pig. It is excreted in saliva and acts as a pheromone that stimulates sexual behavior in females during estrus [19]. Androstenone is stored in the fat tissue because of its lipophilic properties. A high concentration of androstenone produces a pronounced urine-like flavor in meat, also known as boar taint, which is disliked by consumers and hence poses a problem of meat quality [20]. This is the main reason for the castration of male pigs reared for meat production. This practice is very widespread in European countries [21] but generates severe physiological and behavioral signs of pain [22]. Therefore, surgical castration in male pigs is highly debated in Europe and, finding solutions to rear entire males without boar taint is of primary interest. A better understanding of the biological mechanisms involved in the production of androstenone should help to address this problem.

Taking into account the particularities of steroid production and their possible consequences for meat quality, our study focused on steroid synthesis in the boar testis. Our primary objective was to describe all possible steroid synthesis pathways in the pig, to update the pathways originally described in 1986 [1] with particular focus on Δ16-androstenes and a comparative analysis with human. Our second objective was to complete the characterization of porcine genes involved in steroid synthesis, especially genes *CYP11B* and *AKR1*.

2. Materials and methods

Information on human genes was obtained from Ensembl (human release 72; http://www.ensembl.org/) and NCBI (Nov. 2012, human annotation release 104; http://www.ncbi.nlm.nih.gov/). The human reference transcripts were used in a BLAST procedure against the pig databases RefSeq RNA or/and Non-RefSeq RNA or/and EST at NCBI to characterize the porcine transcripts. To compile all the transcripts documented in porcine databases, a BLASTN procedure was applied using each reference transcript listed in Table 1 against EST and transcript databases for porcine species (update 2012-10-01). Each possible transcript was compared to the reference transcript by analyzing BLAST results using a dedicated python script to detect new transcripts. The corresponding porcine genomic sequences were retrieved from the pig database using the NCBI nucleotide *megablast* search tool (genome reference *Sus scrofa* 10.2). Each new transcript was examined to determine the exact exon/intron structure. To identify potential exons in a genomic sequence or to determine the exon/intron structure of a gene, the transcript sequence was projected on the genomic sequence using (1) *Sim4* (http://pbil.univ-lyon1.fr/members/duret/cours/inserm210604/exercise4/sim4.html) and (2) *GeneSeqer* (http://www.plantgdb.org/cgi-bin/GeneSeqer/index.cgi). Annotations of the alternative splicing pattern of human genes were obtained through the

Table 1
Available data on porcine genes involved in steroid pathways.

Gene	Main transcript	Genomic sequences
CYP11A1	NM_214427 (X13768)	NW_003537258 & NW_003538139
CYP11B	~~D38590~~ (1) KF314683	KF314688
CYP17	~~NM_214428 (M63507)~~ (2) AK343431	NW_003536297 (3)
CYP19A1	NM_214429	NW_003538376 & NW_003540886 (4)
CYP19A2	NM_214430	NW_003609189 (SSC1)
CYP19A3	NM_214431	
CYP21	~~NM_214433 (M83939)~~ (2) AK343420	NW_003610615 (SSC7) (3)
HSD3B1	NM_001004049 (AF232699)	NW_003534677 (SSC4) (3)
HSD11B1	NM_214248 (AF414424)	NW_003539307
HSD11B2	NM_213913 (AF374414)	NW_003610403 (SSC6) (3)
HSD17B1	~~NM_001128472 (EF581989)~~ (2) EU429459	NW_003611499 (SSC12) (3)
HSD17B2	NM_001167649 (AB529535)	NW_003610375 (SSC6) & NW_003537949
HSD17B3	NM_001244790 (AK238558)	NW_003611244 (SSC10)
HSD17B4	NM_214306 (X78201)	NW_003534350 (SSC2)
HSD17B7	NM_001185137 (AB529536)	NW_003534660 (SSC4) (3)
HSD17B12	~~XM_003353892~~ (5) AK396540	NW_003609538 (SSC2)
SRD5A1	XM_003134156 (AK350108)	NW_003301585 (SSC16) (3)
SRD5A2	NM_213988 (AF008440)	NW_003537808 & NW_003609920 (SSC3)
STAR	NM_213755 (AY800265)	NW_003612200 (SSC15) (3)
AKR1C-pig1	~~DQ474065 (NM_001044569)~~ (6) AK391133	CU972427 (SSC10) (3)
AKR1CLP	KF314684 6 (7)	CU972427 (SSC10) (3)
AKR1C-pig3	NM_001044570 (DQ474066)(8)	CU972427 (SSC10) (3)
AKR1C-pig4	NM_001044618 (DQ474067)(8)	CU972427 (SSC10) (3)
AKR1C-pig6	NM_001038626 (DQ474068)(8)	CU972427 (SSC10) (3)
AKR1E2	NM_001044568 (DQ474064)(8)	CU972427 (SSC10) (3)
CYB5A	NM_001001770 (AF016388)	NW_003609242 (SSC1) (3)
CYB5B	NM_001159592 (AY609739)	NW_003534899 (SSC6) (3)
CYB5R1	NM_001243918 (AK231742)	NW_003611243 (SSC10) (3)
CYB5R3	XM_003125982 (AK392329)	NW_003610195 (SSC5) (3)
FDX1	~~NM_214065~~ (2) AY610208 + AK343486 (9)	NW_003535539 (SSC9)
FDXR	NM_001244727 (AK236711)	NW_003611480 (SSC12) (3)
POR	NM_001129959 (L33893)	NW_003538795

When the transcript of reference proposed by the NCBI is not correct, we proposed another sequence and we have striped its name.
(1) This sequence is not correct: see Section 3.2.2.2.
(2) The sequence proposed as the reference transcript does not contain 5′NC and/or 3′NC sequences.
(3) This gene was fully sequenced.
(4) Redundant sequences.
(5) The genomic sequence of this gene was not complete and the proposed annotation was incorrect; as a result, the predicted transcript included several errors.
(6) This sequence is not correct: see Section 3.2.3.4.
(7) The sequence of this non-coding transcript was probably incomplete in 5′ and in 3′.
(8) See Section 3.2.3.4.
(9) Assembly of the two transcripts.