Regulation of 3β-Hydroxysteroid Dehydrogenase/Δ^5-Δ^4 Isomerase: A Review

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Abstract: This review focuses on the expression and regulation of 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD), with emphasis on the porcine version. 3β-HSD is often associated with steroidogenesis, but its function in the metabolism of both steroids and xenobiotics is more obscure. Based on currently available literature covering humans, rodents and pigs, this review provides an overview of the present knowledge concerning the regulatory mechanisms for 3β-HSD at all omic levels. The HSD isoenzymes are essential in steroid hormone metabolism, both in the synthesis and degradation of steroids. They display tissue-specific expression and factors influencing their activity, which therefore indicates their tissue-specific responses. 3β-HSD is involved in the synthesis of a number of natural steroid hormones, including progesterone and testosterone, and the hepatic degradation of the pheromone androstenone. In general, a number of signaling and regulatory pathways have been demonstrated to influence 3β-HSD transcription and activity, e.g., JAK-STAT, LH/hCG, ERα, AR, SF-1 and PPARα. The expression and enzymic activity of 3β-HSD are also influenced by external factors, such as dietary composition. Much of the research conducted on porcine 3β-HSD is motivated by its importance for the occurrence of the boar taint phenomenon that results from high concentrations of steroids such as androstenone. This topic is also examined in this review.
1. Introduction

The elimination of xenobiotics is usually conducted in two phases: Phase I and Phase II. The majority of xenobiotics are Phase I metabolized in the liver by the cytochrome P450 (CYP) enzyme family. However, though receiving less attention, other enzymes are also able to perform Phase I metabolism. One example of such an enzyme is 3β-hydroxysteroid dehydrogenase (3β-HSD), mostly known for its role in steroidogenesis and steroid metabolism. In addition, the porcine version of 3β-HSD has received some attention because of its importance for hepatic clearance of the steroid androstenone in sexually mature male pigs. The accumulation of steroids, especially androstenone, is associated with a negative perception of the meat from pigs.

The enzyme 3β-HSD is a key enzyme in the biosynthesis of all active steroid hormones. Several different isoforms have been identified in several species. Some of these isoforms are expressed in the liver and kidneys or exclusively in the liver [1]. The expression of 3β-HSD in different organs is dependent upon developmental stages [2]. In the liver, 3β-HSD functions in steroid degradation and the regulation of steroid hormone levels in plasma, as a steroid reductase, and also in the Phase I degradation of the male pheromone androstenone [3]. However, its function is not restricted to the inactivation of intrinsic steroid hormones [1]. The activity of 3β-HSD is affected by external factors, such as diet and environmental toxins. Isoflavones, such as genistein and daidzein, and their metabolites have been demonstrated to affect the enzyme kinetics of 3β-HSD in some species [4].

There are also reports detailing the direct metabolic activity of 3β-HSD on xenobiotics, such as the estrogenic mycotoxin zearalenone [5]. Therefore, the regulation of 3β-HSD expression and activity, especially in the liver, is an important aspect of the environmental toxicology of xenobiotics. The body of humans and animals are challenged with zearalenone via the ingestion of foods such as cereals and corn. This compound at high concentrations causes hyperestrogenic effects, such as abortion and infertility. For a review of the acute and long-term toxic effects of zearalenone, see Zinedine et al. [6]. A portion of the ingested zearalenone has been demonstrated to be converted into metabolites by 3β-hydroxysteroid dehydrogenase in the livers of pigs and rats [5,7,8].

2. 3β-Hydroxysteroid Dehydrogenase

The membrane-bound enzyme 3β-hydroxysteroid dehydrogenase was first described by Samuels et al. [9] in 1951 and later fully characterised by The et al. [10]. It belongs to the family of oxidoreductases, which act on the CH–OH group with NAD+ or NADP+ as an acceptor. 3β-HSD is located both in the endoplasmic reticulum and mitochondria, where it catalyses 3β-hydroxysteroid dehydrogenation and Δ5- to Δ4-isomerisation of the Δ5-steroid precursors. This was first demonstrated using plasmid transfection to induce the expression of human 3β-HSD isoenzymes [11].

3β-HSD belongs to a large protein family called the short-chain dehydrogenases/reductases, which includes many representatives with diverse functions and a wide-spread occurrence among living
organisms [12–14]. This is in contrast to 3α-HSD, which is a member of the aldo-keto reductase family, characterized by a TIM barrel fold [15–17], which is involved in oxidative cholesterol metabolism and regulated via the LXRα-receptor (a “cholesterol sensor”) [18].

3β-HSD is required for the biosynthesis of all classes of steroid hormones, such as glucocorticoids, mineralocorticoids, progesterone, androgens and estrogens [13]. Enzymes of the 3β-HSD family also catalyze the formation and/or degradation of 5α-androstanes and 5α-pregnanes [2]. Thus, 3β-HSD controls critical steroid hormone-related reactions in the adrenal cortex, gonads, placenta, liver, and other peripheral target tissues [19,20].

It is hypothesized that for every sex hormone, a pair of HSDs exists, which will either convert a potent hormone into its cognate inactive metabolite or vice-versa. HSDs often catalyse stereoselective reactions at specific positions of the steroid so that for each sex hormone there is an isoform that either will inactivate a hormone or produce an active ligand. This is achieved either by the reduction of a steroid ketone to a steroid alcohol (reductase activity) or by the oxidation of a steroid alcohol to a steroid ketone (oxidase activity) [21].

3. Subcellular Localization

3β-HSD has a dual subcellular localization in that it is found both in the endoplasmic reticulum (ER) and in the mitochondria [22,23]. Within these compartments, histochemical techniques have localized 3β-HSD activity to the smooth ER and mitochondrial cristae [24–26]. 3β-HSD’s subcellular localization pattern is unique in that it displays various degrees of ER and mitochondrial distribution. The relevance of the dual localization is unclear, yet it can be hypothesized that smooth ER-localized 3β-HSD would have greater access to cytosolic steroid precursors.

4. Transcriptional Regulation of 3β-HSD

Most studies of 3β-HSD transcriptional regulation have been conducted on human HSD genes [13]. In humans, there are two 3β-HSD isozymes encoded by the HSD3B1 and HSD3B2 genes. In mice, six 3β-HSD isozymes have been found [27], and in rats, two isoforms have been found [28]. At present, only one isoform of 3β-HSD has been described in pigs [29].

The gene regulation of 3β-HSD is complex and involves several different factors. During development, testicular 3β-HSD expression appears to be separated from luteinizing hormone (LH) regulation [30]. In early studies it was determined that cyclic adenosine monophosphate (cAMP) and protein kinase-C play a role in the regulation mechanism of 3β-HSD in human choriocarcinoma cells [31], potentially linking the mechanism to a number of endocrine and paracrine regulatory mechanisms. In adult rat testes, primary control of 3β-HSD expression occurs through the action of the LH receptor and the induction of the cAMP second messenger system. LH, forskolin, cAMP, and cholera toxin all induce 3β-HSD mRNA in adult rat Leydig cells [32,33].

The activation of the protein kinase-C pathway induces the generation of cAMP but also causes a near-complete inhibition of the stimulatory effects of human chorion gonadotropin (hCG), LH, forskolin, cholera toxin, and cAMP analogues on 3β-HSD mRNA levels in porcine granulosa cells in culture [34]. The expression of 3β-HSD in the adrenals has long been known to be influenced by adrenocorticotropic hormone and angiotensin II [35].
On the receptor level a number of mechanisms have been suggested:

- It has been demonstrated that the nuclear orphan receptor steroidogenic factor 1 (SF-1) controls the expression of human 3β-HSD type II [36]. Moreover, it has been observed that the overexpression of the SF-1 repressor DAX-1 decreases the expression of 3β-HSD [37].

- Another nuclear receptor known to influence 3β-HSD expression is fetoprotein transcription factor, also known as liver receptor homologue-1 (LRH-1, NR5A2) [38], which is found in the ovaries, testes and adipose tissue. Using a gene-reporter assay, a study demonstrated that co-transfection of LRH-1 with a reporter containing the promoter region of the 3β-HSD gene resulted in greater reporter activity [39].

- GATA4 and 6 have been demonstrated to be potential activators of the human 3β-HSD promoter [40]. Moreover, it has been demonstrated that co-transfection of GATA with SF-1 and LRH-1 induce greater promoter activation than SF-1 and LRH-1 alone [41].

- In Leydig cells, 3β-HSD transcription has been demonstrated to be regulated via the LH/hCG-receptor and the promoter androgen response element [42] and regulated in the liver via the receptors estrogen receptor α (ERα), androgen receptor (AR), and cyclin D1 expression [43]. Steroids have been demonstrated to be correlated with the expression of 3β-HSD in pig follicles [44], and in rainbow trout ovaries, 3β-HSD I is down-regulated by estradiol-17β in vivo [45]. 3β-HSD expression has also been demonstrated to be induced by peroxisome proliferator-activated receptor α (PPARα) activation in human liver cell lines [46].

- It has been demonstrated using knockout mice that the liver X receptor (LXR) represses 3β-HSD expression in very much the same manner that the constitutive androstane receptor is represses the expression of some of the CYPs. Therefore, the binding of LXR agonists can lead to increased 3β-HSD expression [47].

- Havelock et al. [48] found that follicle stimulating hormone (FSH) could stimulate the expression of nerve growth factor-induced clone B, which, in turn, up-regulates 3β-HSD type 2 in the human ovary.

Studies have suggested that androgens can inhibit androgen production by the testes and that this repression can occur at the level of 3β-HSD regulation [49]. Rats treated with hCG displayed an increase in 3β-HSD activity, but R1881 (an androgen agonist) decreased hCG induction, whereas cyproterone acetate (an androgen antagonist) increased activity [50]. Similarly, dexamethasone (a glucocorticoid agonist) addition reduced both basal and cAMP stimulatory effects on 3β-HSD mRNA, but not cytochrome P450c17 activity in mouse Leydig cells [51].

The expression of 3β-HSD is altered by several different compounds, both of endogenous and exogenous origin. The presence of steroids and their regulating factors has been demonstrated to change the expression of 3β-HSD. In a study using porcine hepatocytes isolated from intact male pigs weighing 70 and 92 kg, different response patterns to steroid treatment depending on weight were discovered. In low-weight pigs, estrone sulfate produced no effect on 3β-HSD protein expression, whereas a significant induction was present in the hepatocytes from heavy-weight pigs [52]. Similarly, androstenone produced an increase in protein expression in heavy-weight pigs, whereas it had no effect on low-weight pigs. The suggested agonistic effect of steroids on 3β-HSD expression is not consistent with in vivo finding. In pigs, both surgical castration and injection with anti-gonadotropin-releasing
hormone (immunocastration) have been demonstrated to increase the mRNA and protein expression of hepatic 3β-HSD [53]. Both castration methods lowered the circulating concentration of testosterone and androstenone.

Compounds such as glucocorticoids and interleukins have also been demonstrated to regulate 3β-HSD expression [54–56].

In summary, these findings indicate that the regulation of 3β-HSD is very complex, affected by several compounds and most likely different between tissues, different ages of organisms and species.

5. 3β-HSD Isoforms in the Liver

3β-HSD was purified from human liver by Takikawa et al. [57,58]. The adult mouse liver expresses 3β-HSD types II, III, and IV, with type III predominating. Mouse type I expression ends at postnatal Day 20, whereas type V is detected at postnatal Day 40 and is male specific, and type II is expressed at low levels throughout development. These data suggest that the mouse liver enzyme might play a key role during foetal development [59]. Rat type III is expressed in the male rat liver and has a function in inactivating steroids in this tissue [60–62].

Studies have also examined the regulation of 3β-HSD in the rodent liver. It appears that isoform-specific, sexually dimorphic regulation of 3β-HSD occurs in rats and mice [61,63].

In the mouse and rat liver, sexually dimorphic GH expression regulates many drug-metabolizing and steroid-metabolizing enzymes [64–68]. GH expression in transgenic mice down-regulates the male-specific isoform of 3β-HSD [69]. These data suggest that circulating levels of steroids might affect the regulation of 3β-HSD activity in the liver, principally through altering GH and PRL levels, thereby resulting in steroid degradation feedback. This periodic variation in GH levels as a wave-formed transmitter-receiver signaling pattern is a general principle for gender differences and should be related to intracellular receiving “oscillators” with specific resonance characters [67,68].

Immunohistochemical studies localized 3β-HSD protein to the bile duct epithelium, and 3β-HSD activity has been mostly associated with the microsomal fraction (containing e.g., ER) [70,71]. Porcine hepatic 3β-HSD cDNA has been sequenced (GenBank accession number: NM_001004049). The gene has been mapped to the chromosome 4q16–4q21 [29]. The structure of the porcine 3β-HSD gene was described by Cue et al. [72]. The protein encoded by the porcine gene displays most similarity to human 3β-HSD type I (373 aa) and murine types I, II, II and IV.

6. 3β-HSD in Testicular Leydig Cells

The fluctuations in testicular steroid synthesis might be due to the changes in steroidogenic enzymes. As we have stated above, 3β-HSD is one of the key enzymes in the biosynthesis of androgens and almost all other biologically active steroids. Therefore, high 3β-HSD activity in the testes is essential for normal steroidogenesis and subsequently for reproduction.

The majority of studies on the steroidogenic enzymes have used the Leydig cells of mice and rats as a model, whereas less information is available on the regulation of steroidogenic enzymes in porcine Leydig cells. In the Leydig cells of rats, mRNA for 3β-HSD is expressed at relatively high levels [32]. The expression of 3β-HSD during development is an indicator of testicular androgen production. Adult Leydig cells arise postnatally and encompass three developmental stages: progenitor, immature,
and adult cells [73]. Rat testes at postnatal day 15 display 3β-HSD localization to the smooth ER of precursor Leydig cells and endothelial cells near 3β-HSD-positive Leydig cells [74,75]. Pelletier et al. [76] have suggested that in the adult rat testes, 3β-HSD is restricted to the mitochondria.

Clark et al. [77] studied Leydig cells from immature pigs and demonstrated that mRNA expression for 3β-HSD in porcine Leydig cells is lower than that for the CYP side-chain cleavage enzyme and CYP 17α-hydroxylase/C17-20-lyase. The same in vitro study revealed that treatment with hCG resulted in an increase in the mRNA for 3β-HSD in a dose- and time-dependent manner, indicating the regulation of 3β-HSD by gonadotropins as well as by some steroids.

Other localization studies have examined 3β-HSD by immunocytochemistry in cynomolgus monkey testes, showing that 3β-HSD expression is observed in both Leydig and Sertoli cells [78]. Thus, the predominant site of 3β-HSD expression in the testes is in Leydig cells, but evidence exists for a parallel localization in Sertoli cells, at least in some primate species.

The presence of 3β-HSD in pig testicular cytosol was first described by Nakajin et al. [79]. The expression of 3β-HSD in pig Leydig cells was localized to the theca interna and was unaffected by follicle atresia [80]. Therefore, the steroidal regulation of 3β-HSD in pig Leydig cells may act through a mechanism separate from the androgen receptor [77]. The expression of 3β-HSD, CYP450c17 and CYP450scc have been analyzed in several porcine fetal tissues, and the expression of 3β-HSD was found to be greater in fetal testes than in the fetal adrenal gland at all stages of development [81].

Studies have been performed to determine whether 3β-HSD regulation in fetal testes might be mediated through growth factors. Epidermal growth factor (EGF), transforming growth factor β (TGF-β), activin A, and GH have been shown to increase 3β-HSD activity. It has also been demonstrated that EGF treatment of porcine Leydig cells increases 3β-HSD activity [82]. Phospholipids may also play a role in the regulation of 3β-HSD activity as demonstrated in in vitro microsomal studies [83].

7. 3β-HSD in Other Tissues

3β-HSD is expressed in a great number of tissue types in the body: adrenal, ovary, testis, placenta, liver, breast, prostate, skin, brain, kidney, cardiovascular, blood cells, and adipose [13]. Of special interest is the interplay between adrenals and gonads in steroid hormone metabolism [84].

The adrenals undergo a morphological differentiation from the fetal to the adult adrenal cortex. For non-primates, such as rats, guinea pigs and mice, there is no production of androgens in the adrenals due to the lack of cytochrome P450C17 [85]. Therefore, there are no detectable androgens in the serum in these animals when they are castrated. 3β-HSD is, however, expressed in the pig adrenals [81].

For the ovary, differential regulation of 3β-HSD plays an important role in the steroidogenic profile of ovarian tissue, and a complex interplay of pituitary and ovarian factors together maintains a tight control over the enzyme activities involved. Different species have different expression patterns during the development of the various tissues and during the oestrous cycles. The expression of 3β-HSD in pig ovaries was first described by Conley et al. [86] (for a review, see [87]). Similar to rats, 3β-HSD is localized to the theca interna layer of the pig follicle [88,89]. The pituitary control of steroidogenesis in the follicles is mainly mediated by FSH and LH, which causes an increase in cAMP and phosphatidylinositol turnover, resulting in an increase in the expression of 3β-HSD and other
steroidogenic enzymes [90–92]. Other important factors for 3β-HSD expression in the ovaries are progesterone and androgen, and the connection between the receptors for these steroids and the expression of 3β-HSD has been found at some stages in the corpus luteum development [93–95]. Prostaglandin F2α and E2 have been observed to affect the accumulation of progesterone and 3β-HSD mRNA in porcine luteinised granulosa cells [96]. In the corpus luteum, the mitochondrial 3β-HSD is especially important for steroidogenesis because of the co-localization with P450scc [97]. mRNA for 3β-HSD and other proteins has been measured in porcine follicles [88]. Increased expression throughout follicular development was observed.

Adipose tissue plays an important role in the metabolism of sex steroids and has been shown to exhibit 3β-HSD activity [98]. One 3β-HSD isoform was characterized in porcine adipose tissue [29], which strongly implies that androstenone is metabolized in adipose tissue. The observed accumulation of androstenone in adipose tissue will then depend on the balance of supply and local turn-over.

8. Dietary Influence on 3β-HSD

A major gap in the current knowledge of the role of 3β-HSD is the extent to which nutrition interacts with genetics to influence 3β-HSD expression and activity and thus steroid levels. Some dietary components, such as spearmint (Mentha spicata), have been demonstrated to have an anti-androgenic effect on male rats, including depressed 3β-HSD activity [99]. Chen et al. [100] demonstrated that androstenone levels in fat slightly decreased after the consumption of raw potato starch for 2 weeks. Dietary components most likely accelerated androstenone hepatic metabolism and therefore reduced the concentrations in fat. In another study, chicory root affected both global androstenone levels in male pigs and the levels of 3β-HSD expressed in the liver [101].

Wang et al. [102] studied the effects of dietary soybean isoflavone on zearalenone residues in the muscle and liver of young female pigs and demonstrated that addition of zearalenone into the diet increased protein expression of 3α/3β-HSD. Interestingly, the addition of isoflavones to the zearalenone-contaminated diet decreased the hepatic protein expression of 3α/3β-HSD compared with the addition of zearalenone alone. Furthermore, the isoflavones daidzein, genistein, biochanin A and formononetin have been demonstrated to inhibit bovine adrenal 3β-HSD activity [103].

Generally, dietary fats can influence the fatty acid composition of testicular lipids as well as the metabolism of testicular steroids and their concentrations in urine and plasma [104–106]. Testicular 3β-HSD in rats has been demonstrated to be affected by dietary lipid composition [107].

Drugs can also influence 3β-HSD. For example, ciprofibrate fed to male rats dramatically decreases testicular 3β-HSD activity without changing either 3β-HSD protein or mRNA expression [108], which indicates a direct inhibition on enzyme activity level.

9. The Boar Taint Phenomenon

The sensory quality of meat originating from entire non-castrated male pigs may be negatively affected by the presence of high concentrations of skatole and/or androstenone. This phenomenon is known as boar taint [109]. The major factor determining the occurrence of boar taint in pig products is the balance between the biosynthesis and catabolism of androstenone and skatole [110]. Skatole is synthesized in the intestine of pigs and catabolized in the liver [110]. Androstenone is synthesized in
the Leydig cells of the testes [111,112]. The production of androstenone is, like other sex steroids, mainly regulated by LH excreted by the pituitary gland. A portion of the androstenone is catabolized in the testes, whereas the rest is degraded in the liver. The catabolism of androstenone is conducted in two phases. Phase I is conducted by the enzymes \(3\alpha\)- and \(3\beta\)-HSD with the production of \(3\alpha\)- and \(3\beta\)-androstenol, respectively [3]. These metabolites undergo Phase II sulpho-conjugation by hydroxysteroid sulphotransferases (isoform 2A1 and 2B1). Both testicular production and liver metabolism are important in the determination of the final concentrations of androstenone in fat. The enzymes responsible for the Phase I metabolism of skatole are to a great extent regulated by the presence of androstenone [113–119]. Cue et al. [72] detected a number of polymorphisms in the porcine \(3\beta\)-HSD 5'-flanking region. However, none of these has been associated with variations in androstenone levels. This is in contrast to new results indicated that single nucleotide polymorphisms in the full-length gene have an impact on the accumulation of androstenone in Duroc pigs [120].

At present, the regulation of porcine \(3\beta\)-HSD in the testes is not fully elucidated. In principle, there are hypothetically two ways of connecting \(3\beta\)-HSD to androstenone levels in male animals: Either inhibiting testis activity and thereby decreasing androstenone synthesis or increasing liver activity and thereby increasing androstenone degradation. There are indications that this second effect can be achieved via bioactive components in the feed, e.g., chicory root [101].

Interactions between \(3\beta\)-HSD and anabolic steroids in relation to other physiological function in the animals should be established. Recent advances in biochemistry and molecular biology provide us with an excellent opportunity to increase our understanding of \(3\beta\)-HSD regulations and the methods of its manipulation without side-effects.

10. Conclusions

\(3\beta\)-HSD is essential for both steroidogenesis and steroid degradation because of its dual functionality and depending on its localization in Leydig cells or hepatocytes. The molecular regulation of the human and rodent isoenzymes is complex and involves several factors and receptors, whereas the regulatory mechanisms of the porcine \(3\beta\)-HSD are only starting to be elucidated.

The expression and activity of \(3\beta\)-HSD depend on the presence of several endogenous compounds, such as steroids, as well as exogenous toxic compounds, such as zearalenone. Moreover, dietary compounds have been demonstrated to affect the expression of \(3\beta\)-HSD in various organs, including the liver.

\(3\beta\)-HSD is a key enzyme in the metabolism of the boar taint compound androstenone in male pigs. Based on existing data, it is clear that the availability of androstenone depends on the balance between androstenone biosynthesis in the testes and the activities of \(3\beta\)-HSD in the liver.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Payne, A.H.; Clarke, T.R.; Bain, P.A. The murine 3β-hydroxysteroid dehydrogenase multigene family: Structure, function and tissue-specific expression. *J. Steroid Biochem. Mol. Biol.* **1995**, *53*, 111–118.

2. Payne, A.H.; Abbaszade, I.G.; Clarke, T.R.; Bain, P.A.; Park, C.H. The multiple murine 3β-hydroxysteroid dehydrogenase isoforms: Structure, function, and tissue- and developmentally specific expression. *Steroids* **1997**, *6*, 169–175.

3. Doran, E.; Whittington, F.M.; Wood, J.D.; McGivan, J.D. Characterisation of androstenone metabolism in pig liver microsomes. *Chem. Biol. Interact.* **2004**, *141*, 141–149.

4. Hu, G.X.; Zhao, B.H.; Chu, Y.H.; Zhou, H.Y.; Akingbemi, B.T.; Zheng, Z.Q.; Ge, R.S. Effects of genistein and equol on human and rat testicular 3 beta-hydroxysteroid dehydrogenase and 17 beta-hydroxysteroid dehydrogenase 3 activities. *Asian J. Androl.* **2010**, *4*, 519–526.

5. Fink-Gremmels, J.; Malekinejad, H. Clinical effects and biochemical mechanisms associated with exposure to the mycoestrogen zearalenone. *Anim. Feed Sci. Technol.* **2007**, *137*, 326–341.

6. Zinedine, A.; Soriano, J.M.; Moltó, J.C.; Mañes, J. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food Chem. Toxicol.* **2007**, *45*, 1–18.

7. Kiessling, K.H.; Pettersson, H. Metabolism of zearalenone in rat-liver. *Acta Pharm Toxicol.* **1978**, *4*, 285–290.

8. Hasson, M.; Roel, F.M.-B.; Johanna, F. Bioactivation of zearalenone by porcine hepatic biotransformation. *Vet. Res.* **2005**, *36*, 799–810.

9. Samuels, L.T.; Helmreich, M.L.; Lasater, M.B.; Reich, H. An enzyme in endocrine tissues which oxidizes delta 5–3 hydroxy steroids to α, β unsaturated ketones. *Science* **1951**, *2939*, 490–491.

10. The, V.L.; Lachance, Y.; Labrie, C.; Leblanc, G.; Thomas, J.L.; Strickler, R.C.; Labrie, F. Full length cDNA structure and deduced amino acid sequence of human β-hydroxy-5-ene steroid dehydrogenase. *Mol. Endocrinol.* **1989**, *5*, 1310–1312.

11. Lorence, M.C.; Murry, B.A.; Trant, J.M.; Mason, J.I. Human 3β-hydroxysteroid dehydrogenase/5-4 isomerase from placenta: Expression in nonsteroidogenic cells of a protein that catalyzes the dehydrogenation/isomerization of C21 and C19 steroids. *Endocrinology* **1990**, *5*, 2493–2498.

12. Kallberg, Y.; Oppermann, U.; Jörnvall, H.; Persson, B. Short-chain dehydrogenase/reductase (SDR) relationships: A large family with eight clusters common to human, animal, and plant genomes. *Protein Sci.* **2002**, *3*, 636–641.

13. Simard, J.; Ricketts, M.L.; Gingras, S.; Soucy, P.; Feltus, F.A.; Melner, M.H. Molecular biology of the 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase gene family. *Endocr. Rev.* **2005**, *4*, 525–582.

14. Joernvall, H.; Persson, B.; Krook, M.; Atrian, S.; Gonzalez-Duarte, R.; Jeffery, J.; Ghosh, D. Short-chain dehydrogenases/reductases (SDR). *Biochemistry* **1995**, *18*, 6003–6013.
15. Banner, D.W.; Bloomer, A.C.; Petsko, G.A.; Phillips, D.C.; Pogson, C.I.; Wilson, I.A.; Corran, P.H.; Furth, A.J.; Milman, J.D.; Offord, R.E.; et al. Structure of chicken muscle triose phosphate isomerase determined crystallographically at 2.5[Å] resolution: Using amino acid sequence data. *Nature* 1975, 5510, 609–614.

16. Hoog, S.S.; Pawlowski, J.E.; Alzari, P.M.; Penning, T.M.; Lewis, M. Three-dimensional structure of rat liver 3 alpha-hydroxysteroid/dihydrodiol dehydrogenase: A member of the aldo-keto reductase superfamily. *Proc. Natl. Acad. Sci. USA* 1994, 7, 2517–2521.

17. Mizrachi, D.; Auchus, R.J. Androgens, estrogens, and hydroxysteroid dehydrogenases. *Mol. Cell. Endocrinol.* 2009, 301, 37–42.

18. Stayrook, K.R.; Rogers, P.M.; Savkur, R.S.; Wang, Y.; Su, C.; Varga, G.; Bu, X.; Wei, T.; Nagpal, S.; Liu, X.S.; et al. Regulation of human 3β-hydroxysteroid dehydrogenase (AKR1C4) expression by the liver X receptor α. *Mol. Pharm.* 2008, 2, 607–612.

19. Mason, J.I.; Naville, D.; Evans, B.W.; Thomas, J.L. Functional activity of 3beta-hydroxysteroid dehydrogenase/isomerase. *Endocr. Res.* 1998, 24, 549–557.

20. Labrie, F.; Luu-The, V.; Lin, S.X.; Simard, J.; Labrie, C.; El-Alfy, M.; Pelletier, G.; Belanger, A. Intracrinology: Role of the family of 17 beta-hydroxysteroid dehydrogenases in human physiology and disease. *J. Mol. Endocrinol.* 2000, 1, 1–16.

21. Penning, T.M. Hydroxysteroid dehydrogenases and pre-receptor regulation of steroid hormone action. *Hum. Reprod. Update* 2003, 3, 193–205.

22. Cherradi, N.; Defaye, G.; Chambaz, E.M. Dual subcellular localization of the 3β-hydroxysteroid dehydrogenase isomerase: Characterization of the mitochondrial enzyme in the bovine adrenal cortex. *J. Steroid Biochem. Mol. Biol.* 1993, 6, 773–779.

23. Sauer, L.A.; Chapman, J.C.; Dauchy, R.T. Topology of 3 beta-hydroxy-5-ene-steroid dehydrogenase/delta 5-delta 4-isomerase in adrenal cortex mitochondria and microsomes. *Endocrinology* 1994, 2, 751–759.

24. Berchtold, J.P. Ultracytochemical demonstration and probable localization of 3β-hydroxysteroid dehydrogenase activity with a ferricyanide technique. *Histochemistry* 1977, 3, 175–190.

25. Headon, D.R.; Hsiao, J.; Ungar, F. The intracellular localization of adrenal 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase by density gradient perturbation. *Biochem. Biophys. Res. Commun.* 1978, 3, 1006–1012.

26. Dupont, E.; Luu-The, V.; Labrie, F.; Pelletier, G. Light microscopic immuno cytocytochemical localization of 3β-hydroxy-5-ene-steroid dehydrogenase/Δ5-Δ4-isomerase in the gonads and adrenal glands of the guinea pig. *Endocrinology* 1990, 6, 2906–2909.

27. Abbaszade, I.G.; Arensburg, J.; Park, C.H.; Kasa-Vubu, J.Z.; Orly, J.; Payne, A.H. Isolation of a new mouse β-Hydroxysteroid dehydrogenase isoform, 3β-HSD VI, expressed during early pregnancy. *Endocrinology* 1997, 4, 1392–1399.

28. Simard, J.; Couet, J.; Durocher, F.; Labrie, Y.; Sanchez, R.; Breton, N.; Turgeon, C.; Labrie, F. Structure and tissue-specific expression of a novel member of the rat 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase (3 beta-HSD) family. The exclusive 3 beta-HSD gene expression in the skin. *J. Biol. Chem.* 1993, 26, 19659–19668.
29. Von Teichman, A.; Joerg, H.; Werner, P.; Brenig, B.; Stranzinger, G. cDNA cloning and physical mapping of porcine 3β-hydroxysteroid dehydrogenase/Δ⁵-Δ⁴ isomerase. *Anim. Genet.* **2001**, *5*, 298–302.

30. Siiteri, P.K.; Wilson, J.D. Testosterone formation and metabolism during male sexual differentiation in the human embryo. *J. Clin. Endocrinol. Metab.* **1974**, *1*, 113–125.

31. Tremblay, Y.; Beaudoin, C. Regulation of 3 beta-hydroxysteroid dehydrogenase and 17 beta-hydroxysteroid dehydrogenase messenger ribonucleic acid levels by cyclic adenosine 3′,5′-monophosphate and phorbol myristate acetate in human choriocarcinoma cells. *Mol. Endocrinol.* **1993**, *3*, 355–364.

32. Keeney, D.S.; Mason, J.I. Expression of testicular 3 beta-hydroxysteroid dehydrogenase/delta 5–4-isomerase: Regulation by luteinizing hormone and forskolin in Leydig cells of adult rats. *Endocrinology* **1992**, *11*, 2007–2015.

33. Saez, J.M. Leydig cells: Endocrine, paracrine, and autocrine regulation. *Endocr. Rev.* **1994**, *5*, 574–626.

34. Chedrese, P.J.; Zhang, D.; The, V.L.; Labrie, F.; Juorio, A.V.; Murphy, B.D. Regulation of mRNA expression of 3β-hydroxy-5-ene steroid dehydrogenase in porcine granulosa cells in culture: A role for the protein kinase-c pathway. *Mol. Endocrinol.* **1990**, *12*, 1532–1538.

35. Coulter, C.L.; Goldsmith, P.C.; Mesiano, S.; Voytek, C.C.; Martin, M.C.; Mason, J.I.; Jaffe, R.B. Functional maturation of the primate fetal adrenal *in vivo*. II. Ontogeny of corticosteroid synthesis is dependent upon specific zonal expression of 3 beta-hydroxysteroid dehydrogenase/isomerase. *Endocrinology* **1996**, *11*, 4953–4959.

36. Leers-Sucheta, S.; Morohashi, K.I.; Mason, I.; Melner, M.H. Synergistic activation of the human type II 3β-Hydroxysteroid dehydrogenase/Δ⁵-Δ⁴ isomerase promoter by the transcription factor steroidogenic factor-1/adrenal 4-binding protein and phorbol ester. *J. Biol. Chem.* **1997**, *12*, 7960–7967.

37. Val, P.; Lefrancois-Martinez, A.M.; Veyssiere, G.; Martinez, A. SF-1 a key player in the development and differentiation of steroidogenic tissues. *Nucl. Recept.* **2003**, *1*, 8.

38. Labelle-Dumais, C.; Paré, J.F.; Bélanger, L.; Farookhi, R.; Dufort, D. Impaired progesterone production in *Nr5a2*−/− mice leads to a reduction in female reproductive function. *Biol. Reprod.* **2007**, *2*, 217–225.

39. Sirianni, R.; Seely, J.B.; Attia, G.; Stooco, D.M.; Carr, B.R.; Pezzi, V.; Rainey, W.E. Liver receptor homologue-1 is expressed in human steroidogenic tissues and activates transcription of genes encoding steroidogenic enzymes. *J. Endocrinol.* **2002**, *3*, R13–R17.

40. LaVoie, H.A.; King, S.R. Transcriptional regulation of steroidogenic genes: *STARD1*, *CYP11A1* and *HSD3B*. *Exp. Biol. Med.* **2009**, *8*, 880–907.

41. Martin, L.J.; Taniguchi, H.; Robert, N.M.; Simard, J.; Tremblay, J.J.; Viger, R.S. GATA factors and the nuclear receptors, steroidogenic factor 1/liver receptor homolog 1, are key mutual partners in the regulation of the human 3β-hydroxysteroid dehydrogenase type 2 promoter. *Mol. Endocrinol.* **2005**, *9*, 2358–2370.

42. Tang, P.Z.; Tsai-Morris, C.H.; Dufau, M.L. Regulation of 3β-hydroxysteroid dehydrogenase in gonadotropin-induced steroidogenic desensitization of Leydig cells. *Endocrinology* **1998**, *11*, 4496–4505.
43. Mullany, L.K.; Hanse, E.A.; Romano, A.; Blomquist, C.H.; Mason, I.; Delvoux, B.; Anttila, C.; Albrecht, J.H. Cyclin D1 regulates hepatic estrogen and androgen metabolism. *Am. J. Physiol.* 2010, 6, G884–G895.

44. Sun, Y.L.; Zhang, J.; Ping, Z.G.; Fan, L.N.; Wang, C.Q.; Li, W.H.; Lu, C.; Zheng, L.W.; Zhou, X. Expression of 3 beta-hydroxysteroid dehydrogenase (3 beta-HSD) in normal and cystic follicles in sows. *Afr. J. Biotechnol.* 2011, 11, 6184–6189.

45. Nakamura, I.; Kusakabe, M.; Young, G. Differential suppressive effects of low physiological doses of estradiol-17 beta in vivo on levels of mRNAs encoding steroidogenic acute regulatory protein and three steroidogenic enzymes in previtellogenic ovarian follicles of rainbow trout. *Gen. Comp. Endocrinol.* 2009, 3, 318–323.

46. Matsunaga, T.; Endo, S.; Maeda, S.; Ishikura, S.; Tajima, K.; Tanaka, N.; Nakamura, K.T.; Imamura, Y.; Hara, A. Characterization of human DHRS4: An inducible short-chain dehydrogenase/reductase enzyme with 3 beta-hydroxysteroid dehydrogenase activity. *Arch. Biochem. Biophys.* 2008, 2, 339–347.

47. Beltowski, J.; Semczuk, A. Liver X receptor (LXR) and the reproductive system—A potential novel target for therapeutic intervention. *Pharm. Rep.* 2010, 1, 15–27.

48. Havelock, J.C.; Smith, A.L.; Seely, J.B.; Dooley, C.A.; Rodgers, R.J.; Rainey, W.E.; Carr, B.R. The NGFI-B family of transcription factors regulates expression of 3 beta-hydroxysteroid dehydrogenase type 2 in the human ovary. *Mol. Hum. Reprod.* 2005, 2, 79–85.

49. Kostic, T.S.; Stojkov, N.J.; Bjelic, M.M.; Mihajlovic, A.I.; Janjic, M.M.; Andric, S.A. Pharmacological doses of testosterone upregulated androgen receptor and 3-beta-hydroxysteroid dehydrogenase/delta-5-delta-4 isomerase and impaired Leydig cells steroidogenesis in adult rats. *Toxicol. Sci.* 2011, 2, 397–407.

50. Galarreta, C.; Fanjul, L.; Adashi, E.; Hsueh, A. Regulation of 3-beta-hydroxysteroid dehydrogenase activity by human chorionic gonadotropin, androgens, and antiandrogens in cultured testicular cells. *Ann. N. Y. Acad. Sci.* 1984, 1, 663–665.

51. Payne, A.H.; Sha, L.I. Multiple mechanisms for regulation of 3 beta-hydroxysteroid dehydrogenase/d5-d4-isomerase, 17a-hydroxylase/c17-20 lyase cytochrome p450, and cholesterol side-chain cleavage cytochrome p450 messenger ribonucleic acid levels in primary cultures of mouse Leydig cells. *Endocrinology* 1991, 3, 1429–1435.

52. Nicolau-Solano, S.I.; Doran, O. Effect of testosterone, estrone sulphate and androstenone on 3beta-hydroxysteroid dehydrogenase protein expression in primary cultured hepatocytes. *Livest. Sci.* 2008, 114, 202–210.

53. Rasmussen, M.; Brunius, C.; Ekstrand, B.; Zamaratskaia, G. Expression of hepatic 3beta-hydroxysteroid dehydrogenase and sulfotransferase 2A1 in entire and castrated male pigs. *Mol. Biol. Rep. Rep.* 2012, 7927–7932.

54. Gingras, S.; Moriggl, R.; Groner, B.; Simard, J. Induction of 3beta-hydroxysteroid dehydrogenase/D5-D4 isomerase type 1 gene transcription in human breast cancer cell lines and in normal mammary epithelial cells by interleukin-4 and interleukin-13. *Mol. Endocrinol.* 1999, 1, 66–81.
55. Gingras, S.; Côté, S.; Simard, J. Multiple signaling pathways mediate interleukin-4-induced 3β-hydroxysteroid dehydrogenase/Δ⁵-Δ⁴ isomerase type 1 gene expression in human breast cancer cells. *Mol. Endocrinol.* 2000, 2, 229–240.

56. Papacleovoulou, G.; Hogg, K.; Fegan, K.S.; Critchley, H.O.D.; Hillier, S.G.; Mason, J.I. Regulation of 3β-hydroxysteroid dehydrogenase type 1 and type 2 gene expression and function in the human ovarian surface epithelium by cytokines. *Mol. Hum. Reprod.* 2009, 6, 379–392.

57. Takikawa, H.; Stolz, A.; Sugiyama, Y.; Yoshida, H.; Yamanaka, M.; Kaplowitz, N. Relationship between the newly identified bile-acid binder and bile-acid oxidoreductases in human-liver. *J. Biol. Chem.* 1990, 4, 2132–2136.

58. Takikawa, H.; Fujiyoshi, M.; Nishikawa, K.; Yamanaka, M. Purification of 3-alpha-hydroxysteroid and 3-beta-hydroxysteroid dehydrogenases from human liver cytosol. *Hepatology* 1992, 2, 365–371.

59. Park, C.H.; Abbaszade, I.G.; Payne, A.H. Expression of multiple forms of 3β-hydroxysteroid dehydrogenase in the mouse liver during fetal and postnatal development. *Mol. Cell. Endocrinol.* 1996, 2, 157–164.

60. Zhao, H.F.; Rhgaurae, E.; Trudel, C.; Couet, J.; Labrie, F.; Simard, J. Structure and sexual dimorphic expression of a liver-specific rat 3β-hydroxysteroid dehydrogenase/isomerase. *Endocrinology* 1990, 6, 3237–3239.

61. Couet, J.; Simard, J.; Martel, C.; Trudel, C.; Labrie, Y.; Labrie, F. Regulation of 3-ketosteroid reductase messenger ribonucleic acid levels and 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase activity in rat liver by sex steroids and pituitary hormones. *Endocrinology* 1992, 6, 3034–3044.

62. De Launoit, Y.; Zhao, H.F.; Belanger, A.; Labrie, F.; Simard, J. Expression of liver-specific member of the 3 beta-hydroxysteroid dehydrogenase family, an isoform possessing an almost exclusive 3-ketosteroid reductase activity. *J. Biol. Chem.* 1992, 7, 4513–4517.

63. Naville, D.; Keeney, D.S.; Jenkin, G.; Murry, B.A.; Head, J.R.; Mason, J.I. Regulation of expression of male-specific rat liver microsomal 3β-hydroxysteroid dehydrogenase. *Mol. Endocrinol.* 1991, 8, 1090–1100.

64. Shapiro, B.H.; Agrawal, A.K.; Pampori, N.A. Gender differences in drug metabolism regulated by growth hormone. *Int. J. Biochem. Cell Biol.* 1995, 1, 9–20.

65. Mason, J.I.; Keeney, D.S.; Bird, I.M.; Rainey, W.E.; Morohashi, K.I.; Leers-Sucheta, S.; Melner, M.H. The regulation of 3β-hydroxysteroid dehydrogenase expression. *Steroids* 1997, 1, 164–168.

66. Mode, A.; Gustafsson, J. Sex and the liver—A journey through five decades. *Drug Metab. Rev.* 2006, 38, 197–207.

67. Waxman, D.J.; Holloway, M.G. Sex differences in the expression of hepatic drug metabolizing enzymes. *Mol. Pharm.* 2009, 2, 215–228.

68. Waxman, D.J.; O’Connor, C. Growth hormone regulation of sex-dependent liver gene expression. *Mol. Endocrinol.* 2006, 11, 2613–2629.

69. Keeney, D.S.; Murry, B.A.; Bartke, A.; Wagner, T.E.; Mason, J.I. Growth hormone transgenes regulate the expression of sex-specific isoforms of 3 beta-hydroxysteroid dehydrogenase/Δ⁵-Δ⁴-isomerase in mouse liver and gonads. *Endocrinology* 1993, 3, 1131–1138.
70. Furster, C.; Zhang, J.; Toll, A. Purification of a 3β-hydroxy-Δ5-C27-steroid dehydrogenase from pig liver microsomes active in major and alternative pathways of bile acid biosynthesis. *J. Biol. Chem.* 1996, 34, 20903–20907.

71. Furster, C. Hepatic and extrahepatic dehydrogenation/isomerization of 5-cholesten-3β,7α-diol: localization of 3β-hydroxy-Δ5-C27-steroid dehydrogenase in pig tissues and subcellular fractions. *Biochim. Biophys. Acta* 1999, 3, 343–353.

72. Cue, R.A.; Nicolau-Solano, S.I.; McGivan, J.D.; Wood, J.D.; Doran, O. Breed-associated variations in the sequence of the pig 3β-hydroxysteroid dehydrogenase gene. *J. Anim. Sci.* 2007, 3, 571–576.

73. Benton, L.; Shan, L.X.; Hardy, M.P. Differentiation of adult Leydig cells. *J. Steroid Biochem. Mol. Biol.* 1995, 53, 61–68.

74. Majdic, G.; Millar, M.R.; Saunders, P.T.K. Immunolocalisation of androgen receptor to interstitial cells in fetal rat testes and to mesenchymal and epithelial cells of associated ducts. *J. Endocrinol.* 1995, 2, 285–293.

75. Haider, S.G.; Servos, G. Ultracytochemistry of 3 beta-hydroxysteroid dehydrogenase in Leydig cell precursors and vascular endothelial cells of the postnatal rat testis. *Anat. Embryol.* 1998, 2, 101–110.

76. Pelletier, G.; Li, S.; Luu-The, V.; Tremblay, Y.; Belanger, A.; Labrie, F. Immunoelectron microscopic localization of three key steroidogenic enzymes (cytochrome P450(scc), 3 beta-hydroxysteroid dehydrogenase and cytochrome P450(c17)) in rat adrenal cortex and gonads. *J. Endocrinol.* 2001, 2, 373–383.

77. Clark, A.M.; Chuzel, F.; Sanchez, P.; Saez, J.M. Regulation by gonadotropins of the messenger ribonucleic acid for P450 side-chain cleavage, P450(17) alpha-hydroxylase/C17,20-lyase, and 3 beta-hydroxysteroid dehydrogenase in cultured pig Leydig cells. *Biol. Reprod.* 1996, 2, 347–354.

78. Liang, J.H.; Sankai, T.; Yoshida, T.; Cho, F.; Yoshikawa, Y. Localization of testosterone and 3 beta-hydroxysteroid dehydrogenase Delta(5)-Delta(4)-isomerase in cynomolgus monkey (Macaca fascicularis) testes. *J. Med. Primatol.* 1998, 1, 10–14.

79. Nakajin, S.; Ishii, A.; Shinoda, M. Purification and characterization of 5-alpha-dihydrotestosterone 3-beta-hydroxysteroid dehydrogenase from mature pig testicular cytosol. *Biol. Pharm. Bull.* 1994, 9, 1155–1160.

80. Garrett, W.M.; Guthrie, H.D. Expression of androgen receptors and steroidogenic enzymes in relation to follicular growth and atresia following ovulation in pigs. *Biol. Reprod.* 1996, 5, 949–955.

81. Conley, A.J.; Rainey, W.E.; Mason, J.I. Ontogeny of steroidogenic enzyme expression in the porcine conceptus. *J. Mol. Endocrinol.* 1994, 2, 155–165.

82. Sordoillet, M.; Chauvin, M.; Peretti, E.; Morera, A.; Benahmed, M. Epidermal growth factor directly stimulates steroidogenesis in primary cultures of porcine Leydig cells: Actions and Sites of Action. *Endocrinology* 1991, 4, 2160–2168.

83. Cooke, G.M. Identification of phospholipids capable of modulating the activities of some enzymes involved in androgen and 16-androstene biosynthesis in the immature pig testis. *J. Steroid Biochem. Mol. Biol.* 1992, 2, 151–159.
84. Conley, A.J.; Bird, I.M. The role of cytochrome P450 17 alpha-hydroxylase and 3 beta-hydroxysteroid dehydrogenase in the integration of gonadal and adrenal steroidogenesis via the delta 5 and delta 4 pathways of steroidogenesis in mammals. *Biol. Reprod.* **1997**, *4*, 789–799.

85. Belanger, B.; Belanger, A.; Labrie, F.; Dupont, A.; Cusan, L.; Monfette, G. Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: Unique importance of extratesticular androgens in men. *J. Steroid Biochem.* **1989**, *5*, 695–698.

86. Conley, A.J.; Kaminski, M.A.; Dubowsky, S.A.; Jablonka-Shariff, A.; Redmer, D.A.; Reynolds, L.P. Immunohistochemical localization of 3 beta-hydroxysteroid dehydrogenase and P450 17 alpha-hydroxylase during follicular and luteal development in pigs, sheep, and cows. *Biol. Reprod.* **1995**, *5*, 1081–1094.

87. Kozłowska, A.; Majewski, M.; Jana, B. Expression of steroidogenic enzymes in porcine polycystic ovaries. *Folia Histochem. Cytobiol.* **2009**, *2*, 257–264.

88. Yuan, W.; Lucy, M.C. Messenger ribonucleic acid expression for growth hormone receptor, luteinizing hormone receptor, and steroidogenic enzymes during the estrous cycle and pregnancy in porcine and bovine corpora lutea. *Domest. Anim. Endocrinol.* **1996**, *5*, 431–444.

89. Garrett, W.M.; Guthrie, H.D. Steroidogenic enzyme expression during preovulatory follicle maturation in pigs. *Biol. Reprod.* **1997**, *6*, 1424–1431.

90. Picon, R.; Darmoul, D.; Rouiller, V.; Duranteau, L. Activity of 3β-hydroxysteroid dehydrogenase/isomerase in the fetal rat ovary. *J. Steroid Biochem.* **1988**, *5*, 839–843.

91. McAllister, J.M.; Kerin, J.F.P.; Trant, J.M.; Estabrook, R.W.; Mason, J.I.; Waterman, M.R.; Simpson, E.R. Regulation of cholesterol side-chain cleavage and 17α-hydroxylase/lyase activities in proliferating human theca interna cells in long term monolayer culture. *Endocrinology* **1989**, *4*, 2192–2200.

92. Kaynard, A.H.; Periman, L.M.; Simard, J.; Melner, M.H. Ovarian 3 beta-hydroxysteroid dehydrogenase and sulfated glycoprotein-2 gene expression are differentially regulated by the induction of ovulation, pseudopregnancy, and luteolysis in the immature rat. *Endocrinology* **1992**, *4*, 2192–2200.

93. Hild-Petito, S.; West, N.B.; Brenner, R.M.; Stouffer, R.L. Localization of androgen receptor in the follicle and corpus luteum of the primate ovary during the menstrual cycle. *Biol. Reprod.* **1991**, *3*, 561–568.

94. Suzuki, T.; Sasano, H.; Kimura, N.; Tamura, M.; Fukaya, T.; Yajima, A.; Nagura, H. Physiology: Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: relationship to expression of steroidogenic enzymes. *Hum. Reprod.* **1994**, *9*, 1589–1595.

95. Sasano, H.; Suzuki, T. Localization of steroidogenesis and steroid receptors in human corpus luteum—Classification of human corpus luteum (CL) into estrogen-producing CL, steroid-producing degenerating CL, and nonsteroid-producing degenerating CL. *Semin. Reprod. Endocrinol.* **1997**, *4*, 345–351.

96. Li, X.M.; Juorio, A.V.; Murphy, B.D. Prostaglandins alter the abundance of messenger ribonucleic acid for steroidogenic enzymes in cultured porcine granulosa cells. *Biol. Reprod.* **1993**, *6*, 1360–1366.
97. Chapman, J.; Polanco, J.; Min, S.; Michael, S. Mitochondrial 3 beta-hydroxysteroid dehydrogenase (HSD) is essential for the synthesis of progesterone by corpora lutea: An hypothesis. Reprod. Biol. Endocrinol. 2005, 1, 11.

98. Kershaw, E.E.; Flier, J.S. Adipose tissue as an endocrine organ. J. Clin. Endocrinol. Metab. 2004, 6, 2548–2556.

99. Kumar, V.; Kural, M.R.; Pereira, B.M.J.; Roy, P. Spearmint induced hypothalamic oxidative stress and testicular anti-androgenicity in male rats—Altered levels of gene expression, enzymes and hormones. Food Chem. Toxicol. 2008, 12, 3563–3570.

100. Chen, G.; Bourneuf, E.; Marklund, S.; Zamaratskaia, G.; Madej, A.; Lundström, K. Gene expression of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase in relation to androstenone, testosterone, and estrone sulphate in gonadally intact male and castrated pigs. J. Anim. Sci. 2007, 10, 2457–2463.

101. Rasmussen, M.K.; Brunius, C.; Zamaratskaia, G.; Ekstrand, B. Feeding dried chicory root to pigs decrease androstenone accumulation in fat by increasing hepatic 3β hydroxysteroid dehydrogenase expression. J. Steroid Biochem. Mol. Biol. 2012, 130, 90–95.

102. Wang, D.F.; Zhou, H.L.; Hou, G.Y.; Qi, D.S.; Zhang, N.Y. Soybean isoflavone reduces the residue of zearalenone in the muscle and liver of prepubertal gilts. Animal 2013, 4, 699–703.

103. Wong, C.K.; Keung, W.M. Bovine adrenal 3β-hydroxysteroid dehydrogenase (E.C. 1.1.1.145)/5-ene-4-ene isomerase (E.C. 5.3.3.1): characterization and its inhibition by isoflavones. J. Steroid Biochem. Mol. Biol. 1999, 71, 191–202.

104. Coniglio, J.G. Testicular lipids. Prog. Lipid Res. 1994, 4, 387–401.

105. Gromadzka-Ostrowska, J.; Przepiorka, M.; Romanowicz, K. Influence of dietary fatty acids composition, level of dietary fat and feeding period on some parameters of androgen metabolism in male rats. Reprod. Biol. 2002, 3, 277–293.

106. Dorgan, J.F.; Judd, J.T.; Longcope, C.; Brown, C.; Schatzkin, A.; Clevidence, B.A.; Campbell, W.S.; Nair, P.P.; Franz, C.; Kahle, L.; et al. Effects of dietary fat and fiber on plasma and urine androgens and estrogens in men: A controlled feeding study. Am. J. Clin. Nutr. 1996, 6, 850–855.

107. Catalfo, G.; Alaniz, M.; Marra, C. Influence of commercial dietary oils on lipid composition and testosterone production in interstitial cells isolated from rat testis. Lipids 2009, 4, 345–357.

108. Hierlihy, A.M.; Cooke, G.M.; Curran, I.H.A.; Mehta, R.; Karamanos, L.; Price, C.A. Effects of ciprofibrate on testicular and adrenal steroidogenic enzymes in the rat. Reprod. Toxicol. 2006, 1, 37–43.

109. Walstra, P.; Claudi-Magnussen, C.; Chevillon, P.; von Seth, G.; Diestre, A.; Matthews, K.R.; Homer, D.B.; Bonneau, M. An international study on the importance of androstenone and skatole for boar taint: levels of androstenone and skatole by country and season. Livest. Prod. Sci. 1999, 1, 15–28.

110. Zamaratskaia, G.; Squires, E.J. Biochemical, nutritional and genetic effects on boar taint in entire male pigs. Animal 2009, 11, 1508–1521.

111. Meadus, W.J.; Mason, J.I.; Squires, E.J. Cytochrome P450c17 from porcine and bovine adrenal catalyses the formation of 5,16-androstadien-3β-ol from pregnenolone in the presence of cytochrome b5. J. Steroid Biochem. Mol. Biol. 1993, 5, 565–572.
112. Davis, S.M.; Squires, E.J. Association of cytochrome b5 with 16-androstene steroid synthesis in the testis and accumulation in the fat of male pigs. *J. Anim. Sci.* 1999, 5, 1230–1235.

113. Brunius, C.; Rasmussen, M.K.; Lacoutière, H.; Andersson, K.; Ekstrand, B.; Zamaratskaia, G. Expression and activities of hepatic cytochrome P450 (CYP1A, CYP2A and CYP2E1) in entire and castrated male pigs. *Animal* 2012, 2, 271–277.

114. Chen, G.; Cue, R.A.; Lundstrom, K.; Wood, J.D.; Doran, O. Regulation of CYP2A6 protein expression by skatole, indole, and testicular steroids in primary cultured pig hepatocytes. *Drug Metab. Dispos.* 2008, 1, 56–60.

115. Doran, E.; Whittington, F.W.; Wood, J.D.; McGivan, J.D. Cytochrome P450IIIE1 (CYP2E1) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes. *Chem.-Biol. Interac.* 2002, 1, 81–92.

116. Tomankova, J.; Rasmussen, M.K.; Andersson, K.; Ekstrand, B.; Zamaratskaia, G. Improvac does not modify the expression and activities of the major drug metabolizing enzymes cytochrome P450 3A and 2C in pigs. *Vaccine* 2012, 24, 3515–3518.

117. Rasmussen, M.K.; Zamaratskaia, G.; Ekstrand, B. Gender-related differences in cytochrome P450 in porcine liver—Implication for activity, expression and inhibition by testicular steroids. *Reprod. Domest. Anim.* 2011, 1, 616–623.

118. Rasmussen, M.K.; Zamaratskaia, G.; Ekstrand, B. *In vitro* cytochrome P450 2E1 and 2A activities in the presence of testicular steroids. *Reprod. Domest. Anim.* 2011, 1, 149–154.

119. Zamaratskaia, G.; Gilmore, W.J.; Lundstrom, K.; Squires, E.J. Effect of testicular steroids on catalytic activities of cytochrome P450 enzymes in porcine liver microsomes. *Food Chem. Toxicol.* 2007, 4, 676–681.

120. Kim, H.; Lee, S.K.; Hong, M.W.; Park, S.R.; Lee, Y.S.; Kim, J.W.; Lee, H.K.; Jeong, D.K.; Song, Y.H.; Lee, S.J. Association of a single nucleotide polymorphism in the akirin 2 gene with economically important traits in Korean native cattle. *Anim. Genet.* 2013, doi:10.1111/age.12055.

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