Expression of Oestrogen Receptors $\alpha$ and $\beta$
and of Aromatase in the Testis of Immature
and Mature Boars

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The boar testis secretes high amounts of oestrogens. In order to test for a likely local significance, we investigated the expression of oestrogen receptors (ER) in immature and mature boar testes using immunohistochemistry (IHC), in vitro and in situ reverse transcription-polymerase chain reaction (RT-PCR). Samples were from 25 boars castrated at ages of 50, 100, 150, 200 and 250 days. Mouse monoclonal primary antibodies against porcine ERα (clone HT227), human ERβ1 (clone PPG5/10) and human P450 aromatase (clone SM1671P) were used. Expression of the mRNA was tested utilizing primers specific for the respective porcine mRNA sequences. ER immunoreactivity was exclusively localized to the nuclei. In immature boars, 90.6 ± 1.2% of prespermatogonia and 71.0 ± 2.6% of the Leydig cells showed a strong staining for ERα; 95.5 ± 3.5% of the prespermatogonia but none of the Leydig and Sertoli cells were ERβ-positive. In mature boars a strong staining for the ERβ was observed in virtually all Sertoli, Leydig and germ cells, except for the elongating/ed spermatids, which were clearly negative; for the ERα, strong immunoreaction signals were restricted to spermatogonia and primary spermatocytes with 93.6 ± 2.7% of these cells being positive; distinctly less intensive signals were observed in 51.4 ± 0.27% of the secondary spermatocytes, round spermatids and Leydig cells. In vitro RT-PCR was positive for both receptors and results of in situ RT-PCR matched those obtained by IHC. P450 aromatase immunoreaction was restricted to the cytoplasm of Leydig cells. These findings suggest that testicular ER may be important factors contributing to onset and maintenance of spermatogenesis in the boar.

Introduction
Manifestation of genomic oestrogenic activity is dependent upon ligand interaction with nuclear oestrogen receptors (ER) in target cells. Both, the ERα and ERβ, are widely distributed in the epididymis of the male reproductive tract (Sharpe 1997; Hess et al. 1997, 2001; O'Donnell et al. 2001; Carreau et al. 2003; Hess 2003; Hess and Carnes 2004). They are also expressed in the testis as was shown in humans (Enmark et al. 1997; Durkee et al. 1997; Saunders et al. 2001; Carreau et al. 2003; Hess 2003; Hess and Carnes 2004). With the ERβ being the predominant receptor isotype in most of these species, localization of ERα seemed to be restricted to the Leydig cells in dogs and cats (Nie et al. 2002) and was present also in germ cells in the rat (Lubahn et al. 1993; Saunders et al. 1998; Oliveira et al. 2004). The ER are ligand-dependent transcription factors that regulate expression of target genes containing ER responsive elements (Parker 1995; Katzenellenbogen and Katzenellenbogen 2000; Robyr et al. 2000). The two ER isoforms are highly homologous in their DNA binding sites (over 90%) while homogeneity in their ligand-binding domain is <60% (Cowley et al. 1997); they form homodimers and heterodimers during their action which exhibit a similar affinity for oestradiol-17β but not for other ligands (Muramatsu and Inoue 2000), allowing them to act as selective oestrogen receptor modulators (Riggs and Hartmann 2003).

Observations in ERα-knockout mice have clearly shown that oestrogens regulate reabsorption of epididymal fluid, a process necessary to maintain normal fertility (Lubahn et al. 1993; Korach 1994; Hess et al. 2000, 2001; Hess and Carnes 2004). However, the actual role of ER in testicular cells still needs further clarification. Administration of oestrogens during the neonatal period or adulthood impaired sperm production and maturation (Steinberger and Duckett 1965; McLachlan et al. 1975; Stillman 1982; Raman-Wilms et al. 1995; Toppari et al. 1996; Cheek and McLachlan 1998; Toppari and Skakkebaek 1998; vom Saal et al. 1998; Akingbemi and Hardy 2001; Norgil Damgaard et al. 2002), indirectly indicating that oestrogens may have the ability to perturb spermatogenesis (Parvinen 1982; Franca et al. 1998). However, these effects must be considered as compound, dose and possibly species specific and they do not preclude an active role of oestrogens in supporting spermatogenesis.

A specific feature of the boar testis is the secretion of high amounts of oestrogens, in particular oestrone sulphate (Claus and Hoffmann 1980; Raeside and Renaud 1983; Claus et al. 1987; Raeside et al. 1993, 1999; Rostalski et al. 2000) and the tremendous daily spermatozoa output (about 30 billion per day) (Swierstra 1968a,b). This may be indicative of special regulatory mechanisms in the boar, warranting further studies on the involvement of testicular oestrogens in testicular function. To our knowledge this question has not been addressed and in order to set the basis for further studies, the present experiments were designed to test for the expression of the ERα and ERβ in the testis of immature and mature boars. In order to identify oestrogen-producing cells, we also tested for the expression of P450 aromatase.

Materials and Methods
Tissue collection and sample preparation

Testes were collected from 25 healthy boars. The animals (Large White), randomly obtained from five litters, were allotted to five age groups (five animals per group) and castrated under general barbiturate anaesthesia at ages of 50, 100, 150, 200 and 250 days. Following removal, the testes were trimmed off the epididymides and excess connective tissue and cut longitudinally. Five to six approximately 1 cm³ parenchyma samples were taken from the area between the tunica albuginea and the mediastium testis. Samples for immunohistochemistry (IHC) and in situ reverse transcription-polymerase chain reaction (RT-PCR) were fixed for 24 h at 4°C in neutral phosphate buffered 10% formalin with gentle agitation. After washing in phosphate-buffered saline and dehydration through a graded ethanol series (30, 50 and 70%), the samples were infiltrated with paraffin (60°C) overnight, embedded in paraffin wax (Histo-Comp-Vogel, Giessen, Germany) and mounted onto blocks.

For in vitro RT-PCR testicular parenchyma samples were immediately quick-frozen in liquid nitrogen and stored at 80°C until RNA extraction.

Immunohistochemical staining procedure

Indirect immunoperoxidase staining methods were applied using the strepavidin–biotin technique for signal enhancement following standard procedures (Schuler et al. 2002). Negative controls were set up using the irrelevant murine monoclonal isotype-specific antibody MsIgG₂a (Beckman Coulter, Miami, FL, USA) for ERβ and aromatase, and MsIgG₁ (Dianova GmbH, Hamburg, Germany) for ERα at an equal concentration of the respective primary antibody. For the receptor isoforms, porcine uterus was used as a positive control. Tissue sections of 5 μm were mounted onto Super Frost-plus glass slides (Menzel Glaeser, Braunschweig, Germany) and mouse monoclonal primary antibodies raised against a peptide mapping at the carboxy terminus of the porcine ERα (clone HT277) (Sierralta and Thole 1996; Qualmann et al. 2000) and against the highly conserved carboxy terminus of the ERβ1 of human origin (clone PPG5/10; Serotec, Düsseldorf, Germany) were used for localization of ERα and ERβ, respectively, while mouse monoclonal antibody against a conserved epitope within human P450 aromatase (cone SM1671P; Acris, Hiddenhausen, Germany) was used to detect P450 aromatase.

Quantitative determination of ER positive cells and statistical evaluation

For quantitative assessment of ER positive cells, a person, blinded for group and animal information evaluated three immunostained sections from each animal for each receptor isoform. The types of positive staining cells were identified and in each of the three sections for each cell type the total number and the number of ER positive cells were counted at a 200-fold magnification. In the case of sexually mature boars, from each slide, at least three different arbitrarily views corresponding to different stages of germ cell development (see below) were chosen. In immature boars three arbitrarily chosen views of approximately 200 cells each were evaluated. Based on the results obtained, expression of ER in the testis was assessed in relation to the sexual development (immature and mature), location of the cells (seminiferous epithelium and interstitium) and stage of spermatogenesis. The histological appearance of the seminiferous tubules was used to define animals as immature (prespermatogonia and Sertoli cells only) or
mature (fully developed spermatogenesis). In respect to the stage of spermatogenesis, staining by
IHC did not allow a complete application of Swierstra's criteria to divide spermatogenesis into
the eight stages (Swierstra 1968a,b). Hence and as described by Kohler (2004) spermatogenesis
was grossly divided into three arbitrary phases, the first immediately after spermatogenesis without
elongated spermatids, the second during elongation of the spermatids and the third just prior to
spermiat with fully elongated spermatids.

For statistical evaluation, a three-factorial partial hierarchical analysis of variance (age group
[G], animal within age group [A (G)], section within animal and age group [S (AG)] using
BMDP statistical software, program BMDP8V (Dixon 1993) was performed.

**In vitro RT-PCR**

Coarse pieces of deep-frozen (−80°C) testicular parenchyma tissue were enveloped in sterile
aluminium foil and grounded into smaller particles by hammering against a solid surface. The
resulting particles were then powdered under liquid nitrogen using a pestle in a mortar pre-
chilled to approximately −80°C. Approximately 200 mg of the ground tissue were then
immersed in 3 ml lysis buffer containing 30 μl β-mercaptoethanol and homogenized by three
times 30 s bursts using an Ultra Turrax T25 (IKA-Werke GmbH and Co KG, Staufen i. Br.,
Germany). Subsequently, a silica gel based RNA extraction was performed according to the
instructions of the kit supplier (RNeasy Mini Kit; Qiagen, Hilden, Germany).

GeneAmp RNA PCR Kit (Perkin Elmer, Foster City, CA, USA) was used for RT-PCR and first
strand cDNA was synthesized from 10 ng of total RNA (1.5 μl of 200 ng/30), using 8.5 μl of the
RT-mix prepared according to the instructions of the kit supplier (Perkin Elmer). A one-step
reverse transcription was carried out in an automated program (8 min at 21°C, 15 min at 42°C,
5 min at 99°C, 5 min at 5°C). The primer pairs for ERα and ERβ were deduced from published
porcine ER sequences (Table 1) and used in PCR reactions to amplify a 305 and a 239 base pair
cDNA fragment in case of the ERα and ERβ, respectively, in a T1-Thermo Cycler (Biometra
GmbH, Göttingen, Germany). All primers were ordered from MWG-Biotech (Ebensburg,
Germany). The PCR reaction mixture per tube consisted of 10 μl of cDNA sample added to 40 μl
of a premixed solution containing 1 μl primer-mix (forward and reverse; each 15 pmol/μl),
0.25 μl Amplitaq Gold DNA polymerase (5 U/μl), 2 μl MgCl2 (25 mm), 4 μl 10× PCR-buffer
(500 mm KCl, 100 mm Tris-HCl) and 32.75 μl double distilled autoclaved water; this solution
was prepared for a set of 15 samples. β-actin was used as an internal control and negative
controls were set up using autoclaved water instead of RNA in the RT reaction mix. The hot-start
PCR reaction was run for 10 min at 95°C (activation of Amplitaq Gold DNA polymerase)
followed by the program comprising of 35 cycles of denaturing at 94°C for 1 min, annealing at
55°C (ERβ) and at 58.5°C (ERα) for 1 min and extension at 72°C for 1 min. The amplicons were
separated and visualized on a 2% ethidium bromide stained agarose gel and visualized under UV
transillumination.

Table 1. Primers used in RT-PCR for detection of mRNA specific for ER and aromatase, their
nucleotide positions (nt) in published porcine mRNA sequences and the length of resulting DNA
fragments
### Primer Sequence utilized

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence utilized</th>
<th>Position (nt)</th>
<th>Amplicons length (nt)</th>
<th>Accession No.</th>
</tr>
</thead>
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<td>ERα-forward</td>
<td>5′-AGG GAG AGG AGT TTG TGT G-3′</td>
<td>170–189</td>
<td>305</td>
<td>AF035775</td>
</tr>
<tr>
<td>ERα-reverse</td>
<td>5′-TCT CCA GCA GCA GGT CAT AG-3′</td>
<td>475–455</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERβ-forward</td>
<td>5′-ACA CCT CTC TCC TTT AGC C-3′</td>
<td>243–262</td>
<td>239</td>
<td>AF267736</td>
</tr>
<tr>
<td>ERβ-reverse</td>
<td>5′-CCT GAC GCA TAA TCA CTG-3′</td>
<td>482–464</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P450arom-forward</td>
<td>5′-TTA GCA AGT CCT CAA GTG TG-3′</td>
<td>421–440</td>
<td>324</td>
<td>U37311</td>
</tr>
<tr>
<td>P450arom-reverse</td>
<td>5′-CCA GGA AGA GGT TGT TAG AG-3′</td>
<td>723–744</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### One-step in situ RT-PCR

This method detects incorporated digoxigenin (DIG)-labelled uridine triphosphate (11-dUTP) in the DNA amplified *in situ*. Sections of 5 μm prepared from formalin-fixed paraffin-embedded tissue were mounted onto TESAP (3-triethoxysilyl-propylamine) coated Superior glass slides with cavities (Paul Marienfeld GmbH and Co. KG, Lauda-Koenigshofen, Germany) and dried for 1 h at 37°C. They were then deparaffinized in xylene and rehydrated in descending isopropanol (100%, 70%) series. Permeabilization was improved with protease K (Sigma, Deisenhofen, Germany) at a concentration of 2 μg/ml in 0.1 n HCl at 25°C for 5 min. To digest genomic DNA, the slides were treated overnight at 37°C in an RNase-free DNase solution (Roche Molecular Biochemicals, Mannheim, Germany) at a concentration of 1 U/μl in DNase buffer (100 mm NaAc, pH 5.0; 5 mm MgSO₄), omitting this step constituted the positive controls. The *in situ* RT-PCR step was performed with the GeneAmp *in situ* PCR thermal cycler (System 1000; Perkin Elmer). Slides were placed on an assembly tool (Perkin Elmer) and overlaid with 50 μl of the reaction mix containing 29 μl DEPC-H₂O, 1 μl deoxynucleotide triphosphate 10 mm, 1 μl Dig-UTP 10 nm (Roche Diagnostics GmbH, Steinheim, Germany), 6 μl Mn (OAc) 225 mm, 1 μl RNAse inhibitor (Roche Diagnostics GmbH), 10 μl 5′-EZ buffer (Perkin Elmer), 20 nm each of forward and reverse primers and 2 μl recombinant thermus thermophilus (rTth)-DNA polymerase (2.5 U/μl). They were then covered with mineral oil and incubated at 62°C for 45 min to allow for the reverse transcription. Cycling conditions and the primers were the same as described for *in vitro* PCR ([Table 1](#table_1)). To visualize the amplified PCR products, the DIG-labelled cDNA segments were detected by alkaline phosphatase-conjugated sheep anti-DIG Fab fragments (Roche Molecular Biochemicals) diluted 1 : 3000 in washing buffer. The sections were equilibrated in TRIS buffer (1 m Tris-HCl, pH 9.5; 1 m NaCl) and reacted with the substrate 5-bromo-4-chloro-3-indolyl phosphate in the presence of nitroblue tetrazolium (Roche Molecular Biochemicals) to form a dark blue precipitate at the site of the label. To stop the reaction, slides were washed in distilled water and embedded in glycerol gelatine.

### Results


**Immunolocalization of ER by IHC**

The ER immunoreaction signals were observed only in nuclei. In the porcine uterus used as positive control, a strong immunostaining for the ERα and ERβ was found in the glandular epithelium, weak signals were detected in the luminal epithelium (Fig. 1a,b). In the negative controls, apart from the occasional traces of a weak non-specific staining in the lumen of blood vessels obviously associated with residual serum components, there were no other non-specific signals (Fig. 1c–e). ER expression showed qualitative and quantitative differences between and within immature (50 days old) and sexually mature (100–250 days old) animals.

In immature boars, 95.5 ± 3.5% of the prespermatogonia cells showed a strong immunoreactivity for the ERβ; a similarly intensive immunoreactivity for ERα was observed in 90.6 ± 1.2% of the prespermatogonia and 71.0 ± 2.6% of the Leydig cells. The Sertoli Leydig cells were negative for both receptor isotypes (Fig. 2a,b).

For the ERα, a strong immunoreactivity was found in 93.6 ± 2.7% of the spermatogonia and primary spermatocytes. With the exception of a few cells, a distinctly less intensive staining was observed in 51.4 ± 0.27% of the other germ cells except elongating/ed spermatids, which stained clearly negative. Most of the Sertoli cells stained weakly positive and 61.1 ± 3.4% of the Leydig cells exhibited a weak to distinct staining (Fig. 3d–f). The immunoreactivity observed showed no
qualitative and quantitative changes in respect to age and to the stage of spermatogenesis (Fig. 3d–f).

**Detection of ER-specific mRNA**

One specific band of the expected base pairs corresponding to the respective ER isotype mRNA (*Table 1*) was detected during *in vitro* RT-PCR in all testicular samples from all age groups and porcine uterus (Fig. 4). Sequencing of PCR products (Qiagen) yielded 95 and 92% similarity with the respective porcine mRNA sequences.

**Figure 4.** Ethidium bromide-stained agarose gel of *in vitro* RT-PCR analysis for the presence of mRNA specific to (a) ERα and (b) ERβ. 1 = 50 days, 2 = 100 days, 3 = 150 days, 4 = 200 days and 5 = 250 days testicular samples; M, DNA ladder; P, porcine uterus (positive control tissue); N, autoclaved double distilled water (negative control sample)

When applying *in situ* RT-PCR, strong positive signals for the ERβ mRNA (Fig. 5a) were found in Sertoli and Leydig cells and germ cells except elongating/ed spermatids. In case of the ERα (Fig. 5b) the staining pattern observed corresponded to the results obtained by IHC; distinct cytoplasmic signals were restricted to the spermatogonia and primary spermatocytes, only weak and fewer signals showed up in round spermatids and Leydig cells, elongated spermatids were negative. Identification of the Sertoli cells was not possible. Negative controls were devoid of non-specific staining (Fig. 5c) while very strong nuclear signals from the genomic DNA were observed in the positive controls (Fig. 5d).

**Figure 5.** *In situ* RT-PCR for oestrogen receptor mRNA in mature boar testis (200 days). (a) distinct signals for ERβ-mRNA in spermatogonia, spermatocytes and round spermatids (blue arrow), (b) strong cytoplasmic signals for ERα-mRNA in spermatogonia and primary spermatocytes (blue arrow), (c) negative control: no staining after *in situ*-RT-PCR (primers omitted), (d) positive control: omission of DNAse treatment showing nuclear signals due to amplification of genomic DNA sequences (blue arrow). Bars = 0.05 mm

**Expression of P450 aromatase**

The P450 aromatase immunoreactivity was found exclusively in the cytoplasm of Leydig cells and an increase in both the number of positive cells and the intensity of the staining was observed with increasing age of the boars (Fig. 6a,b). *In vitro* RT-PCR detected one specific band of aromatase mRNA with the expected number of base pairs (*Table 1*) in samples from all age groups (Fig. 7).
Figure 6. Immunolocalization of P450 aromatase showing positive signals in the cytoplasm of Leydig cells (blue arrows). (a) immature, (b) mature boar testis. Bars = 0.05 mm

Figure 7. Ethidium bromide-stained agarose gel of in vitro RT-PCR analysis for the presence of mRNA specific to P450 aromatase. 1 = 50 days, 2 = 100 days, 3 = 150 days, 4 = 200 days and 5 = 250 days testicular samples; M, DNA ladder; N, autoclaved double distilled water (negative control sample)

Discussion

In the present study expression of the ERα and ERβ in the porcine testis was demonstrated at both the protein and mRNA level (Table 2). Conventional RT-PCR detected mRNA for both receptor isotypes in all age groups and the type and distribution pattern of ER mRNA and ER protein expressing cells was identical when applying in situ RT-PCR and IHC. This indicates that both transcription and translation occur in the same cells; it also points to the specificity of the IHC method applied. Specificity of IHC was further confirmed by lack of non-specific signals in the negative controls and by the results observed in the positive control (porcine uterus), which were consistent with the findings described previously for this tissue (Sierralta and Thole 1996; Qualmann et al. 2000).

Table 2. Summarized expression of ERα and ERβ in boar testis

<table>
<thead>
<tr>
<th>Immature testis cell type</th>
<th>ERα</th>
<th>ERβ</th>
<th>Mature testis cell type</th>
<th>ERα</th>
<th>ERβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prespermatogonia</td>
<td>+++</td>
<td>+++</td>
<td>Spermatogonia</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Primary spermatocytes</td>
<td>+++</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Round spermatids</td>
<td>+</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elongated spermatids</td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sertoli cell</td>
<td>−</td>
<td>−</td>
<td>Leydig cell</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Sertoli cell</td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The observed expression of the ERβ in the nucleus of prespermatogonia in the testis of immature boars is consistent with similar observation in neonatal rodents and prepubertal humans. However, other than in the neonatal rodent, with ERβ expressing Sertoli and Leydig cells (Saunders et al. 1998; Katzenellenbogen and Katzenellenbogen 2000), or in the prepubertal human, with ERβ expressing Sertoli cells (Enmark et al. 1997; Pelletier and El-Alfy 2000; Takeyama et al. 2001), in the immature boar Leydig and Sertoli cells were negative.

Different from ERβ, ERα was detected not only in the prespermatogonia but also in Leydig cells of the immature boar. This conforms to observation in the juvenile rat (O'Donnell et al. 2001) and prepubertal human (Enmark et al. 1997; Pelletier and El-Alfy 2000; Takeyama et al. 2001)
where expression of ERα in prespermatogonia had been reported. However, unlike in the boar, the Leydig cells were negative in these two species (Enmark et al. 1997; Pelletier and El-Alfy 2000; O'Donnell et al. 2001; Takeyama et al. 2001). Another specific observation relates to the fact that ERβ seems to be the main ER isoform in the testis of immature rats and humans (Enmark et al. 1997; Pelletier and El-Alfy 2000; O'Donnell et al. 2001; Takeyama et al. 2001), while in the immature boar no differences were observed in the expression of the ERα and ERβ in prespermatogonia. These observations point towards differences between species. Thus in the immature boar both ER isotypes may be important factors during the postnatal testicular development and onset of spermatogenesis, but only the ERα influences Leydig cell functions.

With exceptions of elongating/ed spermatids, in sexually mature boars, the ERβ was clearly expressed in virtually all germ cells undergoing the development from spermatogonia to round spermatids. Also the majority of Sertoli and Leydig cells were ERβ-positive. This observation is in agreement with results reported for adult rodents, primates, goats, dogs and cats (Goyal et al. 1997; Takeyama et al. 2001; Nie et al. 2002; Hess and Carnes 2004). Although there were little variations in staining intensity, expression of ERβ seemed rather homogeneous within and between cell types and there were no apparent relations to the stage of spermatogenesis. These data obtained on the protein level were confirmed on the mRNA level indicating, that transcription and translation of ERβ occurs in the positive staining cells.

With respect to the ERα, spermatogonia and primary spermatocytes showed a strong immunostaining with 93.6 ± 2.7% of these cells being positive while the secondary spermatocytes and round spermatids exhibited weak staining with 51.4 ± 0.27% of the cells being positive; again elongating/ed spermatids were negative. In situ RT-PCR confirmed the expression pattern for both the ERβ and ERα. These observations indicate that transcription and translation of the ERα in secondary spermatocytes and round spermatids may be down-regulated and/or that a part of the weakly immunostaining positive cells when applying IHC might be a result of a carry over of the receptor protein from the preceding developmental stages. Again, no relationship was observed between the IHC-staining pattern for the ERα and the stage of spermatogenesis. To our knowledge, these are the first data reporting expression of ERα in germ cells of mammals. The predominant expression of ERα in the spermatogonia and primary spermatocytes of the boar so far seems to be unique for this species. However, in accordance with observations in other species (Hess and Carnes 2004), also in the boar Leydig and Sertoli cells express the ERα.

In agreement with previous observations (Conley et al. 1996; Carreau et al. 1999; Fraczek et al. 2000), our data confirm that the expression of aromatase is restricted to the Leydig cells, which also express the ERα and ERβ.

Thus, in the boar testicular oestrogens seem to be autocrine and/or intracrine factors controlling Leydig cell function while Sertoli cell function and spermatogenesis might be affected by paracrine mechanisms. The distinct difference in the expression pattern of ERα and ERβ in germ cells seems to point towards selective roles of oestrogens with respect to spermatogenesis. These observations warrant further studies.

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