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Expression and localization of aromatase during fetal mouse testis development

Caroline Borday¹,²,³†, Jorge Merlet¹,²,³†, Chrystèle Racine¹,²,³ and René Habert¹,²,³∗

Abstract

Background: Both androgens and estrogens are necessary to ensure proper testis development and function. Studies on endocrine disruptors have highlighted the importance of maintaining the balance between androgens and estrogens during fetal development, when testis is highly sensitive to environmental disturbances. This balance is regulated mainly through an enzymatic cascade that converts irreversibly androgens into estrogens. The most important and regulated component of this cascade is its terminal enzyme: the cytochrome p450 19A1 (aromatase hereafter). This study was conducted to improve our knowledge about its expression during mouse testis development.

Findings: By RT-PCR and western blotting, we show that full-length aromatase is expressed as early as 12.5 day post-coitum (dpc) with maximal expression at 17.5 dpc. Two additional truncated transcripts were also detected by RT-PCR. Immunostaining of fetal testis sections and of gonocyte-enriched cell cultures revealed that aromatase is strongly expressed in fetal Leydig cells and at variable levels in gonocytes. Conversely, it was not detected in Sertoli cells.

Conclusions: This study shows for the first time that i) aromatase is expressed from the early stages of fetal testis development, ii) it is expressed in mouse gonocytes suggesting that fetal germ cells exert an endocrine function in this species and that the ratio between estrogens and androgens may be higher inside gonocytes than in the interstitial fluid. Furthermore, we emphasized a species-specific cell localization. Indeed, previous works found that in the rat aromatase is expressed both in Sertoli and Leydig cells. We propose to take into account this species difference as a new concept to better understand the changes in susceptibility to Endocrine Disruptors from one species to another.

Keywords: Cyp19a1, Aromatase, Testis, Fetus, Mouse, Gonocytes, Development, Endocrine disruptors, Leydig cells, Souris, Développement, Perturbateurs endocriniens, Cellules de Leydig

Résumé

Les androgènes et les oestrogènes sont indispensables au développement et aux fonctions du testicule. Le testicule est particulièrement sensible aux perturbateurs endocriniens pendant le développement foetal et beaucoup de perturbateurs endocriniens agissent en modifiant la balance oestrogènes/androgènes. Physiologiquement, cette balance est régulée par une cascade enzymatique qui convertit irréversiblement les androgènes en oestrogènes. Le composant principal de cette cascade est le cytochrome p450 19A1 (appelé couramment aromatase). Le but de ce travail a été d’étudier l’expression de l’aromatase testiculaire au cours du développement foetal chez la souris.

(Continued on next page)
To determine if aromatase is translated in mouse testis, western blot analysis was performed using a specific anti-aromatase antibody (MCA2077T, Serotec, France) (Figure 1D). Two proteins around 54 kDa and one around 27 kDa were detected. The protein of 54 kDa was also present in the ovary extract and it approximately corresponded to the aromatase expected size. We thus suppose that the two heaviest proteins derived from the full-length form of aromatase (T1) with the highest form corresponding to a testis-specific post-translational modification that remains to be identified. In order to understand the origin of the 27 kDa protein, we analysed sequences of the T2 and T3 variants. It revealed that the splicing of exon 3 in T2 would change the ORF and create a precocious codon stop leading to a probably not detected protein of 6 kDa. Splicing of exons 3 and 4 in T3 would not change the ORF allowing in theory the synthesis of a truncated protein of 46 kDa. No protein at this expected size was detected in the western blot (Figure 1D). However, the use of an alternative start codon located later in T2 and T3 sequences may lead to a protein of 27 kDa containing the C-terminal part of aromatase.

These findings are different from those of the only previously published paper on this topic showing that, in the mouse, aromatase expression starts at 17.5 dpc and reaches the highest level at day 1 post-partum [3]. In our study, we detected aromatase expression as early as 12.5 dpc. This discrepancy probably results from the improvement of the methods of detection made since 1994. This is an important point because it shows that estrogens can be produced by mouse fetal testes very early and throughout development.

Our findings indicate that different aromatase transcripts are generated in fetal mouse testes. Previous studies in different mammalian species (including the mouse) reported
that tissue-specific aromatase expression is driven by specific promoters [4-6]. Each tissue-specific promoter is associated with a specific untranslated first exon. In mice testis Golovine et al. have shown that aromatase transcripts may emerge from a specific promoter called Ptes [4]. Our study showed that aromatase expression is also regulated at a second transcriptional level generating two additional truncated variants T2 and T3 by mRNA splicing. Our results suggest that there are several forms of aromatase protein however the nature and the physiological function of these isoforms remain to be investigated.

### Table 1 Sequences of aromatase primers used in RT-PCR and qRT-PCR

|                        | Sequence 5′-3′ | Tm  |
|------------------------|---------------|-----|
| **RT-PCR all transcripts** |               | 55°C |
| forward                 | AACCCCATGCAGTATAATGTC |     |
| reverse                 | CATCTCTCTCAAGTTTCA |     |
| **T1 qRT-PCR**          |               | 60°C |
| forward                 | GCCCTCCTCTCCTGAATTTGA |     |
| reverse                 | CTGCCATGGGAATGAGGG |     |
| internal probe          | TACGGTCCTGGCTACT |     |
| **T2 qRT-PCR**          |               | 60°C |
| forward                 | GCCCTCCTCTCCTGAATTTGA |     |
| reverse                 | CGGAAATCGGGAATGAGGG |     |
| internal probe          | TCAATACCCAGCTCTCGAC |     |
| **T3 qRT-PCR**          |               | 60°C |
| forward                 | CATGCCACTCTCCTGCTGAT |     |
| reverse                 | CCACCATCCGGAACAGCCAG |     |
| internal probe          | TCTTCAATACCCAGCTCTGAC |     |

**Aromatase cell localization in mouse fetal testes**

Immunohistochemical analysis of aromatase localization in 17.5 dpc mouse testes using a specific anti-aromatase antibody (MCA2077T, Serotec, France) showed a strong staining in Leydig cells. Importantly, there was no detectable staining in Sertoli cells (Figure 2A). Conversely, previous studies in fetal and neonatal testes showed that aromatase was expressed in both Leydig cells and Sertoli cells in the rat [7,8]. This and other previous reports indicate that aromatase cell localization in fetal testis is quite variable in mammalian species. Indeed, aromatase is
expressed in Leydig cells and not in Sertoli cells in the fetal testis of the Plains Vizcacha rodent [9], is totally absent in the deer [10], and is detected in both Sertoli cells and Leydig cells in fetal baboon and human testes [11,12]. In addition, our immunohistochemical analysis showed that aromatase was also expressed in gonocytes, but the intensity of the signal was not uniform: in some cells the signal was very strong, whereas in others it was faint or undetectable (Figure 2A, arrowheads). Similar results were previously described for Retinoic Acid Receptor alpha [14]. As aromatase localization in germ cells was quite unexpected, aromatase immunostaining was also performed in enriched gonocyte cultures that were prepared from 17.5 dpc mouse testes as previously described [15]. Similarly, aromatase was detected in some germ cell VASA-positive cells, a germ cell-specific marker (Figure 2B). This result identifies a sub-population of gonocytes with endocrine function. Aromatase expression was previously reported in adult rat and human germ cells [16,17] and in pig gonocytes during development [18]. Aromatase expression was also detected in gonocytes of human fetal testes [12].

In conclusion, aromatase cell localization in fetal testis appears to differ from one species to another and as
consequence also the intracellular estrogen concentration. These differences should be taken into account to explain the variations in the susceptibility of fetal testis to estrogenic and anti-androgenic endocrine disruptors in different mammalian species that has been recently lightened [2].

Additional file

Additional file 1: Sequencing results of T1, T2 and T3. The three different transcripts were isolated on gel and sequenced with the following primers: forward 5'-AACCCTAGCGATAATGTC-3' (located in exon II); reverse 5'-CACAATGACCTTCTTGCCA-3' (located in exon V). Each different exon is highlighted in a different color (red exon II, black exon III, and blue exon IV and green exon V. In addition, sequencing from exons VI to X were performed using other primers and showed no difference in T1, T2 and T3 (data not shown).

Competing interests

All authors declare that they have no competing interests.

Authors’ contributions

Conceived the study: RH. Conceived and designed the experiments: RH, CR. Performed the experiments: CB, JM, CR. Analyzed the data: CB, JM, RH. Contributed reagents, materials, analysis tools and financial supports: RH. Wrote the paper: RH, CR. Improved the redaction: CB, JM. All authors read and approved the final manuscript.

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