Expression levels of Mullerian-inhibiting substance, GATA4 and 17α-hydroxylase/17,20-lyase cytochrome P450 during embryonic gonadal development in two diverse breeds of swine

S A McCoard, T H Wise and J J Ford

United States Department of Agriculture, Agricultural Research Service, US Meat Animal Research Center, Clay Center, Nebraska 68933, USA

(Requests for offprints should be addressed to S A McCoard who is now at Nutrition and Behaviour Group, AgResearch Limited, Private Bag 11008, Tennent Drive, Palmerston North, New Zealand; Email: sue.mccoard@agresearch.co.nz)

Abstract

Sexual differentiation and early embryonic/fetal gonad development is a tightly regulated process controlled by numerous endocrine and molecular signals. These signals ensure appropriate structural organization and subsequent development of gonads and accessory organs. Substantial differences exist in adult reproductive characteristics in Meishan (MS) and White Composite (WC) pig breeds. This study compared the timing of embryonic sexual differentiation in MS and WC pigs. Embryos/fetuses were evaluated on 26, 28, 30, 35, 40 and 50 days postcoitum (dpc). Gonadal differentiation was based on morphological criteria and on localization of GATA4, Mullerian-inhibiting substance (MIS) and 17α-hydroxylase/17,20-lyase cytochrome P450 (P450c17). The timing of testicular cord formation and functional differentiation of Sertoli and Leydig cells were similar between breeds. Levels of GATA4, MIS and P450c17 proteins increased with advancing gestation, with greater levels of MIS and P450c17 in testes of MS compared with WC embryos. Organization of ovarian medullary cords and formation of egg nests was evident at similar ages in both breeds; however, a greater number of MS compared with WC embryos exhibited signs of ovarian differentiation at 30 dpc. In summary, despite breed differences in MIS and P450c17 levels in the testis, which may be related to Sertoli and Leydig cell function, the timing of testicular differentiation did not differ between breeds and is unlikely to impact reproductive performance in adult boars. In contrast, female MS embryos exhibited advanced ovarian differentiation compared with WC embryos which may be related to the earlier reproductive maturity observed in this breed.

Journal of Endocrinology (2002) 175, 365–374

Introduction

In occidental pig breeds, genetically determined gonads begin to form on the ventromedial surfaces of the mesonephroi around 20–21 days postcoitum (dpc) (Pelliniemi 1975, McCoard et al. 2001a). Male sexual differentiation occurs around 26 dpc (Pelliniemi 1975, Tung et al. 1984, Tung & Fritz 1987, McCoard et al. 2001a), characterized by the formation of testicular cords. Shortly thereafter, Leydig cells of the testicular interstitium differentiate, resulting in androgen-stimulated development of the male phenotype. Female sexual differentiation is initiated later in development (~33 dpc), characterized by the formation of medullary cords (McCoard et al. 2001a). By 40 dpc, abundant egg cell nests and well-organized medullary and cortical regions of the ovary are present (McCoard et al. 2001a) and by 44 dpc the medullary cords have degenerated, indicating the completion of ovarian differentiation (Pelliniemi & Lauteala 1981).

Gonadogenesis is a tightly regulated process that involves co-ordinated expression of several developmentally expressed genes. Several genes are known to play important roles in gonadal development and sexual differentiation including SRY (Koopman et al. 1990), SOX9 (Kent et al. 1996, Morais da Silva et al. 1996), Mullerian-inhibiting substance (MIS) (Munsterberg & Lovell-Badge 1991), GATA4 (Heikinheimo et al. 1997, Viger et al. 1998, Ketola et al. 1999, McCoard et al. 2001a), steroidogenic factor 1 (SF1) (Ikeda et al. 1994), DAX1 (Swain et al. 1996) and Wilms tumor factor-1 (WTI) (Pritchard-Jones et al. 1990). In pigs, GATA4 is expressed prior to the morphological differentiation of the bipotential gonads (McCoard et al. 2001a), followed by the expression of SOX9, SF1 and WTI in both sexes (Parma et al. 1999). Coincident with male sexual differentiation, Sertoli cells begin to produce MIS transcripts and protein (Tran et al. 1977, Parma et al. 1999, McCoard et al. 2001a), initiating regression of the Mullerian ducts (Knebelmann et al. 1991,
Behringer et al. 1994, Imbeaud et al. 1996, Mishina et al. 1996), thereby inhibiting development of the female duct system. MIS activity continues to increase in the testis from 27 to 33 dpc (Tran et al. 1977) suggesting a potential role in testicular cord development. Thus, MIS is widely used as a functional marker of Sertoli cell differentiation. Subsequent to secretion of MIS, differentiated Leydig cells produce several steroidogenic enzymes including 17α-hydroxylase/17,20-lyase cytochrome P450 (P450c17) (Conley et al. 1994, Greco & Payne 1994, Kaminski et al. 1999), which functions as an enzymatic catalyst for testosterone synthesis (Moon et al. 1973, Lejeune et al. 1998), facilitating androgen-stimulated development of the Wolffian ducts.

Chinese Meishan (MS) boars reach puberty earlier, but have smaller testes due to reduced Sertoli cell numbers and lower sperm production than White Composite (WC) boars (Okwun et al. 1996a, b, Lunstra et al. 1997). MS females achieve puberty at a younger age, and have a higher ovulation rate and larger litters than WC females (Bolet et al. 1986, Ashworth et al. 1990, Christenson 1993, Faillace et al. 1994, Hunter et al. 1994). Differential seminiferous tubule development and timing of Leydig cell differentiation have been reported in MS and WC pigs (Kaminski et al. 1999), indicating that early developmental events influence future reproductive performance. However, these conclusions were based on only one litter of MS pigs per day of pregnancy. Furthermore, in males, marked differences in Sertoli cell development between these two breeds are not evident until early neonatal life (McCoard et al. 2001b). Breed differences in the timing of female development have not been investigated. The objective of this study was therefore to utilize both morphological criteria and molecular markers of sexual differentiation and gonadal development to compare the timing of sexual differentiation and subsequent gonadal development in male and female MS and WC embryos. Profiles and levels of various gene products implicated in sexual differentiation and early gonadal development were examined using densitometric quantification.

### Materials and Methods

#### Sample collection

Embryos were collected from pure-bred MS and WC (Yorkshire × Landrace) sows (minimum of second parity) bred to MS and WC boars, respectively. A subset of WC embryos was also used in an independent study (McCoard et al. 2001a). Estrus was checked daily and sows were mated naturally on the first and second day of detected estrus, using different boars on each day. Day 0 corresponds to the first day of mating. Embryos/fetuses were collected on 26, 28, 30, 35, 40 and 50 dpc. Immediately after slaughter of the sows, body weight and crown–rump length (CRL) were recorded for each conceptus. Embryonic length is a reliable indicator of gestational age (McCoard et al. 2001a) and was used to confirm embryonic age. Embryos from 26 to 30 dpc were left intact, but paired mesonephroi with attached gonads were dissected from fetuses collected between 35 and 50 dpc. The number of fetuses and the total number of embryos/fetuses evaluated at each gestational age are presented in

### Table 1  Total numbers of animals used and the proportion of embryonic/fetal gonads that exhibit signs of Sertoli and Leydig cell differentiation (male sexual differentiation) or the formation of ovarian medullary cords and formation of egg cell nests (female sexual differentiation)

<table>
<thead>
<tr>
<th>Breed</th>
<th>Age (dpc)</th>
<th>Litters</th>
<th>Total fetuses</th>
<th>Males</th>
<th>P450c17 (%)</th>
<th>MIS (%)</th>
<th>Females</th>
<th>Nests (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC</td>
<td>28</td>
<td>6</td>
<td>74</td>
<td>100</td>
<td>46</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>4</td>
<td>42</td>
<td>100</td>
<td>100</td>
<td>15*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>6</td>
<td>43</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>6</td>
<td>71</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>8</td>
<td>59</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MS</td>
<td>28</td>
<td>6</td>
<td>54</td>
<td>100</td>
<td>57</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>4</td>
<td>43</td>
<td>100</td>
<td>100</td>
<td>76*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>5</td>
<td>47</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>3</td>
<td>31</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>83</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2</td>
<td>25</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*P<0.01, WC compared with MS.
MIS (%): the proportion of embryonic/fetal gonads that produce MIS protein, a marker of Sertoli cell differentiation.
P450c17 (%): the proportion of embryonic/fetal gonads that produce P450 c17, a marker of Leydig cell differentiation.
MC (%): the proportion of embryonic/fetal gonads that contain medullary cords (MC) indicative of the initiation of female sexual differentiation.
Nests (%): the proportion of embryonic/fetal gonads that contain egg cell nests (nests) indicative of female sexual differentiation.

Note: data for 26 dpc fetuses are not presented here. Despite initiation of male sexual differentiation in both breeds at this age, accurate identification of all males and females was not achievable at this age. These data are presented in the text in relation to all embryos irrespective of karyotype.
Table 1. Procedures for handling all animals in this study complied with those specified in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS 1999).

**Sample preparation and immunohistochemistry**

All samples were fixed in fresh 4% paraformaldehyde in Dulbecco’s phosphate-buffered saline (PBS) overnight at
4 °C with gentle agitation (fixative was changed once during incubation). Each sample was washed in PBS (2 × 1 h), dehydrated through graded ethanol (50, 70, 80, 90, 100%; 2 × 1 h each), cleared in xylene (2 × 1 h; Sigma, St Louis, MO, USA), infiltrated with paraffin wax (60 °C; 4 × 1 h), and embedded in paraffin wax. All samples were subjected to the same processing conditions. Sections (5 µm) were made through the longitudinal axis of each embryo and mesonephroi/gonad. Cross-sections were dried overnight onto glass slides at 37 °C and stained the following day. Sections were stained immune-histochemically for GATA4 and MIS (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as previously described (McCoard et al. 2001a,b). The same immuno-histochemical procedure was also used for the anti-P450c17 antibody (courtesy of Dr Anita Payne, Stanford University, Palo Alto, CA, USA) used at a dilution of 1:1000. Non-immune serum or absence of the primary antibodies was used to conclude that non-specific binding was not problematic. For each protein evaluated, all slides were subject to identical staining conditions. Slides were stored at room temperature in the dark until densitometric analysis. To account for interassay variation in staining intensity, testicular tissue from a boar at 105 dpc was selected as control tissue. One section of this tissue was processed with each assay irrespective of breed, sex, age or protein evaluated, and used to correct for interassay and intra-assay variation in staining intensity as described below.

Densitometric measurements

Average density measurements were made using a Bioquant Nova color imaging system (Bioquant Nova Advanced Image Analysis 2000; R&M Biometrics, Inc.) and used to quantify the amount of protein present for each gene examined using brightfield microscopy. Average density measures the average color value of a group of pixels and is the most common way to compare the amount of light-absorbing stain present in one object with others. Since average density depends only on color value, the average density value is independent of the size of the object under examination, allowing valid comparison of structures differing in size.

One full cross-section from near the middle of each gonad was used in these evaluations. Average density values were obtained for each protein in the entire cross-section of each gonad. For each assay, immunoreactive cells were defined by pre-setting a threshold value. In this way, sections from each animal and the control slide were treated in an identical manner. Due to interassay variation in staining intensity, slightly different threshold values were sometimes required to identify all immunoreactive cells. To account for interassay variation in staining intensity and threshold values, the control density value was subtracted from the treatment density values within each assay. This approach enabled the valid comparison of staining intensity between breeds, sexes and across ages for each protein evaluated.

To correct for small fluctuations in either the specimen light source or in ambient room lighting, a stored background image was used to standardize each captured specimen image. Computers operate on a color scale from 0 to 255 (0 is black and 255 is white), the inverse of conventional ‘absorption’ scales. Thus, the data are presented as the inverse of these arbitrary units to correspond to conventional ‘absorption’ scales.

Statistical analysis

Differences between breeds and sexes in all measurements were tested using a mixed model procedure (SAS 1999). For traits expressed in both sexes, the model included fixed effects of breed, sex and age, and two-way interactions, and the random effect of litter nested within breed. Sex was dropped from the model for those traits expressed in only one sex. For body weight, data were transformed to logarithms before statistical analyses to adjust for heterogeneity of variances. Paired comparisons were made using the Tukey–Kramer procedure. Data are presented as least square means (LSM) and standard errors (body weight and CRL data) or inverse least square means (densitometric data).

Results

The presence of MIS protein in the cytoplasm of Sertoli cells (Fig. 1A) and formation of morphologically distinguishable early testicular cords identified by GATA4 immunoreactivity (Fig. 1B, see McCoard et al. 2001a) indicated functional and morphological differentiation of the testis by 26 dpc in both breeds. At this stage of development, 20–25% of all embryonic gonads examined in both breeds contained MIS protein (Fig. 1A) and exhibited morphological signs of seminiferous tubule formation (Fig. 1B). GATA4 protein was present in the somatic cells of both the undifferentiated (absence of testicular cords and MIS protein) and differentiated gonads (Fig. 1B and E) but was never observed in germ cells or endothelial cells of the vasculature. GATA4 was strongly expressed in differentiated Sertoli cells enabling rapid histomorphometric identification of male sexual differentiation (Fig. 1B and E). GATA4 was also present in Leydig cells of the interstitium (Fig. 1E), coelomic epithelial cells and some undifferentiated interstitial cells. Morphological and functional differentiation of testicular cords of all males, based on the presence of MIS and morphologically differentiated testicular cords, was evident by 28 dpc (Table 1). In both breeds, P450c17 protein was first identified in the cytoplasm of Leydig cells at 28 dpc (Table 1), indicative of their functional differentiation. By 30 dpc, the presence of P450c17 and MIS in all testes irrespective of breed (Table 1) illustrated the presence of functionally
differentiated Leydig cells (Fig. 1C) and Sertoli cells (Fig. 1D and 1F) respectively.

Somatic cells of the ovaries contained GATA4 protein (Fig. 1 G–I). Intense GATA4 immunoreactivity was observed in early ovarian medullary cords enabling rapid identification of early signs of sexual differentiation of the ovary (Fig. 1H, see McCaig et al. 2001a). Intense GATA4 immunoreactivity of somatic cells and absence of staining in germ cells enabled the rapid identification of egg cell nests (Fig. 1I). Based on the formation of ovarian medullary cords, detected by histological examination of GATA4 immunoreactivity, the first signs of sexual differentiation of the female gonad were not evident until 30 dpc in both breeds (Table 1). At this stage of development, a greater proportion (P<0.001) of MS (76%) compared with WC (15%) female embryos exhibited early morphological features of sexual differentiation. Female embryos at this stage of development were distinguished from testes by the relatively unorganized structure of the ovarian tissue (Fig. 1 G) and the absence of MIS and P450c17 protein. Ovarian medullary cords were present in all female gonads of both breeds by 35 dpc (Table 1). By 40 dpc, all ovaries from WC fetuses and 83% of MS fetal ovaries contained egg cell nests indicative of sexual differentiation (Table 1). At this stage of development, distinctive regionalization of the ovaries into cortex and medulla regions was evident in both breeds and all ovaries from both breeds contained numerous egg cell nests. Ovaries continued to increase in size up to 50 dpc, as did the total number of germ cells and egg cell nests (Fig. 1I).

Levels of MIS protein in testes increased rapidly from the onset of sexual differentiation (26 dpc) to 50 dpc in both breeds (Fig. 2A). Testes from MS embryos/fetuses contained greater levels of MIS protein compared with WC fetuses (Fig. 2A). GATA4 protein levels also increased from the onset of sexual differentiation in both males and females (Fig. 2B), but did not differ between the breeds or between the sexes at any age evaluated (Fig. 2B). Similar patterns of GATA4 and MIS protein in the testes of both breeds were observed (Fig. 1A and B). Levels of P450c17 protein in the embryonic testes of both breeds increased from the onset of Leydig cell differentiation (Fig. 2C), and testes from MS embryos/fetuses contained greater levels of P450c17 protein than testes from WC embryos/fetuses (Fig. 2C).

Body weight of both MS and WC embryos and fetuses increased with advancing gestational age (Fig. 3A). A divergence in body weight was observed between 40 and 50 dpc in favor of WC compared with MS fetuses, but breed differences in body weight were not observed at any other stage evaluated. In both breeds, male fetuses had greater body weights compared with female fetuses (Fig. 3A). CRL also increased with advancing age in both breeds, but did not differ between breeds (Fig. 3B). However, there was a tendency for males to have greater CRL values than females in both breeds (Fig. 3B).

Discussion

This study describes the timing of sexual differentiation, spatial and temporal localization patterns and levels of molecular markers associated with sexual differentiation and early gonad development in MS and WC conceptuses. Sexual differentiation is accompanied by changes in the structural organization of the gonad and onset of expression of various developmentally expressed genes.

Testicular cords were detected morphologically by 26 dpc in both breeds, consistent with previous reports in domestic pigs (Pelliniemi 1975, McCaig et al. 2001a). Coincident with the formation of testicular cords, MIS protein was observed in the cytoplasm of Sertoli cells in embryos of both breeds at 26 dpc, indicative of their functional differentiation (Tran et al. 1977). Well-organized testicular cords and high levels of MIS protein were observed in large proportions of the gonads of both breeds by 28 dpc. Differentiated Leydig cells (P450c17 immunoreactive) were also detected in the testicular interstitium of both breeds from 28 dpc onwards, consistent with previous reports (Moon et al. 1973, Pelliniemi 1975, 1976). Collectively, these observations indicate that the timing of differentiation of testicular cords and steroidogenic machinery of the testis do not differ between MS and WC breeds. These observations contrast with the report by Kaminski et al. (1999), where testicular cord formation was delayed and P450c17 expression was not observed until 34 dpc in MS embryos compared with 30 dpc in WC (i.e. a 4-day delay). Discrepancies between findings of the present study and the previous report are unlikely to be the result of differences in genetic background of MS pigs as they were derived from the same base population. Rather, discrepancies are likely to result from the number of MS embryos evaluated between 26 and 35 dpc. In the present study, 43–54 embryos from four to six MS litters were evaluated at each gestational age, and embryonic age was confirmed by CRL, a reliable indicator of gestational age (McCaig et al. 2001a). In the previous study by Kaminski et al. (1999), only one MS litter (the number of embryos per litter was not reported) was evaluated at each age.

Early signs of structural organization of fetal ovaries were detected in a greater proportion of MS compared with WC ovaries at 30 dpc, indicating that MS embryos initiate ovarian differentiation earlier (up to 5 days) than WC embryos. However, by 35 dpc, all ovaries from both breeds contained early ovarian cords. Egg cell nests were not detected until 40 dpc, at which time all WC ovaries and the vast majority of MS ovaries contained egg nests indicative of sexual differentiation (Byskov 1986). Organization of cortical cords and degeneration of medullary cords characterizes final differentiation of the ovary (Pelliniemi & Lautela 1981). Medullary cords were present in some ovaries of both breeds at 40 dpc but, by 50 dpc, medullary cords were absent from both breeds.
Collectively, these observations illustrate that MS female embryos initiate ovarian differentiation earlier than WC embryos which may be associated with advanced morphological development in MS ovaries (S A McCoard, unpublished observations) and subsequent earlier onset of puberty and enhanced reproductive performance (Bolet

Figure 2  (A) Densitometric values (arbitrary units) indicating the amount of Mullerian-inhibiting substance (MIS) protein in the testes of Meishan (MS) and White Composite (WC) embryos during late embryonic and early fetal life. NS, not significant. (B) Densitometric values (arbitrary units) indicating the amount of GATA4 protein in the gonads of male and female MS and WC embryos during late embryonic and early fetal life. (C) Densitometric values (arbitrary units) indicating the amount of 17α-hydroxylase/17,20-lyase cytochrome P450 (P450c17) protein in the testes of male MS and WC embryos during late embryonic and early fetal life. *P<0.05.

In males, sexual differentiation is an active process requiring initiation by SRY resulting in the differentiation of Sertoli cells and Leydig cells which produce the hormones MIS and testosterone respectively. Mullerian duct regression requires MIS, and maintenance of the Wolffian duct system, later developing into the vas deferens and epididymis, requires testosterone (Behringer et al. 1990, Mishina et al. 1996, Racine et al. 1998). However, the profiles of MIS and P450c17 protein in the present study do not support a role for MIS as a negative regulator of P450c17 expression in the embryonic pig. This is likely a reflection of the variety of species- and tissue-specific mechanisms of P450c17 gene regulation (Chung et al. 1987, DiBlasio et al. 1987, Mellon & Vaisse 1989, Mesiano et al. 1993, Staels et al. 1993, Givens et al. 1994, Greco & Payne 1994, Miller et al. 1997, Zhang & Mellon 1997). Profiles of both MIS and P450c17 protein exhibit similar patterns; however, MIS and P450c17 were both elevated in MS compared with WC testes. Observed differential secretory profiles were not associated with timing of initiation of Sertoli and Leydig cell differentiation. However, elevated production of MIS in MS testes may be related to MIS function, i.e. Mullerian duct regression, which was not evaluated in this study. Similarly, elevated P450c17 in MS boars may be associated with increased testosterone synthesis and secretion from Leydig cells (Moon et al. 1973, Ford et al. 1980). Potential association of differential production of MIS and P450c17 with cell function, and relationship to future reproductive characteristics in these two diverse breeds of pigs remain to be elucidated.


MIS and P450c17 are essential for normal male development. Increased MIS protein with advancing age is consistent with the requirement of high levels of MIS to cause regression of Mullerian ducts. Similarly, increased P450c17 with age is consistent with the requirement for testosterone production from the testis for maintenance of Wolffian ducts and subsequent development of male genitalia. In addition to the vital role of MIS on the Mullerian ducts, MIS has been implicated as a negative modulator of Leydig cell differentiation and function (Behringer et al. 1990, Mishina et al. 1996, Racine et al. 1998). However, the profiles of MIS and P450c17 protein in the present study do not support a role for MIS as a negative regulator of P450c17 expression in the embryonic pig. This is likely a reflection of the variety of species- and tissue-specific mechanisms of P450c17 gene regulation (Chung et al. 1987, DiBlasio et al. 1987, Mellon & Vaisse 1989, Mesiano et al. 1993, Staels et al. 1993, Givens et al. 1994, Greco & Payne 1994, Miller et al. 1997, Zhang & Mellon 1997). Profiles of both MIS and P450c17 protein exhibit similar patterns; however, MIS and P450c17 were both elevated in MS compared with WC testes. Observed differential secretory profiles were not associated with timing of initiation of Sertoli and Leydig cell differentiation. However, elevated production of MIS in MS testes may be related to MIS function, i.e. Mullerian duct regression, which was not evaluated in this study. Similarly, elevated P450c17 in MS boars may be associated with increased testosterone synthesis and secretion from Leydig cells (Moon et al. 1973, Ford et al. 1980). Potential association of differential production of MIS and P450c17 with cell function, and relationship to future reproductive characteristics in these two diverse breeds of pigs remain to be elucidated.

Figure 3 (A) Body weight and (B) crown–rump length (CRL) (LSM ± s.e.) of MS and WC embryos during late embryonic and early fetal life. ***P<0.001.
developmental expression patterns of GATA4 and MIS previously observed (McCoard et al. 2001a), further supporting a role for GATA4 in porcine embryonic gonadogenesis.

In conclusion, highly co-ordinated morphological changes and strict spatial and temporal patterns of MIS, GATA4 and P450<sub>c17</sub>, production illustrate that embryonic sexual differentiation is a tightly regulated process in the pig. Male MS and WC embryos exhibited similar temporal and spatial patterns of morphological development indicating that the timing of differentiation of testicular cords and steroidogenic machinery of the testis does not differ between these two breeds, and is unlikely to impact future reproductive performance in pigs. However, while differential expression of MIS and P450<sub>c17</sub>, two key proteins associated with sexual differentiation, did not influence the timing of sexual differentiation, implications for Sertoli and Leydig cell function warrant further investigation. In contrast to males, female MS embryos exhibited advanced sexual differentiation compared with WC embryos, which may be related to future reproductive characteristics, such as early onset of puberty observed for this breed.

Acknowledgements

The authors thank Susan Hassler and Alan Kruger for their skilful technical assistance, the personnel of the USMARC Swine Operations for care of animals, and Donna Gries for secretarial assistance.

References


Chung BC, Picado-Leonard J, Maniu M, Bienkowski M, Hall PF, Shively JE & Miller WL 1987 Cytochrome P450c17 (steroid 17 alpha-hydroxylase/17,20-lyase); cloning of human adrenal and testis cDNAs indicates the same gene is expressed in both tissues. PNAS 84 407–411.


FASS 1999 Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, revised ed. 1, Savoy, IL: Federation of Animal Science Societies.


FASS 1999 Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, revised ed. 1, Savoy, IL: Federation of Animal Science Societies.


McCoard SA, Lunstra DD, Wise TH & Ford JJ 2001b Specific staining of Sertoli cell nuclei and evaluation of Sertoli cell number and proliferative activity in Meishan and White Composite boars during the neonatal period. *Biologoy of Reproduction* 64 689–695.


Tremblay JJ & Viger RS 1999 Transcription factor GATA-4 enhances the testis anti-Mullerian hormone gene expression of the mouse anti-Mullerian hormone gene suggests a role in both male and female sexual differentiation. *Development* 113 613–624.

interaction with the nuclear receptor SF-1. Molecular Endocrinology 13 1388–1401.


Received 26 June 2002
Accepted 10 July 2002