

Comparative Study of Uterine Morphogenesis and Protein Secretion in Neonatal White Crossbred and Meishan Gilts¹

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ABSTRACT

Thirty-five crossbred and 22 Meishan contemporary gilts were necropsied on Day 1, 14, 28, 42, or 56 of age (birth = Day 0). At necropsy, a cross section of one uterine horn was fixed for histomorphometric study, and minced uterine tissue was cultured with 50 μ Ci [³H]leucine. Secreted proteins were identified by two-dimensional PAGE, fluorography, and incorporation of radioactivity. Body weights at necropsy were similar for the two breeds and increased ($p < 0.01$) between Days 1 and 56 of age. Ovarian and uterine weights, as well as histomorphometric areas, were similar for the two breeds on Day 1 but increased markedly ($p < 0.01$) in Meishan gilts on Day 56. In gilts of both breeds, secretion of uterine proteins 1 ($M_r \times 10^{-3}/pI$; 45/6.0), 2a and 2b (doublet, 25/6.2), and 3 (20/5.5) increased in association with endometrial gland development. A fourth protein (97/4.0) was observed in gilts of both breeds but was more abundant in Meishan; a fifth protein (13/6.0) was detected only in crossbred gilts on Day 56. Although specific regulatory roles for locally produced uterine proteins remain to be defined, the increase in specific uterine proteins and breed differences in uterine protein secretion suggest that uterine proteins may influence early uterine development.

INTRODUCTION

Ovulation rate, fertilization rate, early embryo survival, and uterine capacity are four components that affect litter size in swine [1, 2]. The first three traits have been studied extensively, but the factors that influence uterine capacity in swine are largely unknown. The Chinese Meishan pig is among the world's most prolific breeds [3–6], but the biological basis for this hyperprolificacy is not fully defined. Several reports show that Meishan gilts have a greater ovulation rate than most occidental breeds of swine when measured at the same age [7–9], and this superiority is maintained in Meishan sows [9]. Early embryo survival (before Day 30 of gestation) in Meishan gilts may or may not exceed that of occidental breeds of swine [8, 9]. However, number of piglets born alive as a percentage of ovulation rate, which is influenced by both early embryo survival and uterine capacity, is greater in Meishan gilts [7]. The extent to which patterns of uterine development may differ between Meishan and occidental breeds of pigs is not known. However, such early events in the organization of uterine tissues likely influence uterine capacity and/or function during pregnancy [10].

Organizational events associated with growth, morphogenesis, and cytodifferentiation of uterine tissue have been described [11–15]. It is likely that the success of these events will determine the potential of the uterus to support conceptus development. In turn, interruption or acceleration of these events may compromise adult uterine function [10, 16], thereby contributing to reproductive inefficiency.

At birth, the uterus consists of an outer epithelial layer, a single myometrial layer, and an endometrial layer made up of stromal cells covered by a single layer of luminal epithelial cells. No endometrial glands are present [11, 12]. Within 1 wk, endometrial glands begin to differentiate, and they continue to differentiate for the next several months [11, 12]. An understanding of histological and biochemical factors that affect neonatal uterine growth, including endometrial gland development, may provide insight into events that are essential for establishment of maximal uterine capacity and may help identify biological markers of hyperprolificacy.

The objectives of this study were to compare, in crossbred and Meishan gilts from birth to 56 days of age, 1) ovarian, uterine, and body weight development; 2) uterine gland development; and 3) proteins secreted by uterine explants.

MATERIALS AND METHODS

Animals

Gilts from 10 White composite crossbred (1/4 each Chester White, Landrace, Large White, and Yorkshire) and 8 Chinese Meishan sows were used. Thirty-five crossbred ($n = 7$ gilts per necropsy age) and 22 Meishan ($n = 4$ or 5 gilts per necropsy age) neonatal contemporary gilts born in January and February were randomly assigned at birth (Day 0) to be necropsied on Day 1, 14, 28, 42, or 56 of age. Those gilts necropsied on Day 42 or 56 of age were weaned with littermates at 28–32 days of age and allowed ad libitum access to an 18% crude protein diet in a nursery. At necropsy, all gilts were weighed, stunned with a captive bolt device, and exsanguinated, and the uterus and ovaries were removed aseptically. All procedures involving animals were approved by the Animal Care and Use Committee at the Roman L. Hruska U.S. Meat Animal Research Center.

Tissue Collection and Processing

Ovaries were removed, weighed, and by gross and histological examination classified according to stage of development as follows: type 1, no follicles on the surface; type 2, fewer than five protruding follicles (larger than 2 mm in diameter); type 3, five or more protruding follicles and a grapelike cluster appearance; and type 4, having corpora lutea [17]. Each uterus was immediately trimmed of mesometrium and weighed, uterine horn length was recorded, and a 0.5- to 1-cm cross section of the right uterine

Accepted December 5, 1996.

Received March 18, 1996.

¹Mention of names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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horn proximal to the external uterine bifurcation was fixed in 4% paraformaldehyde for histomorphometric study. The remaining uterine tissue was minced and prepared for culture.

Histology and Histomorphometry

Fixed tissue was dehydrated, embedded in paraffin (56°C melting point), sectioned (8 µm), mounted on slides, and stained with hematoxylin and eosin. Before assessment of morphological and glandular development, a preliminary examination of the same neonatal uterine cross sections showed that three repeated determinations of seven morphological traits, described below, resulted in coefficients of variation of 1% or less. Furthermore, in a separate study, Vallet et al. [18] determined repeatability of histomorphometric measurements of neonatal uterine cross sections, and reported correlations of two repeated determinations of 0.96 or more for six different histomorphometric measurements. These results indicate that morphometric measurements of uterine cross sections are highly repeatable. Therefore, to assess morphological and glandular development, a single uterine cross section from each gilt was evaluated for uterine diameter and total uterine cross-sectional, longitudinal myometrial, circular myometrial, endometrial, total endometrial gland, gland epithelial, and luminal epithelial areas. Morphometric analysis was performed using a Microcomp Integrated Image Analysis Software System for Planar Morphometry (Southern Micro Instruments, Atlanta, GA). The system was calibrated using a slide micrometer. To obtain the measurements, digitized images of the external border of the uterus, the border between the longitudinal and circular myometrium, the border between the myometrium and endometrium, the basal border of the luminal epithelium, and the luminal border were delineated, and the areas within these borders were calculated. Longitudinal myometrial area was defined as the uterine area minus the area within the longitudinal/circular myometrial border. Circular myometrial area was defined as the area within the longitudinal/circular myometrial border minus the area within the circular myometrium/endometrial border. Endometrial area was defined as the area within the border between the myometrium and endometrium minus the area within the luminal border. Luminal epithelial area was calculated by subtracting the area within the luminal border from the area within the basal border of the luminal epithelial layer. Endometrial glands were defined as groups of epithelial cells that surrounded a closed lumen separated from the uterine lumen by interposed cells [19]. To determine glandular epithelial area, the area within the lumen of each gland was determined, summed, and subtracted from the sum of the areas within the basal borders of each gland.

Tissue Culture and Analysis of Uterine Proteins

Minced tissue from each uterus (Day 1 gilts, 135 ± 12 mg; Day 14, 28, 42, or 56 gilts, 200 mg ± 10 mg) was cultured in 5 ml of Minimum Essential Medium with 50 µCi [³H]leucine. Tissues were cultured for 24 h at 37°C under an atmosphere of 50% N₂:45% O₂:5% CO₂. After incubation, each culture was centrifuged (2000 × g) to facilitate separation of conditioned medium and tissue. Conditioned medium was frozen in liquid nitrogen and stored at -70°C for later analysis. The conditioned medium was dialyzed for 72 h against three 4-L volumes of 10 mM Tris, 0.02% sodium azide, pH 8.2, and nondialyzed radioactivity was determined. An aliquot containing 200 000 dpm of

nondialyzable radioactivity from each culture was lyophilized and subjected to two-dimensional PAGE and fluorography for a 28-day exposure [20]. A visual inspection of all fluorographs was made according to breed and age. After alignment of fluorographs and dried two-dimensional gels for each gilt, proteins of interest were punched from each gel using a sharp 7-mm-i.d. cork borer [21]. Gel samples were placed in scintillation vials, digested for 72 h at 80°C with 0.5 ml 30% hydrogen peroxide, and neutralized with 0.5 ml 50 mM ascorbic acid. Then 20 ml of scintillation fluid was added to each vial, and samples were counted for 10 min on a liquid scintillation counter to determine counts per minute (cpm). A protein-deficient region ($M_r \times 10^{-3}/pI$; 14/6.5) was selected to obtain background radioactivity. A background gel sample was collected from five randomly selected two-dimensional PAGE gels representing each breed. All background cpm values were averaged, and the average was subtracted from sample values to adjust for background radioactivity.

Statistical Analysis

Data were analyzed using the General Linear Models least-square ANOVA procedure [22]. Body weight, ovarian weight, uterine weight, total uterine horn length, eight morphometric traits, and tritium-labeled uterine proteins from crossbred and Meishan gilts were initially analyzed using a statistical model that included the main effects of breed, age, and their interaction. To determine developmental age changes, polynomial regressions of traits on age were fitted and appropriate functions were determined for each breed. The heterogeneity of regression was used to determine breed differences in developmental changes. Chi-square analysis was used to compare gilts of the two breeds for presence of vesicular follicles.

RESULTS

Birth weight was greater ($p < 0.05$) for crossbred gilts (1.23 ± 0.04 kg; $n = 35$) than for Meishan gilts (1.06 ± 0.05 kg; $n = 22$). However, a test of heterogeneity of regression indicated that body weight did not differ ($p > 0.15$) for crossbred and Meishan gilts at the five necropsy ages (Fig. 1A). Body weight increased ($p < 0.01$) with age, and the pattern of body weight increase was quadratic for gilts of both breeds.

Ovarian weights for crossbred and Meishan gilts are presented in Figure 1B. Ovarian weight increase from Day 1 to 42 was minimal in both breeds. However, the interaction of breed and age was highly significant ($p < 0.01$), and heterogeneity of regression indicated that patterns of change in ovarian weight from Day 1 to 56 differed ($p < 0.01$) for crossbred and Meishan gilts. Examination of the data indicated that ovarian weight in Meishan gilts was markedly greater at 56 days of age than in crossbred gilts. Gross and histological examination of ovaries collected at 2-wk intervals from 1 to 56 days of age showed that type 1 (no follicles on the surface) ovaries were observed in all crossbred gilts throughout the study and in Meishan gilts from Day 1 to 28. One of five Day 42 Meishan gilts had type 2 ovaries (fewer than five vesicular follicles), and four of four Day 56 Meishan gilts had type 3 ovaries (5 or more vesicular follicles). Chi-square analysis indicated that number of gilts with vesicular follicles was greater ($p < 0.01$) for Meishan than for crossbred gilts at 56 days of age.

Uterine weights for crossbred and Meishan gilts are presented in Figure 1C. The interaction of breed and age was

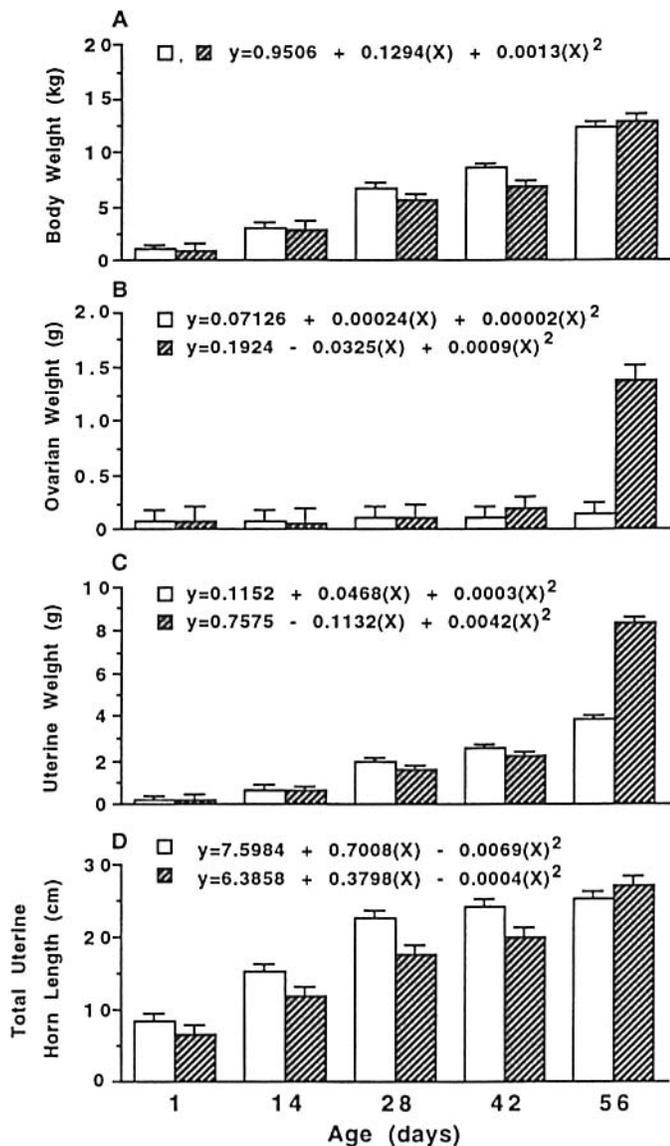


FIG. 1. Body weight (A), ovarian weight (B), uterine weight (C), and total uterine horn length (D) with increasing age for crossbred (open bars) and Meishan (cross-hatched bars) gilts. Data were analyzed by heterogeneity of regression. Changes with day were best fitted ($p < 0.05$) using quadratic equations. If patterns of change differed ($p < 0.01$) for breeds, an equation for each breed is indicated on the graph.

significant ($p < 0.01$), and age-related patterns of change in uterine weight differed for crossbred and Meishan gilts (heterogeneity of regression; $p < 0.01$). The data indicated that uterine weight increased steadily for both breeds through 42 days of age with a slight advantage observed for crossbred gilts on Days 28 and 42. However, by 56 days of age there was a marked increase in uterine weight for Meishan gilts.

Total uterine horn lengths for crossbred and Meishan gilts are presented in Figure 1D. The interaction of breed and age was significant ($p < 0.05$), and patterns of change in uterine horn length differed ($p < 0.01$) with increasing age for crossbred and Meishan gilts. Examination of the data indicated that the interaction of breed and age resulted from the fact that uterine horn length was numerically greater for crossbred than for Meishan gilts on Days 1, 14, 28, and 42; but by Day 56, this relationship was reversed.

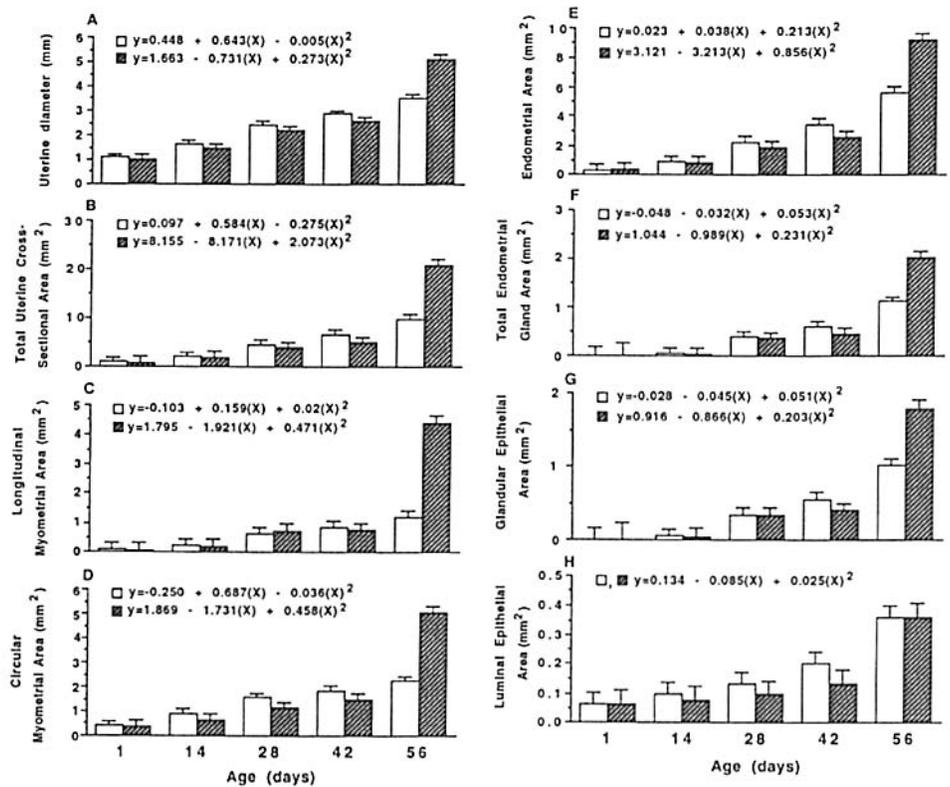
Morphometric analysis of uterine wall cross sections for

crossbred and Meishan gilts with increasing age are presented in Figure 2. Interactions of breed and age were significant ($p < 0.01$) for all eight histomorphometric traits except for luminal epithelial area (Fig. 2H), for which patterns of change did not differ between breeds. All eight histomorphometric traits were best described by quadratic polynomial regression equations and, with the exception of luminal epithelial area mentioned above, differed ($p < 0.01$) in patterns of change between crossbred and Meishan gilts. As illustrated in Figure 2 (A-G), seven of the uterine traits increased from Day 1 for both breeds but increased markedly at 56 days of age in Meishan gilts.

Histological preparations of uterine wall cross sections from crossbred and Meishan gilts with increasing age are shown in Figure 3. The presence of uterine glands was similar for crossbred and Meishan gilts. Uterine glands were detected in two of seven crossbred and one of four Meishan gilts on Day 1 of age. On Day 14, all crossbred and Meishan gilts had uterine glands present, and rapid uterine gland development was evident in both breeds through Day 56 of age.

Means for nondialyzable radioactivity per gram of endometrial tissue cultured are illustrated in Figure 4. In vitro incorporation of [3 H]leucine into secreted endometrial proteins was not influenced by breed, and a breed \times gilt age interaction was not detected. However, age did affect mean nondialyzable radioactivity ($p < 0.01$) as illustrated (Fig. 4). Visual inspection of fluorographs indicated that increased secretion of uterine proteins 1 ($M_r \times 10^{-3}/pI$; 45/6.0), 2a and 2b (doublet, 25/6.2), and 3 (20/5.5) was observed in both breeds for all neonatal gilt uteri collected between Days 1 and 56 of age (Fig. 5). A fourth protein (97/4.0) was observed in all gilts of both breeds but was greater in Meishan gilts, and a fifth protein (13/6.0) was detected only in crossbred gilts on Day 56. To confirm visual inspection of fluorographs, radioactivity associated with these uterine proteins was determined, and counts per minute minus background are shown in Table 1. Counts associated with uterine protein 1 were greater ($p < 0.01$) in crossbred gilts and increased ($p < 0.01$) with neonatal age. Uterine protein 2, a doublet, was counted separately as proteins 2a and 2b. For uterine protein 2a, the breed \times age interaction was significant ($p < 0.05$), and this protein appeared to increase with neonatal age. Secretion of uterine protein 2b was greater ($p < 0.01$) in Meishan than in crossbred gilts and increased ($p < 0.01$) with neonatal age. Heterogeneity of regression indicated that patterns of change in secretion of uterine protein 3 differed ($p < 0.05$) for crossbred and Meishan gilts. As indicated by examination of the data, secretion of uterine protein 3 was greater and increased at a faster rate in Meishan than in crossbred gilts. Uterine protein 4 was greater ($p < 0.01$) in Meishan than in crossbred gilts, but significant differences were not detected for age of neonatal gilts. Uterine protein 5 was not abundantly secreted, and heterogeneity of regression indicated that patterns of change in secretion of uterine protein 5 differed ($p < 0.05$) for crossbred and Meishan gilts. The data indicated that secretion of uterine protein 5 was greater on Day 56 in crossbred than in Meishan gilts. Overall, a correlation analysis indicated significant ($p < 0.01$) positive associations ($r = 0.47$ to 0.68) between uterine weight and uterine secreted proteins 1, 2a, 2b, 3, 4, and 5 across all neonatal ages for crossbred gilts. In Meishan gilts, in contrast, only uterine protein 3 was significantly ($p < 0.05$) associated ($r = 0.48$) with uterine weight across all neonatal ages. Examination of the data indicated that secretion

FIG. 2. Morphometrial analysis of uterine diameter (A), total uterine cross-sectional area (B), longitudinal (C) and circular (D) myometrial area, endometrial area (E), total endometrial gland area (F), glandular epithelial area (G), and luminal epithelial area (H) with increasing age for crossbred (open bars) and Meishan (cross-hatched bars) gilts. Data were analyzed by heterogeneity of regression. Changes with day were best fitted ($p < 0.05$) using quadratic equations. If patterns of change differed ($p < 0.01$) for breeds, an equation for each breed is indicated on the graph.



of uterine proteins increased in both breeds from Day 1 to 28 and then plateaued or was reduced in Meishan gilts on Day 42 or 56 of age.

DISCUSSION

Neonatal uterine growth and development may influence subsequent adult uterine function and have a significant effect on prenatal survival during both early gestation (maternal recognition of pregnancy) and during the later fetal period (uterine capacity) [10]. Characteristics of neonatal uterine development between birth and puberty have been described at gross [10, 14, 23], histological [10–12, 15, 24], and biochemical levels [10, 15, 16, 18, 25] in occidental

breeds of pigs. Neonatal porcine uterine growth, as measured by uterine horn length and weight, occurs in an ovary-independent manner before, and in an ovary-dependent manner after, Day 60 of neonatal life [10, 23]. During the ovary-independent stage, the porcine uterine wall undergoes dramatic remodeling that includes appearance and proliferation of endometrial glands, formation of endometrial folds, and growth and development of the myometrium [11, 12, 14, 15, 24]. A variety of morphological events and biochemical factors are known to be involved in the early development of the uterus of neonatal gilts. These have been reviewed by Bartol et al. [10].

Although numerous studies have been conducted in oc-

TABLE 1. Radioactivity (cpm) incorporated into secreted uterine proteins during in vitro culture of uterine explants collected at different neonatal ages of crossbred and Meishan gilts.

Protein ^a	Breed	Age (day)				
		1	14	28	42	56
1 ^{b,c}	Crossbred	197 ± 37	261 ± 37	310 ± 37	307 ± 37	334 ± 37
	Meishan	152 ± 48	167 ± 48	309 ± 43	228 ± 43	187 ± 48
2a ^d	Crossbred	60 ± 26	27 ± 26	105 ± 26	121 ± 26	183 ± 26
	Meishan	36 ± 35	46 ± 35	278 ± 31	181 ± 31	174 ± 35
2b ^{e,f}	Crossbred	79 ± 29	50 ± 29	127 ± 29	187 ± 29	148 ± 29
	Meishan	102 ± 39	112 ± 39	227 ± 35	295 ± 35	199 ± 39
3 ^d	Crossbred	44 ± 33	-6 ± 33	73 ± 33	112 ± 33	170 ± 33
	Meishan	9 ± 44	11 ± 44	174 ± 40	283 ± 40	229 ± 44
4 ^e	Crossbred	25 ± 51	12 ± 51	52 ± 51	108 ± 51	143 ± 51
	Meishan	176 ± 68	220 ± 68	180 ± 60	309 ± 60	172 ± 68
5 ^d	Crossbred	-8 ± 35	-19 ± 35	40 ± 35	71 ± 35	170 ± 35
	Meishan	31 ± 46	17 ± 46	62 ± 42	98 ± 42	2 ± 46

^a Uterine protein description presented in Figure 5.

^b Main effect, breed ($p < 0.05$).

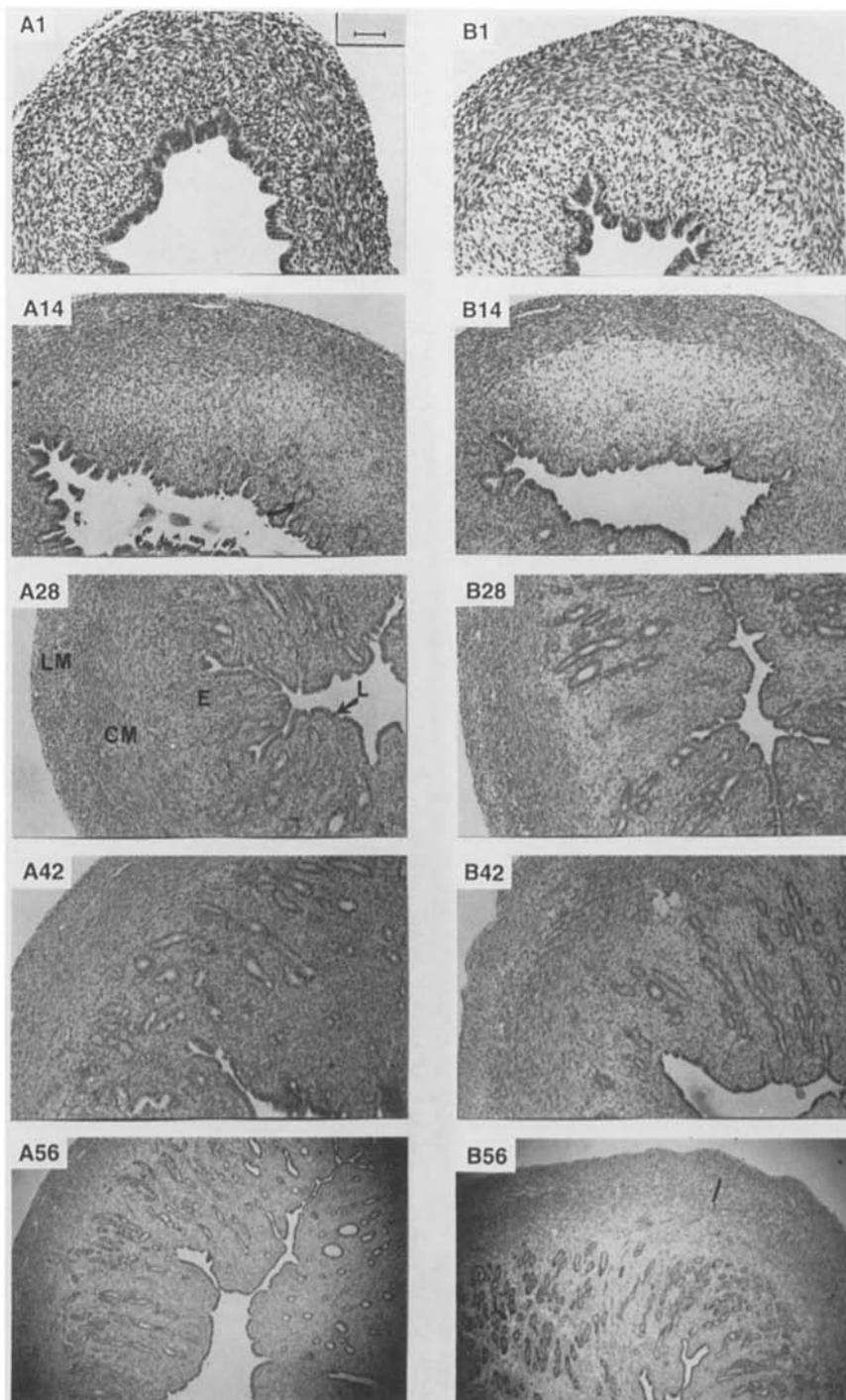
^c Main effect, age ($p < 0.05$).

^d Interaction, breed × age ($p < 0.05$).

^e Main effect, breed ($p < 0.01$).

^f Main effect, age ($p < 0.01$).

FIG. 3. Representative light micrographs of uterine wall cross sections of crossbred (A) and Meishan (B) gilts on Days 1, 14, 28, 42, and 56 of age. Bar length shown for Day 1 (A1) is 50 μm , and it is 100, 100, 100, and 200 μm , respectively, for Days 14, 28, 42, and 56. On Day 1 of age for crossbred and Meishan gilts (A1 and B1), no uterine glands are visible, but on Day 14 (A14 and B14), uterine glands with glandular epithelium are seen (arrows). In A28, luminal epithelium (L), endometrium (E), and circular (CM) and longitudinal (LM) myometrium are depicted.



cidental breeds of pigs, few [17, 26] have compared ovarian and uterine development in neonatal Meishan and occidental breeds of pigs. Current and previous results indicate that body weight is similar or slightly less for Meishan than for occidental breeds of gilts, but the two breeds show a similar pattern of weight increase during the early neonatal period. Ovarian and uterine morphological development (uterine weight and length, endometrial and myometrial thickness) for gilts from birth to about 42–45 days of age were similar for Meishan and occidental breeds. Marked increases in ovarian and uterine horn weight, as well as thickness (cross-sectional area) of the endometrium and both circular and longitudinal myometrial layers, were observed by 56 (this study) to 60 days [26] in Meishan. Miyano et al. [26]

also reported these increases by 90–100 days of age in occidental breeds of gilts. These increases are associated with the first appearance of ovarian vesicular follicles in Meishan gilts at about 42–45 days of age and most likely in occidental breeds of gilts after appearance of ovarian vesicular follicles at about 70–90 days of age [27]. Because of apparent low serum concentrations of estradiol-17 β in neonatal gilts and inadequate sensitivities of estradiol-17 β RIA [28], serum estrogen was not determined in the present study. However, uterine wet weight has been shown to be a sensitive bioassay for circulating estrogens [29], and uterine wet weight was recorded at necropsy in this study. Urinary estrogen, the primary estrogen product produced from ovarian estrone and estradiol-17 β , was used by Camous et

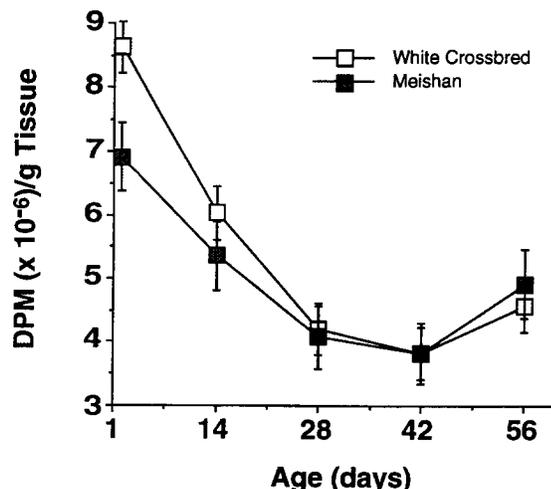


FIG. 4. Mean nondialyzable radioactivity ($\text{dpm} \times 10^{-6}/\text{g}$ tissue) from cultures of uteri collected from 1-, 14-, 28-, 42-, and 56-day-old gilts. Overall, the analysis of variance indicated that the influence of age on mean nondialyzable radioactivity was significant ($p < 0.01$), but the influences of breed and breed \times age were nonsignificant. Heterogeneity of regression analysis indicated that the overall relationship between age and nondialyzable radioactivity was quadratic, and the linear component of the relationship between age and nondialyzable radioactivity differed with breed ($p < 0.05$).

al. [30] to describe the relationship of estrogen secretion and ovarian activity in neonatal gilts. Camous et al. [30] reported that in Large White gilts the pattern of urinary estrone excretion was relatively constant and less than $2.5 \mu\text{g}/24 \text{ h}$ from 40 to 90 days of age; after 90 days of age, urinary estrone increased twofold, coinciding with the appearance of vesicular follicles. Consistent with these observations are reports by Wu and Dziuk [23] and Bartol et al. [10] that ovariectomy (Day 0 or 20 of age) of neonatal crossbred occidental breeds of gilts did not affect uterine growth until after Day 60 of age; uteri of intact controls were heavier by 90 days of age. It has been suggested that in occidental gilts, this period of dramatic, ovary-dependent uterine growth reflects effects of ovarian estrogen [10, 14]. Neonatal porcine uterine tissues are estrogen receptor-positive [31] and estrogen-sensitive [16, 18]. Taken together, these results suggest that the increases in uterine development in Meishan gilts at Day 56 of age are the result of increased ovarian estrogen production associated with the appearance of vesicular follicles.

Using histological procedures, Miyano et al. [26] observed uterine gland differentiation on Day 1 of age in 2 of 2 Meishan and 0 of 2 Landrace gilts. In the present study, uterine gland differentiation was observed in 2 of 7 crossbred and in 1 of 4 Meishan gilts on Day 1 of age. Although the percentage of gilts of both breeds with some gland differentiation is 25%, several other studies report no glandular development in occidental gilts on Day 0 or 1 [12].

In addition to histological evaluation, *in vitro* uterine protein secretion was examined on uteri collected on Day 1, 14, 28, 42, or 56 from crossbred and Meishan gilts. Of the uterine proteins examined, uterine protein 1 was secreted in more abundance in crossbred gilts, while uterine proteins 2b, 3, and 4 were secreted in more abundance in Meishan gilts. However, on the basis of visual inspection and incorporation of [³H]leucine during culture, increases in uterine proteins 1, 2a, 2b, and 3 were evident between 14 and 28 days of age in both breeds. This temporal association between endometrial gland development and the

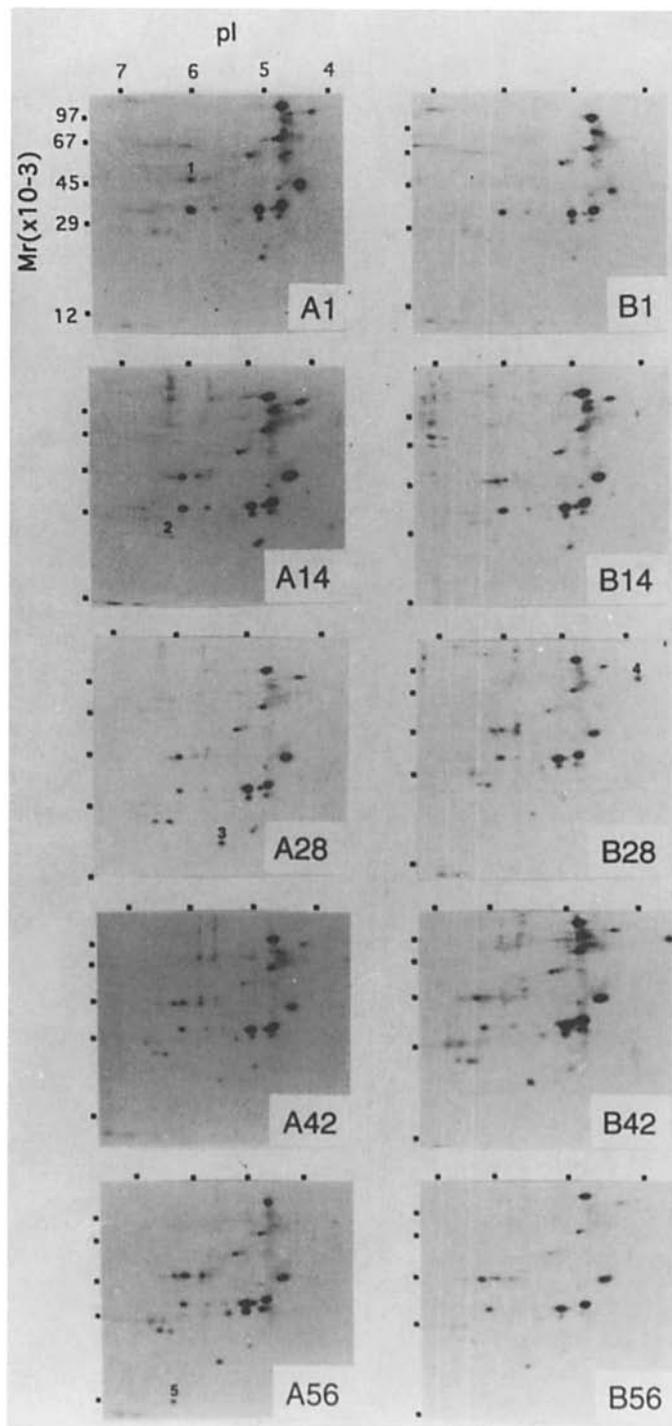


FIG. 5. Representative fluorographs of two-dimensional PAGE gels of proteins secreted in culture by uteri from crossbred (A) and Meishan (B) gilts on Days 1, 14, 28, 42, and 56 of age. For both breeds, secretion of at least four proteins ($M_r \times 10^{-3}/\text{pI}$) shown in A1 and B1, A14 and B14, and A28 and B28 increased in association with uterine gland development (protein 1, $45/6.0$, A1; protein 2a [left] and 2b [right], a doublet, $25/6.2$, A14; protein 3, $20/5.5$, A28). A fourth protein ($M_r \times 10^{-3}/\text{pI}$; $97/4.0$), shown in B28, was greater in Meishan than in crossbred gilts; a fifth protein ($M_r \times 10^{-3}/\text{pI}$; $13/6.0$), shown in A56, increased by Day 56 in crossbred gilts.

marked increase in at least four uterine secreted proteins suggests possible cause-and-effect relationships of uterine secreted proteins and endometrial gland development during the ovary-independent stage. During the increase in vesicular follicles between Day 42 and 56 in Meishan gilts,

follicular estrogen may be responsible for additional uterine development during the ovary-dependent stage. This increased uterine development does not appear to be mediated through the specific uterine proteins reported in the present study. In general, all uterine-secreted proteins appeared to decrease in Meishan and increase in crossbred gilts on Days 42 and 56 of age. It remains to be determined whether the increased abundance of uterine secreted proteins during the ovary-independent stage and the early appearance of vesicular follicles during the ovary-dependent stage in Meishan gilts may be partially responsible for the increased prolificacy of Meishan gilts.

In both breeds, uterine protein 3 ($M_r \times 10^{-3}$ /pI; 20/5.5) has been identified as retinol-binding protein (RBP) in our laboratory by its cross-reactivity with anti-human RBP antiserum [18]. RBP has been considered a major secretory product of the liver. Its function is to transport retinoids from hepatocytes (which take up retinoids from the circulation) to stellate cells (which store retinoids) and secreted retinyl esters in an endocrine fashion from both parenchymal and stellate cells to the general circulation [32]. Recent results indicate that the RBP is expressed in numerous adult extra-hepatic tissues [33, 34] as well as neonatal uterine tissue [35, 36], suggesting that RBP is required for paracrine transport of retinol within developing tissues. Retinoids are required for growth, proliferation of most cell types, and maintenance of differentiated epithelium [37]. Retinoids also modulate cellular effects of mitogens and hormones, alter growth factor receptor populations, and alter cell signal transduction pathways [37, 38]. Identification of RBP ([18]; present study) and its mRNA [35, 36] in developing uterine tissue suggests that retinoid transport and metabolism are present to modulate the effects of vitamin A in events critical to uterine growth and endometrial glandular development.

Specific regulatory roles for locally produced uterine proteins remain to be fully defined. However, breed differences reported here in patterns of production of uterine proteins may reflect critical differences in patterns of uterine cytodifferentiation between a recognized prolific (Meishan) and an occidental breed of pig that ultimately could affect the capacity of adult uterine tissues to support conceptus development.

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