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Serum Estrogen Concentrations during Postnatal Development in Male Pigs (41719)

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Abstract. Serum concentrations of estrone (E_1), estradiol (E_2), estrone sulfate (E_1S), and testosterone (T) were quantified in male pigs at 10 ages from 2 weeks to 2 years. Changes in the concentrations of all four of these hormones were characterized by elevated amounts during neonatal development (1 to 3 weeks of age), a nadir for concentration of each hormone between 1 and 3 months of age, an increase during pubertal development with maximal concentrations at 8 months of age, and no further change by 2 years of age. E_1S was predominant of the three estrogens at all age groups with mean concentrations that ranged from 2 to 33 ng/ml. E_1 was present in higher concentrations during neonatal development than E_2, whereas the reverse situation was observed after 4 months of age. Observations from the present study indicate that early neonatal and pubertal development in male pigs are associated with elevated serum estrogen concentrations.

Male pigs and horses are uniquely characterized by high testicular secretion of estrogens which is reflected by large amounts of these steroids in the urine (1, 2). For adult male pigs, estrone sulfate is the predominant estrogen in peripheral and spermatic venous plasma (3, 4). Changes in peripheral concentrations of testosterone, 5α-androstenone (5α-androst-16-en-3-one) and dehydroepiandrosterone sulfate during pubertal development of male pigs have been described (5, 6). Testosterone is the predominant biologically active androgen in the peripheral circulation, whereas 5α-androstenone is one of the two main C_{19}-Δ-16 unconjugated steroids that act physiologically as pheromones in male pigs (7). Developmental patterns for estrone sulfate have not been described in detail. Allrich *et al.* (8) observed that serum estradiol concentrations were low from 40 to 100 days of age and increased thereafter until 250 days. The aim of the present investigation was to evaluate changes in peripheral serum concentrations of estrone (E_1), estradiol (E_2), and estrone sulfate (E_1S) in male pigs from the neonatal period to adulthood. In addition, these changes were compared to changes in serum testosterone (T) concentrations.

Materials and Methods. Blood samples were obtained by jugular venipuncture from 101 male pigs at 10 different ages during postnatal development ranging from 12 days to 2.4 years of age. Pigs were Duroc, Hampshire, or Yorkshire purebreds which were weaned at 28–31 days of age and were fed standard corn–soybean meal rations. Breeds were randomized across age groups. Blood samples from 28-day-old males were obtained before they were weaned. Males at 1.6 to 2.4 years of age were sexually experienced herd sires.

Serum samples were stored frozen until assayed for E_1 and E_2 concentrations by previously described radioimmunoassay procedures (9). The antiserum (S-310, number 5) was purchased from G. Abraham (Torrance, Calif.). Samples were assayed for E_1S by a modification of the procedures of Wright *et al.* (10) and for T using previously described procedures (11) except that the antiserum was against a testosterone-19-bovine serum albumin antigen (Cambridge Nuclear Corp., Billerica, Mass.). The E_1S antiserum was prepared against an estrone-3-glucosiduronate-bovine thyroglobulin antigen and was a gift from D. Collins (Atlanta, Ga.). For the E_1S assay, 0.05 or 0.1 ml of serum was added to 0.4 ml of 3 *M* NaCl and extracted once with 5 ml of ethyl ether. After discarding the ether phase, 0.1 ml of 1 *M* Na_2CO_3 was added and E_1S was extracted with two additions of 4 ml of ethyl acetate. E_1S concentrations were corrected for procedural losses during extraction. The concentration of E_1S was 1.6 ± 0.06 ng/

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ml in serum from castrated males to which E_1S standard had been added to a concentration of 1.5 ng/ml. When male serum was assayed at volumes of 100, 50, and 25 μl, the concentration of E_1S was 5.7, 5.5, and 5.2 ng/ml, respectively. After solvolysis of 1 ml of serum from castrate males which contained 300 pg of E_1S standard and 50 μl of intact male serum (345 pg of immunoassayable E_1S), E_1 was determined equivalent to 280 and 360 pg of E_1S, respectively.

Concentrations and interassay coefficients of variation were 44.1 pg/ml, 15.4%; 73.8 pg/ml, 11.1%; 7.1 ng/ml, 9.7%, and 4.1 ng/ml, 10.5% for E_1, E_2, E_1S, and T, respectively, in a serum pool from intact males which was included in all assays. Data are presented as mean ± SEM and were evaluated for statistical significance by analysis of variance after transformation to natural logarithms to adjust for heterogeneity of variances. Hormone concentrations at different ages were compared by the Newman–Keuls method for unequal sample size (12).

Results. Serum concentrations of E_1 and E_2 are illustrated in Fig. 1. Both were elevated at 12 days of age. E_2 concentrations were reduced ($P < 0.05$) by Day 21; whereas, E_1 remained elevated at this age but decreased ($P < 0.01$) by 28 days. E_1 concentrations continued to decrease through 2 months of age. E_1 and E_2 concentrations increased ($P < 0.01$)

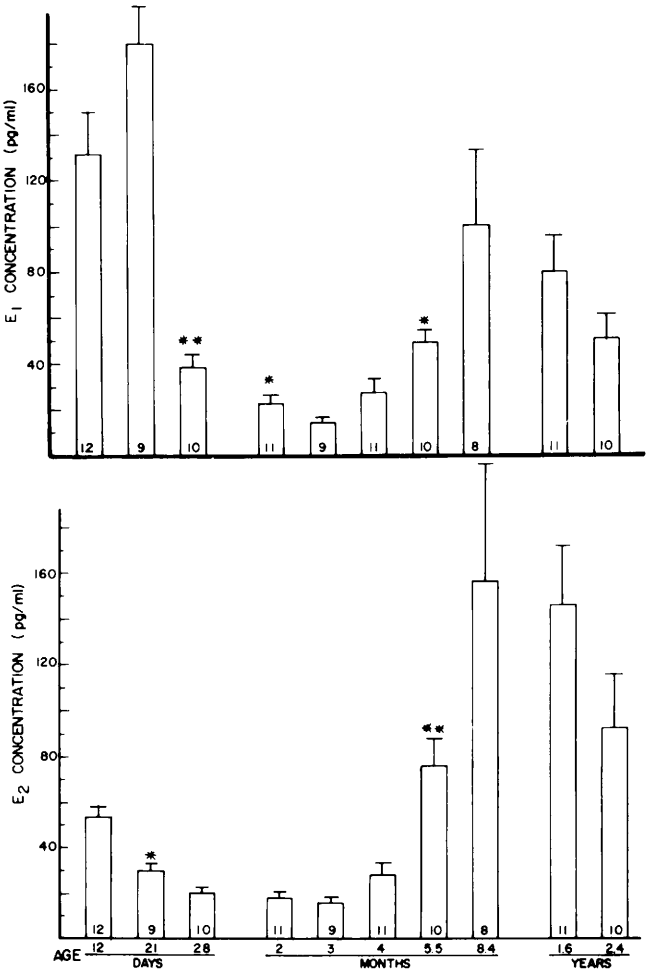

FIG. 1. Serum concentrations of estrone (E_1) and estradiol (E_2) during postnatal development in male pigs. Number of pigs evaluated at each age is indicated by number at base of column. Concentrations that were significantly different from the preceding concentration are indicated; $^*P < 0.05$, $^{**}P < 0.01$.

from 4 to 8.4 months. The pattern of change in serum E_1S concentrations was similar to that observed for E_1 with the exception of an increase from Day 12 to 21 (Fig. 2). Serum T concentrations decreased ($P < 0.01$) from 21 to 28 days of age (Fig. 2). Both E_1S and T concentrations increased ($P < 0.01$) from 4 to 8.4 months. E_1 and E_1S were higher ($P < 0.05$) at their maximal concentrations during neonatal development (Day 21) than at their maximal postpubertal concentrations (8.4 months). In contrast, E_2 and T were lower ($P < 0.05$) at their maximal concentrations during the neonatal period than at their maximal postpubertal concentrations.

Discussion. Based on results from the present study, E_1S concentrations in peripheral serum greatly exceed E_1 and E_2 concentrations throughout all phases of postnatal life in male pigs. Dehydroepiandrosterone sulfate and 5-androstenediol sulfate, as well as E_1S, are found in high concentrations in peripheral serum of male pigs, and these concentrations are reduced greatly after castration (4, 6). The concentrations of sulfa-conjugated steroids in testicular lymph greatly exceed concentrations in testicular venous blood, and 80% of the E_1S in young males and 60% in postpubertal males are estimated to leave the testes in the lymph (13). Testes of pigs secrete abundant amounts of sulfa-conjugated steroids relative to most other mammalian species, and sulfatransferase activity, as well as the aromatase system, resides within the Leydig cells (14). The significance of these conjugated steroids is unresolved. Booth (2, 4) suggested that they may

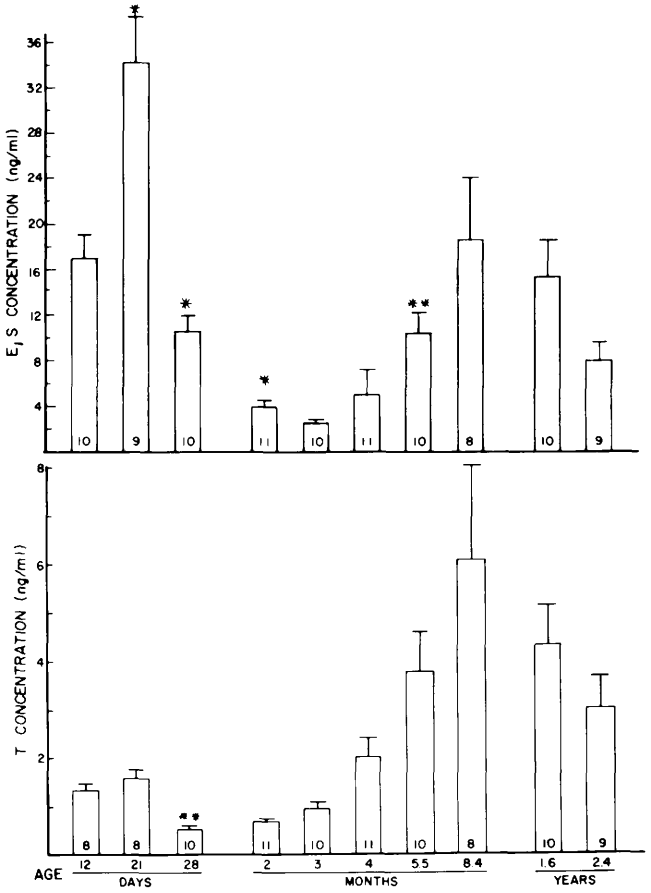

FIG. 2. Serum concentrations of estrone sulfate (E_1S) and testosterone (T) during postnatal development in male pigs. Number of pigs evaluated at each age is indicated by number at base of column. Concentrations that were significantly different from the preceding concentration are indicated; $*P < 0.05$, $**P < 0.01$.

serve as precursors of unconjugated steroids in target organs such as the prostate, seminal vesicles, and submaxillary salivary gland. It seems likely that these serve a role other than excretory products.

Concentrations of E_1, E_2, and E_1S were elevated during the first 3 weeks of life and after 4 months of age. With exception of slight deviations, these changes paralleled those observed in serum testosterone concentrations. From Days 12 to 21, E_2 concentrations were reduced by 50% while E_1S concentrations doubled. This indicates a major increase in sulfatransferase activity during neonatal development. The function of increased secretion of testicular steroids at this time is unknown but is supported by presence of abundant smooth endoplasmic reticulum and hydroxysteroid dehydrogenase activity within the Leydig cells (15, 16). With T as the substrate, higher 17β-hydroxysteroid dehydrogenase activity is observed histochemically during neonatal development than during pubertal development (16). This is an apparent explanation of why E_1 is predominant over E_2 during the neonatal period but not during later stages of development. As yet, no component of the sexual differentiation process can be associated with this neonatal increase in testicular steroidogenesis. Males that are castrated at birth or at 2 months of age show female sexual behavior when treated postpubertally with estrogen (17, 18). In contrast, in males that are castrated at 4 months of age or later, female estrous behavior in response to postpubertal estrogen treatment is diminished or absent (17, 18). These observations indicate that defeminization components of the sexual differentiation process are associated with the pubertal increase in testicular steroidogenesis. This occurs at a much later stage in development than observed for other mammalian species (19). Masculinization of sexual behavior in male pigs appears to also be associated with the pubertal increase in testicular steroidogenesis (20). The relative roles of estrogen and T in differentiation of sexual behavior remain to be evaluated.

The male pig, in common with the male horse, ejaculates a large volume of semen during copulation, and estrogen in pigs acts synergistically with T to maintain seminal volume (21). These authors also proposed a synergistic action of estrogen with T for maintenance of sexual behavior, but this effect was not observed by other investigators (20). Estrone treatment of prepubertally castrated male pigs caused hypertrophy of the prostate, seminal vesicles, and bulbourethral glands (22). It is significant that male pigs have higher concentrations of E_1 and E_2 than estrous females and that long bone growth continues after estrogen secretion begins to increase during pubertal development. Thus, the ability of estrogen to promote epiphyseal closure in rodents and humans (23) is not apparent in male pigs since epiphyseal closure is not complete until after 3 years of age (24). The observations of the present study identify stages during postnatal development when serum concentrations of E_1, E_2, and E_1S are undergoing changes and relate how male pigs differ in serum estrogen concentrations relative to other species.

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