Effects of decreased estradiol-17 β on the serum and anterior pituitary IGF-I system in pigs

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Abstract

To further delineate the role of estradiol in the IGF system an experiment was conducted to determine the dosage of the aromatase inhibitor, anastrozole, needed to decrease serum concentrations of estradiol-17 β (E2) in maturing boars. A second experiment was conducted to determine if administration of anastrozole to growing boars decreased serum concentrations of E2 and affected components of the serum and anterior pituitary gland (AP) IGF system vs untreated boars and barrows. In Experiment 1, 12 crossbred boars (292 days, 158 kg) were administered either 0, 1 or 10 mg/day anastrozole ($n=4$ /group) beginning on day 1. Blood samples were collected every 7–14 days. Mean serum concentrations of E2 were decreased ($P<0.05$) in the 10 mg group vs the 0 and 1 mg groups by day 36; however, no difference ($P>0.05$) existed between the 0 and 1 mg groups. In Experiment 2, 24 crossbred boars and 12 barrows (101 days, 44 kg) were stratified by litter to one of three treatment groups ($n=12$): boars administered 10 mg/day anastrozole, boars administered 0 mg/day, and barrows administered 0 mg/day. Blood samples were collected and pigs were weighed on day 0 and every 14 days thereafter, then killed on day 84 when blood and APs were collected. The 10 mg/day pigs were

fed the anastrozole-amended diet beginning on day 1. Mean serum concentrations of E2 did not differ ($P>0.05$) between the 10 mg/day pigs and 0 mg/day pigs on day 0; however, on day 15 through to 84 mean serum concentrations of E2 were greater ($P<0.05$) in 0 mg/day pigs than in the 10 mg/day pigs. Mean percentage increase in serum concentrations of IGF-I was greater ($P<0.05$) in untreated boars than anastrozole-treated boars and barrows from day 58 through to 84. Mean percentage of basal IGF-I increased ($P<0.05$) from day 29 through to 84 in untreated boars. Mean relative amounts of AP IGF-binding protein (IGFBP)-2 and -5 were less ($P<0.01$) in 10 mg/day pigs than in the 0 mg/day pigs, but each was greater ($P<0.01$) than in barrows administered 0 mg/day. These results indicate anastrozole administered at a dosage of 10 mg/day suppresses serum concentrations of E2 in pigs. Administration of anastrozole to boars reduced the percentage increase in serum concentrations of IGF-I and relative amounts of AP IGFBP-2 and -5. These data further support a role for E2 in regulating components of the IGF system in pigs.

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Introduction

It is well established that boars grow faster and more efficiently than gilts or barrows (Dunshea *et al.* 1993, Owens *et al.* 1999, Clapper *et al.* 2000). Growth differences among boars, barrows and gilts may be due to the differences in circulating concentrations of estradiol-17 β (E2) and insulin-like growth factor-I (IGF-I). Administration of estrogenic compounds (De Wilde & Lauwers 1984) or IGF-I (Klindt *et al.* 1998) has been shown to increase lean gain and efficiency of growth in swine. Clapper *et al.* (2000) found that mean serum concentrations of E2 were greater in boars than in gilts and increased with age in boars. Boars have also been found to have greater circulating concentrations of IGF-I than gilts or barrows (Dunshea *et al.* 1993, Owens *et al.* 1999,

Clapper *et al.* 2000). Administration of E2 implants has been reported to increase circulating concentrations of IGF-I in barrows (Rempel & Clapper 2002), steers (Enright *et al.* 1990, Hays *et al.* 1995), and ovariectomized ewes (Clapper *et al.* 1998). Thus, E2 may mediate its growth-promoting effects through increases in circulating concentrations of IGF-I.

Estradiol has been shown to affect circulating components of the IGF-I system in pigs. Insulin-like growth factor binding proteins (IGFBPs), which are part of the IGF-I system, can regulate IGF actions by stimulating or inhibiting IGF-I activity (Zapf 1995). Previous research has demonstrated that mean serum concentrations of E2 and IGF-I and mean relative amounts of both forms of IGFBP-3 and the 28 kDa IGFBP-4 were greater in boars than barrows and gilts, and mean relative amounts of

serum IGFBP-2 were greater in boars and barrows than gilts (Clapper *et al.* 2000). Administration of E2 implants increased mean serum concentrations of IGF-I, and relative amounts of the 41 kDa IGFBP-3 in barrows (Rempel & Clapper 2002). Barrows implanted with E2 also had greater relative amounts of serum IGFBP-2 vs boars, although mean serum concentrations of IGF-I were greater in boars than in implanted or unimplanted barrows (Rempel & Clapper 2002).

Estradiol has been found to affect components of the porcine anterior pituitary gland (AP) IGF-I system as well. Rempel & Clapper (2002) found E2-implanted barrows had greater AP concentrations of IGF-I compared with boars and unimplanted barrows. However, boars still had greater relative amounts of AP IGFBP-2 and -5 vs implanted and unimplanted barrows (Rempel & Clapper 2002). Because both estradiol and testosterone were increasing in the boars during this time it was not possible to determine which steroid(s) and/or gonadal factors might be responsible for the increase in relative amounts of AP IGFBP-2 and -5.

Estradiol is synthesized from androgens by the aromatase enzyme (Simpson *et al.* 1994). Anastrozole is a non-steroidal aromatase inhibitor that prevents the aromatase enzyme from synthesizing E2 from androgens (Mauras *et al.* 2000). Use of anastrozole has been found to reduce plasma concentrations of E2 by 86% in women (Geisler *et al.* 2001) and serum concentrations of E2 by 48% in men (Mauras *et al.* 2000). Administration of anastrozole may be useful in further defining the effects of decreased E2 on the IGF system in pigs without the confounding effects associated with castration. The objectives of this study were (i) to determine the dosage of anastrozole necessary to decrease serum concentrations of E2 in maturing boars, and (ii) to determine if administration of anastrozole to pre-pubertal growing boars decreases serum concentrations of E2 and affects components of the serum and AP IGF system vs untreated boars and barrows.

Materials and Methods

Animals

All experimental procedures were reviewed and approved by the South Dakota State University Institutional Animal Care and Use Committee.

Experiment 1 Twelve crossbred boars of similar age (292.5 ± 14.3 days) and weight (158.8 ± 4.4 kg) were stratified by litter to one of three treatment groups ($n=4$) on day 0. Boars were penned separately and received free access to water. Treatment groups were 0, 1 and 10 mg anastrozole administered daily. Anastrozole was solubilized in absolute ethanol then applied to 235 g corn-soy-canola meal feed samples, and ethanol was allowed to evaporate overnight. The corn-soy-canola meal diet contained

3.4 Mcal metabolizable energy (ME)/kg diet, 18% crude protein and 0.9% lysine. Each boar was fed the appropriate treatment at approximately 0900 h. After consuming the amended feed, boars were fed the remaining portion of their ration. Boars were fed an amount of feed that contained 3-fold the energy requirements for maintenance each day. Blood samples (10 ml) were collected from all pigs on days 0, 8, 15, 29 and 36 by jugular venipuncture.

Experiment 2 Twenty-four crossbred boars and 12 barrows of similar age (101.7 ± 0.3 days) and weight (44.7 ± 1.0 kg) were stratified by litter to one of three treatment groups ($n=12$) on day 0. Pigs were penned separately and received free access to water and fed a diet daily that contained 2.5-fold their energy requirements for maintenance. Treatment groups were anastrozole-treated boars ($n=12$) administered 10 mg anastrozole daily, untreated boars ($n=12$) and barrows ($n=12$). Anastrozole was solubilized in absolute ethanol and applied to 200 g corn-canola meal feed samples, then ethanol was allowed to evaporate overnight. The corn-canola meal diet contained 3.1 Mcal ME/kg, 17.3% crude protein and 1.1% lysine. Anastrozole-treated boars were fed the anastrozole-amended feed and untreated boars and barrows were fed their rations at approximately 0800 h each day. After consuming the anastrozole-amended feed, treated boars were fed the remaining portion of their ration. Blood samples (10 ml) were collected from all pigs on days 0, 15, 29, 43, 58 and 71 by jugular venipuncture. Previous research has shown this sampling interval is adequate to accurately determine changes in circulating concentrations of steroids, IGF-I and IGFBPs (Clapper *et al.* 2000, Lee *et al.* 2002, Rempel & Clapper 2002). Pigs were slaughtered on day 84, at which time blood samples and APs were collected.

Blood samples were allowed to clot overnight at 4 °C, then serum was collected by centrifugation (1500 g for 30 min at 4 °C) and stored at -20 °C. At slaughter, APs were trimmed of connective tissue, bisected mid-sagittally, wrapped in foil, frozen in liquid nitrogen and stored at -80 °C.

Measurement of E2

Serum concentrations of E2 were determined in duplicate by RIA (Kesler *et al.* 1977, Long & Diekmann 1984) in all samples taken from boars in Experiments 1 and 2. E2 (E-8875; Sigma Chemical Co., St Louis, MO, USA) was the standard, and [³H]E2 (NET 517; NEN, Boston, MA, USA) was the tracer. Antisera (GDN #244 anti-estradiol-17β-6-BSA) was used at a dilution of 1:20 000. Sera (500 μl) were extracted twice with 3 ml diethyl ether. Recovery of [³H]E2 added to porcine serum before extraction averaged $98 \pm 1.5\%$. Sensitivity of the assays was 4.8 pg/tube for Experiment 1 and 4.0 pg/tube for Experiment 2. Intra- and inter-assay coefficients of

variation for Experiment 1 were 9.0 and 16.1% respectively. Intra- and inter-assay coefficients of variation for Experiment 2 were 7.2 and 11.0% respectively.

Measurement of testosterone

Serum concentrations of testosterone were determined in duplicate by RIA (Gay & Kerlan 1978) in all samples taken from anastrozole-treated and untreated boars in Experiment 2. Testosterone (T-6147; Sigma) was the standard, and [^3H]testosterone (TRK 402; Pharmacia Biotech, Piscataway, NJ, USA) was the tracer. Antisera (GDN #250 anti-testosterone-11-BSA) was used at a dilution of 1:10 000. Sera (50 μl) were extracted once with 3 ml diethyl ether. Recovery of [^3H]testosterone added to porcine serum before extraction averaged $99 \pm 1.7\%$. Sensitivity was 4.2 pg/tube. Intra- and inter-assay coefficients of variation for were 14.4 and 16.9% respectively.

Measurement of IGF-I

Serum and AP concentrations of IGF-I were determined in duplicate by RIA (Echternkamp *et al.* 1990, Funston *et al.* 1995) in all blood samples and APs collected in Experiment 2. One half of each AP was placed in a 15 ml polypropylene tube (14-956-1J; Fisher Scientific, Pittsburgh, PA, USA) with 1 ml homogenization buffer (1% cholic acid, 0.1% SDS, 200 μM phenylmethylsulfonylfluoride, 100 μM EDTA, 1 μM leupeptin, 1 μM pepstatin) and homogenized on ice with a T25 Ultra-Turrax tissue disperser (IKA Works, Wilmington, NC, USA) for 30 s at 20 500 r.p.m. AP homogenates were diluted to 100 mg AP tissue/ml homogenization buffer. Homogenates were centrifuged at 15 000 *g* for 10 min at 4 °C and the supernatant removed and stored at -20 °C. Protein content of AP homogenates (1:10 dilution) was determined by the Bradford method using reagents supplied by Bio-Rad (Hercules, CA, USA). IGFBPs were extracted from serum and AP homogenates with a 1:17 ratio of sample to acidified ethanol (12.5% 2 M HCl:87.5% absolute ethanol) (Daughaday *et al.* 1980). Extracted samples were centrifuged (12 000 *g*, 4 °C) to separate the IGFBPs. A portion of the resulting supernatant was removed and neutralized with 0.855 M Tris base, incubated an additional 4 h at 4 °C, then centrifuged again at 12 000 *g* at 4 °C to remove any additional IGFBPs. When samples of this extract, equivalent to the original serum or AP homogenate sample, were subjected to Western ligand blot analysis and subsequent phosphorimager, no detected binding of ^{125}I -IGF-I to IGFBPs was observed. Inhibition curves of the neutralized extracted serum ranging from 12.5 to 50 μl were parallel to standard curves. Recombinant human IGF-I (GF-050; Austral Biological, San Ramon, CA, USA) was used as the standard and radioiodinated antigen. Antisera UB2-495 (National Hormone and Peptide Program,

National Institutes of Diabetes, Digestive and Kidney Diseases (NIDDK), Bethesda, MD, USA) was used at a dilution of 1:100 000. Recovery of ^{125}I -IGF-I added to porcine serum before extraction averaged $89 \pm 5\%$. Sensitivity was 14.8 pg/tube. Intra- and inter-assay coefficients of variation were 7.9 and 8.1% respectively.

Measurement of IGFBP

Relative amounts of serum and AP IGFBP were analyzed by one-dimensional SDS-PAGE (Laemmli 1970) and Western ligand blot analysis (Hossenlopp *et al.* 1986, Howard & Ford 1992) in all samples taken in Experiment 2. Sera (3 μl) were electrophoresed through a 5% stacking gel and 10% resolving gel. Aliquots of AP homogenate (10 μg) were electrophoresed through a 5% stacking gel and 12% resolving gel. Proteins were electrophoretically transferred to nitrocellulose membranes (Protran, 0.22 μm , BA 83; Schleicher & Schuell, Keene, NH, USA), and IGFBP activity was detected by incubating membranes with ^{125}I -IGF-I (500 000 c.p.m./ml Tris-buffered saline, 1% BSA (A7030; Sigma), 0.1% Tween-20). All treatments and bleeding dates were represented on each gel. Relative abundance of each IGFBP was determined by phosphorimager (Bio-Rad). A control sample was electrophoresed on each gel, and relative abundance of each sample IGFBP was normalized to the relative abundance of the corresponding IGFBP in the control sample.

The identity of IGFBP-2 and -5 in AP was confirmed by immunoprecipitation of proteins with specific antibodies (06-107 and 06-110 respectively; Upstate Biotechnologies, Inc., Lake Placid, NY, USA). The resulting supernatants, precipitates and non-immunoprecipitated AP samples were subjected to Western ligand blot analysis (Hossenlopp *et al.* 1986, Howard & Ford 1992).

Measurement of AP luteinizing hormone (LH)

Anterior pituitary concentrations of LH were determined in triplicate by RIA in all APs collected in Experiment 2 (Clapper *et al.* 1998). Porcine LH (AFP11043B; National Hormone and Peptide Program, NIDDK) was used as the radioiodinated antigen and standard. LH antiserum (AFP15103194; National Hormone and Peptide Program, NIDDK) was used at a dilution of 1:300 000. AP homogenates were diluted 1:25 000 in 0.01 M PBS-0.1% gelatin prior to assay. Intra-assay coefficient of variation was 4.6%. Sensitivity was 0.1 ng/tube.

Measurement of AP growth hormone (GH)

Anterior pituitary concentrations of GH were determined in triplicate by RIA in all APs collected in Experiment 2 (Klindt *et al.* 1983). Porcine GH (AFP10864B; National Hormone and Peptide Program, NIDDK) was used as the

radioiodinated antigen and standard. GH anti-serum (AFP422801; National Hormone and Peptide Program, NIDDK) was used at a dilution of 1:60 000. AP homogenates were diluted 1:100 000 in 0.01 M PBS–0.1% gelatin prior to assay. Intra-assay coefficient of variation was 3.4%. Sensitivity was 0.1 ng/tube.

Statistical analysis

Experiment 1 To determine differences among dosages of anastrozole, statistical analyses were performed using the general linearized model of the Statistical Analysis System (1999) (SAS). The model for determining differences in serum concentrations of E2 included $Y_{ijkl} = \mu + \text{Pig}_i + \text{Treatment}_j + \text{Pig}_i(\text{Treatment})_j + \text{Date}_k + \text{Treatment}_j \times \text{Date}_k + \text{Pig}_i(\text{Treatment})_j \times \text{Date}_k$. Pig within treatment was the whole plot error term used to test the effect of treatment. Pig within treatment by date was the subplot error term used to test pig, date and treatment by date effects.

Experiment 2 To determine differences among treatments, statistical analyses were performed using the general linearized model of SAS. Because mean serum concentrations of IGF-I differed among groups on day 0, differences in mean serum concentrations of IGF-I were determined by analyzing the remaining data as a percentage of day 0 (basal) values for each treatment group. The model for determining differences in serum concentrations of E2, testosterone, percentage of basal IGF-I and relative amounts of serum IGFBP was $Y_{ijkl} = \mu + \text{Pig}_i + \text{Treatment}_j + \text{Pig}_i(\text{Treatment})_j + \text{Date}_k + \text{Treatment}_j \times \text{Date}_k + \text{Pig}_i(\text{Treatment})_j \times \text{Date}_k$. Pig within treatment was the whole plot error term used to test the effect of treatment. Pig within treatment by date was the subplot error term used to test pig, date and treatment by date effects. The effect of anastrozole on AP concentrations of LH, GH, IGF-I and relative amounts of AP IGFBP was analyzed by one-way ANOVA of SAS.

Results

Experiment 1

Mean serum concentrations of E2 did not differ ($P > 0.05$) among groups of boars from day 0 through to 8 (Fig. 1). From day 15 through to 29, mean serum concentrations of E2 were greater ($P < 0.01$) in boars administered 0 mg/day anastrozole vs those administered 1 and 10 mg/day. By day 36, mean serum concentrations of E2 were less in the boars administered 10 mg/day anastrozole vs those administered 0 and 1 mg/day. Mean serum concentrations of E2 did not differ ($P > 0.05$) on days 0 and 8 in boars administered 0 mg/day anastrozole; however, mean serum concentrations of E2 were increased ($P < 0.05$) on day 15

Figure 1 Mean serum concentrations of E2 in boars administered 0 ($n=4$), 1 ($n=4$) or 10 mg ($n=4$) anastrozole from day 0 through to 36 in Experiment 1. Means are least-square means $\pm$ S.E.M. ^{ab}Means with different letters differ ($P < 0.05$) by treatment within a day.

through to 36 compared with previous days. Mean serum concentrations of E2 did not differ ($P > 0.05$) on day 0 through to 15 in boars administered 1 mg/day anastrozole; however, mean serum concentrations of E2 were increased ($P < 0.05$) on day 29 through to 36 compared with previous days. Mean serum concentrations of E2 did not differ ($P > 0.05$) from day 0 through to 36 in boars administered 10 mg/day anastrozole.

Experiment 2

Mean serum concentrations of E2 did not differ ($P > 0.05$) between untreated boars and anastrozole-treated boars on day 0 (Fig. 2). On day 15 through to 84, mean serum concentrations of E2 were greater ($P < 0.05$) in the untreated boars vs the anastrozole-treated boars. Mean serum concentrations of E2 did not differ ($P > 0.05$) in untreated boars from day 0 to 29 but increased on day 43 through to 71 (Fig. 2) and were greater ($P < 0.05$) on day 84 than all other days. In anastrozole-treated boars, mean serum concentrations of E2 decreased ($P < 0.05$) on day 15 compared with day 0 and remained decreased ($P < 0.05$) through to day 71. Mean serum concentrations of E2 were greater ($P < 0.05$) on day 84 vs day 0 in anastrozole-treated boars.

Mean serum concentrations of testosterone did not differ ($P > 0.05$) between untreated boars and anastrozole-treated boars on day 0 through to 71 (Fig. 3). On day 84, mean serum concentrations of testosterone were greater ($P < 0.01$) in the untreated boars vs the anastrozole-treated boars. Mean serum concentrations of testosterone were greater ($P < 0.01$) in untreated boars and anastrozole-treated boars on day 84 than on any other day.

Because mean serum concentrations of IGF-I differed ($P < 0.05$) among groups on day 0 (untreated boars,

Figure 2 Mean serum concentrations of E2 in untreated boars ($n=12$) and anastrozole-treated boars ($n=12$) from day 0 through to 84 in Experiment 2. Means are least-square means $\pm$ S.E.M. ^{ab}Means with different letters differ ($P<0.05$) by treatment within a day.

Figure 4 Mean percentage of basal IGF-I in untreated boars ($n=12$), anastrozole boars ($n=12$) and barrows ($n=12$) from day 0 through to 84 of experiment 2. Means are least-square means $\pm$ S.E.M. of percentage of day 0 values for each treatment group. ^{abc}Means with different letters differ ($P<0.05$) by treatment within a day.

104.1 ± 10.7 ; anastrozole-treated boars, 128.3 ± 11.7 ; barrows, 82.3 ± 10.5 ng/ml) differences in mean serum concentrations of IGF-I were determined by analyzing the remaining data as a percentage of day 0 values (basal) for each treatment group (Fig. 4). Mean percentage of basal IGF-I was not different ($P>0.05$) among anastrozole-treated boars, untreated boars and barrows from day 0 through to 29. Mean percentage of basal IGF-I was greater in untreated boars than anastrozole-treated boars and barrows from day 58 through to 84. Mean percentage of basal IGF-I increased ($P<0.05$) in anastrozole-treated boars and barrows from day 0 through to 43; however, mean percentage of basal IGF-I was not different from day

58 through to 84 within anastrozole-treated boars and barrows. Mean percentage of basal IGF-I increased ($P<0.05$) from day 29 through to 84 in untreated boars.

Relative amounts of serum IGFBP were determined by Western ligand blot analysis. Based upon the similarity of molecular masses and results of immunoprecipitation of IGFBP in porcine serum performed by Klindt *et al.* (1998), these serum IGFBPs were identified as 46 and 41 kDa forms of IGFBP-3, 34 kDa IGFBP-2, and 28 and 24 kDa forms of IGFBP-4. No treatment by day interaction was detected ($P>0.05$) for relative amounts of any serum IGFBP. Mean relative amounts of the 46 and 41 kDa forms of IGFBP-3 in serum did not differ ($P>0.05$) between untreated boars and anastrozole-treated boars, but mean relative amounts of each form of IGFBP-3 were greater ($P<0.01$) in untreated and anastrozole-treated boars vs barrows (Table 1). Mean relative amounts of the 24 kDa form of IGFBP-4 in serum were greater ($P<0.01$) in anastrozole-treated boars compared with untreated boars and barrows; however, mean relative amounts in untreated boars and barrows did not differ ($P>0.05$). No treatment effect was detected ($P>0.05$) in mean relative amounts of any other serum IGFBP among treatment groups.

Western ligand blot analysis identified two IGFBPs in the AP, determined as 33 kDa IGFBP-2 and 29 kDa IGFBP-5. Identity was based upon the similarity of results found by Rempel & Clapper (2002) after immunoprecipitation of IGFBP with specific antibodies. Mean relative amounts of AP IGFBP-2 and -5 were decreased ($P<0.01$) in anastrozole-treated boars compared with untreated boars, but mean relative amounts of AP IGFBP-2 and -5 were greater ($P<0.01$) in each group of boars vs barrows (Fig. 5).

Figure 3 Mean serum concentrations of testosterone in untreated boars ($n=12$) and anastrozole-treated boars ($n=12$) from day 0 to 84 in Experiment 2. Means are least-square means $\pm$ S.E.M. ^{ab}Means with different letters differ ($P<0.01$) according to day and (or) treatment.

Table 1 Mean relative amounts of serum IGFBPs in untreated boars (n=12), anastrozole-treated boars (n=12) and barrows (n=12) in experiment 2^a

	Untreated boars	Anastrozole boars	Barrows	P
IGFBP				
IGFBP-3 (46 kDa)	1.42 ± 0.06 ^b	1.49 ± 0.05 ^b	1.16 ± 0.05 ^c	0.01
IGFBP-3 (41 kDa)	1.63 ± 0.08 ^b	1.69 ± 0.06 ^b	1.23 ± 0.04 ^c	0.01
IGFBP-2	1.25 ± 0.04	1.18 ± 0.03	1.29 ± 0.03	0.20
IGFBP-4 (28 kDa)	1.08 ± 0.03	1.11 ± 0.03	1.05 ± 0.02	0.12
IGFBP-4 (24 kDa)	1.03 ± 0.02 ^c	1.10 ± 0.02 ^b	1.04 ± 0.02 ^c	0.01

^aMeans are expressed as least-square means ± S.E.M. Data are expressed in arbitrary densitometric units.
^{b,c}Means within a row with different letters differ (P<0.01) by treatment.

Figure 5 Mean relative amounts of AP IGFBP-2 and -5 in untreated boars (n=12), anastrozole-treated boars (n=12) and barrows (n=12). Means are least-square means ± S.E.M. Data are expressed as arbitrary densitometric units (ADU). ^{abc}Means with different letters differ (P<0.01) by treatment.

Mean AP concentrations of LH, GH and IGF-I did not differ (P>0.05) between untreated boars and anastrozole-treated boars; however, mean AP concentrations of LH, GH and IGF-I were each greater (P<0.01) in untreated and anastrozole-treated boars vs barrows (Table 2).

Discussion

Administration of E2 has been found to affect serum concentrations of IGF-I and relative amounts of IGFBP (Rempel & Clapper 2002). However, other gonadal hormones/factors may also play a role in regulation of the

IGF-I system. By leaving the animal gonadally intact and simultaneously decreasing serum concentrations of E2, it may be possible to further study the influence of E2 on the peripheral and AP IGF system. Anastrozole is a third generation, non-steroidal, competitive aromatase inhibitor that is effective in reducing E2 synthesis by preventing the conversion of androgens to estrogens (Brodie & Long 2001, Dowsett *et al.* 2001).

Administration of 1 mg/day anastrozole is the recommended dosage for aromatase inhibition and suppression of E2 in postmenopausal breast cancer patients (Plourde *et al.* 1994, Geisler *et al.* 1996, Jonat *et al.* 1996, Yates *et al.* 1996). Administration of 10 mg/day anastrozole was effective in preventing the increase in serum concentrations of E2 in maturing boars and decreased serum concentrations of E2 in prepubertal boars. The dosage necessary to decrease E2 synthesis in the pig may differ from that in humans due to activity or amount of the aromatase enzyme present in each species, because testicular estradiol synthesis as well as aromatase activity can vary among species (Kelch *et al.* 1972, Nitta *et al.* 1993, Inkster *et al.* 1995).

Use of anastrozole has been associated with increased circulating concentrations of testosterone in men (Mauras *et al.* 2000, Taxel *et al.* 2001), male monkeys and dogs (Plourde *et al.* 1994). An increase in serum concentrations of testosterone would be expected because administration of anastrozole prevents/reduces the synthesis of E2 from testosterone (Kellis & Vickery 1987). Serum concentrations of testosterone did not differ between untreated boars and anastrozole-treated boars throughout the majority of the experiment but increased in both groups

Table 2 Mean anterior pituitary gland concentrations of LH, GH and IGF-I in untreated boars (n=12), anastrozole-treated boars (n=12) and barrows (n=12) in Experiment 2^a

	Untreated boars	Anastrozole boars	Barrows	P value
LH	2870.5 ± 344.4 ^b	2958.6 ± 363.4 ^b	1509.8 ± 163.6 ^c	0.01
GH	7365.4 ± 386.2 ^b	7200.3 ± 522.2 ^b	5653.4 ± 335.8 ^c	0.01
IGF-I	200.9 ± 5.9 ^b	188.0 ± 10.9 ^b	75.3 ± 7.8 ^c	0.01

^aMeans are expressed as least-square means ± S.E.M. Means are in ng/mg.
^{b,c}Means within a row with different letters differ (P<0.01) by treatment.

of boars near the end of the experiment. Similar to results found by Clapper *et al.* (2000), Rempel & Clapper (2002) and Allrich *et al.* (1982), mean serum concentrations of testosterone increased in both groups of boars at an age when they would be expected to reach puberty and the magnitude of the increase was indicative of that observed at puberty. Circulating concentrations of E2 are much greater in boars than in postmenopausal women (Geisler *et al.* 1996, 2002) and men (Mauras *et al.* 2000). Therefore, sufficient E2 may still have been synthesized in the boars to obviate the increase in serum concentrations of testosterone. The amount of E2 synthesized by the testis is variable among species (Kelch *et al.* 1972), and aromatase enzyme activity has also been reported to vary among species (Nitta *et al.* 1993, Inkster *et al.* 1995). Similar results have been reported in older men where administration of anastrozole decreased serum concentrations of estradiol but failed to increase serum concentrations of testosterone (Veldhuis & Iranmanesh 2005). These authors proposed that these results could be due to a change in the pattern of LH release, decreased LH bioactivity or diminished LH uptake by the testis. Whether this occurs in the boar awaits further investigation.

Rempel & Clapper (2002) previously reported that administration of E2 implants to barrows increased serum concentrations of IGF-I. Therefore, when serum concentrations of E2 decreased, a concomitant decrease in serum concentrations of IGF-I would be expected. In the present study, serum concentrations of E2 and the percentage increase in IGF-I were less in the anastrozole-treated boars compared with untreated boars. Testosterone has been found to increase circulating concentrations of IGF-I in hypogonadal men (Weissberger & Ho 1993) and castrate baboons (Crawford & Handelsman 1996). However, it appears E2 may be a more potent modulator of circulating levels of IGF-I in the boar, because the percentage increase in circulating IGF-I was less in anastrozole-treated boars compared with untreated boars despite both groups having similar circulating concentrations of testosterone. Similar to the findings of Clapper *et al.* (2000), Dunshea *et al.* (1993) and Owens *et al.* (1999), the percentage increase in serum concentrations of IGF-I was greater in each group of boars than in barrows from day 43 through to 84 of the study. The fact that serum concentrations of IGF-I were still greater in anastrozole-treated boars compared with barrows may have been due to the inability of the anastrozole to completely block estradiol synthesis.

Previous research has demonstrated that administration of E2 implants to barrows increased serum concentrations of IGF-I and relative amounts of the 41 kDa IGFBP-3 (Rempel & Clapper 2002). Relative amounts of both forms of IGFBP-3 in serum were greater in untreated boars and anastrozole-treated boars vs barrows. Similarly, Clapper *et al.* (2000) found relative amounts of both forms of serum IGFBP-3, as well as serum concentrations of E2 and IGF-I, were greater in boars than in barrows.

Administration of E2 implants to ovariectomized ewes increased serum concentrations of IGF-I and relative amounts of the 44 kDa form of serum IGFBP-3 (Clapper *et al.* 1998). The lack of a difference in relative amounts of serum IGFBP-3 in untreated and anastrozole-treated boars may be explained by the fact that serum concentrations of E2 may not have been decreased enough in anastrozole-treated boars to affect relative amounts of IGFBP-3. Whether a threshold level of E2 exists to affect serum IGFBP-3 in the pig has not been determined. A similar phenomenon has been reported in postmenopausal women where increasing amounts of E2 have no effect on circulating levels of IGF-I but not IGFBP-3 (Garnero *et al.* 1999).

Clapper *et al.* (2000) found relative amounts of the 24 kDa IGFBP-4 in serum did not differ between boars and barrows. In addition, administration of E2 implants to barrows did not affect relative amounts of the 24 kDa IGFBP-4 in serum compared with untreated barrows, and relative amounts of the 24 kDa IGFBP-4 in the serum did not differ among E2-implanted barrows, boars and unimplanted barrows (Rempel & Clapper 2002). In the present study, relative amounts of the 24 kDa IGFBP-4 in serum were greater in anastrozole-treated boars than in other treatment groups. These apparent disparate results may have been due to changes in aromatase activity or expression. Mazerbourg *et al.* (2001) reported that expression of an intrafollicular IGFBP-4-degrading protease in porcine ovaries was positively correlated with expression of aromatase. When anastrozole decreased circulating levels of estradiol, a compensatory increase in aromatase activity and/or expression would be expected due to loss of negative feedback. Perhaps this increase in aromatase was accompanied by an increase in IGFBP-4 protease activity. Increased relative amounts of the 24 kDa form of IGFBP-4 in anastrozole-treated boars could function to increase the bioavailability of IGF-I because the 24 kDa form of IGFBP-4 has less affinity for IGF-I than the 28 kDa form of IGFBP-4 (Jones & Clemmons 1995). However, exact mechanisms by which this occurred were not within the scope of this study and remain to be determined.

In the present study, decreased serum concentrations of E2 did not affect AP concentrations of LH in anastrozole-treated boars compared with untreated boars. Similarly, administration of E2 implants to barrows did not affect AP concentrations of LH compared with boars and unimplanted barrows (Rempel & Clapper 2002). However, untreated and anastrozole-treated boars had greater mean AP concentrations of LH compared with barrows. Serum concentrations of E2 in both groups of boars may have increased inhibitory control over LH release from the AP, which would allow AP concentrations of LH to be maintained by decreasing its release (Lindzey *et al.* 1998, Hayes *et al.* 2000).

AP concentrations of GH did not differ between untreated boars and anastrozole-treated boars. In a similar

fashion, administration of E2 implants to barrows did not affect AP concentrations of GH compared with boars and unimplanted barrows (Rempel & Clapper 2002). However, in the present study, AP concentrations of GH were greater in both groups of boars in comparison with barrows. E2 has been found to promote GH secretion in baboons (Copeland *et al.* 1984) and increase GH synthesis, content and release from cultured rat AP cells (Simard *et al.* 1986). In wethers, administration of zeranol, an estrogenic compound, increased GH pulse amplitude and serum concentrations of GH (Hufstedler *et al.* 1996). Serum concentrations of E2 may have been elevated enough in anastrozole-treated boars to maintain mean AP concentrations of GH and IGF-I.

It was previously shown that administration of E2 increased expression of IGF-I in the AP in gonadectomized rats (Michels *et al.* 1993) and in the uterus in ovariectomized hypophysectomized rats (Murphy & Friesen 1988) and ovariectomized rats (Murphy *et al.* 1987). Additionally, administration of E2 increased uterine content of IGF-I in ovariectomized rats (Murphy *et al.* 1987), prepubertal gilts and ovariectomized gilts (Simmen *et al.* 1990). Previous research demonstrated that administration of E2 to barrows increased AP concentrations of IGF-I (Rempel & Clapper 2002). The fact that AP concentrations of IGF-I were similar between anastrozole-treated and untreated boars in the present experiment may be due to the fact that although serum concentrations of E2 were decreased in the anastrozole-treated boars, they may not have been reduced enough to affect AP concentrations of IGF-I.

Relative amounts of AP IGFBP-2 and -5 decreased in anastrozole-treated boars compared with untreated boars. Conversely, Rempel & Clapper (2002) found that acute administration of E2 implants to barrows increased relative amounts of IGFBP-2 and -5 in the AP vs unimplanted barrows, although not to the levels observed in untreated boars. E2 has been found to affect the expression and amount of IGFBP-2 and -5 in other species as well. Clapper *et al.* (1998) reported administration of E2 implants to ovariectomized ewes increased expression of IGFBP-2 and tended to increase relative amounts of IGFBP-2 in the AP. Administration of E2 has also been found to increase expression of IGFBP-2 in the AP in rats (Michels *et al.* 1993). In ovariectomized monkeys, administration of E2 increased synthesis of IGFBP-2 and -5 in the uterine myometrium (Adesanya *et al.* 1996). During the estrous cycle in ewes, expression of IGFBP-5 in the uterine luminal epithelium and inner myometrium has been found to increase around ovulation and decrease during the late luteal phase (Gadd *et al.* 2000), times coincident with increased and decreased circulating concentrations of estradiol respectively. Administration of E2 to equine granulosa cells also increased relative amounts of IGFBP-2 (Davidson *et al.* 2002). Thus, E2 appears to be a regulator of relative amounts and expression of IGFBP-2

and -5. IGFBP-2 and -5 in the AP may function to bind IGF-I and enhance IGF-I activity (Michels *et al.* 1993, Adesanya *et al.* 1996). In addition to modulating IGF-I activity, IGFBP-2 and -5 have been found to have growth-promoting effects independently of IGF-I. IGFBP-2 stimulated cell proliferation in rat osteoblast cells *in vitro* (Slootweg *et al.* 1995). Furthermore, overexpression of IGFBP-2 in mouse adrenocortical tumor cells increased cell proliferation in culture (Hoeflich *et al.* 2000). Co-incubation of IGFBP-5 with mouse osteoblast cells increased cell proliferation (Mohan & Baylink 1995), while incubation of IGFBP-5 with human osteoblast cells increased cell differentiation (Richman *et al.* 1999). The current experiment demonstrated that the porcine AP IGF system is sensitive to changes in circulating concentrations of estradiol. However, other gonadal hormones and/or factors may also regulate AP IGFBP because Rempel & Clapper (2002) found boars had greater relative amounts of AP IGFBP-2 and -5 vs E2-implanted and unimplanted barrows.

In the present study, administration of 10 mg/day anastrozole to growing boars decreased mean serum concentrations of E2 and mean relative amounts of AP IGFBP-2 and -5 compared with untreated boars. These data support the role of E2 in regulating components of the AP IGF system in pigs. Although E2 has been found to regulate other components of the circulating and AP IGF-I system, a greater magnitude in E2 reduction may be necessary to affect the peripheral IGF-I system. The possible influences of other gonadal hormones/factors, either alone or in conjunction with E2, to regulate components of the IGF-I system cannot be discounted.

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