Increased ovulation rate in gilts treated with dihydrotestosterone

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Treatment with testosterone increases ovulation rate in pigs. The present study was conducted to examine the effects of 5 α -dihydrotestosterone (DHT), a non-aromatizable androgen receptor ligand, on ovulation rate and amounts of androgen receptor and FSH receptor mRNAs in postpubertal gilts. In Expt 1, ovulation rate in response to daily i.m. injections of 0, 6, 60 or 600 $\mu\text{g DHT kg}^{-1}$ body weight from day 13 of the oestrous cycle (day 0 = day 1 of oestrus) to the following oestrus increased with each dose of DHT ($P < 0.05$). The mean increase in number of corpora lutea ranged from approximately three to 17 over the three dosages of DHT. In Expt 2, gilts treated daily with 60 $\mu\text{g DHT kg}^{-1}$ body weight during the early follicular phase (from day 13 to day 16), coincident with follicular recruitment, or the late follicular phase (day 17 to oestrus), had higher ($P < 0.05$) rates of ovulation compared with gilts

that received vehicle, and were not different from gilts treated with DHT from day 13 to oestrus. Percentage recovery of day 3 embryos was not altered when gilts were treated from day 13 to day 16 or from day 17 to oestrus; however, treatment of gilts with DHT from day 13 to oestrus decreased recovery of day 3 (Expt 1) or day 11 (Expt 2) conceptuses. Daily administration of 6 $\mu\text{g DHT kg}^{-1}$ body weight to gilts from day 13 of the oestrous cycle to the following oestrus (Expt 3) did not affect the relative amounts of androgen receptor mRNA, but increased ($P < 0.05$) the amounts of FSH receptor mRNA in pre-ovulatory follicles as determined by RT-PCR. The results of these experiments indicate that androgens may regulate ovulation rate in gilts. One of the roles of androgens might be regulation of the amounts of FSH receptor mRNA in ovarian follicles.

Introduction

Testosterone and androstenedione serve as intermediaries for the synthesis of oestrogen in ovarian follicles (Peters and McNatty, 1980). Androgens might also exert regulatory effects on follicular development. For example, androgens enhanced the effects of FSH on steroidogenesis *in vitro* (Armstrong and Dorrington, 1976; Daniel and Armstrong, 1980; Harlow *et al.*, 1986) and results from our laboratory indicated that testosterone administered during the follicular phase increased the number of preovulatory follicles, ovulation rate (number of corpora lutea) and the number of blastocysts in pigs (Cárdenas and Pope, 1994, 1997). More recently, it was demonstrated that androgens enhanced the effects of FSH on DNA synthesis in rat granulosa cells (Bley *et al.*, 1997), stimulated early growth of primate follicles (Vendola *et al.*, 1998), augmented the development of mouse follicles *in vitro* (Murray *et al.*, 1998) and increased insulin-like growth factor I (IGF-I) and IGF-I receptor mRNAs in primate follicles and oocytes (Vendola *et al.*, 1999a,b).

The use of testosterone in our previous experiments (Cárdenas and Pope, 1994, 1997) raised the question of whether oestradiol or androgen receptor or both mediated the effects of testosterone treatment on ovulation rate. The androgen receptor protein has been located in granulosa cells of small growing follicles and, in lesser amounts, in

larger follicles of several mammalian species (Tetsuka *et al.*, 1995; Garrett and Guthrie, 1996; Hillier *et al.*, 1997). The mechanisms involved in the effects of testosterone on ovulation rate in pigs are not known and could include ovarian- and gonadotrophin-related factors that influence follicular recruitment and atresia. Follicular atresia in pigs (Guthrie *et al.*, 1995) and other mammals (Kaipia and Hsueh, 1997) occurs primarily through apoptosis. In pigs, follicular recruitment occurs from day 14 to day 16 of the oestrous cycle (Clark *et al.*, 1982; Foxcroft and Hunter, 1985) and appears to be controlled mainly by FSH (Foxcroft and Hunter, 1985). Furthermore, FSH decreases apoptosis in cultured granulosa cells of rats and pigs (Tilly *et al.*, 1991; Chun *et al.*, 1996; Guthrie *et al.*, 1998). In the present experiment, the effects of DHT, a non-aromatizable androgen receptor ligand, on ovulation rate and the relative amounts of FSH receptor and androgen receptor mRNAs in preovulatory follicles of pigs were examined.

Materials and Methods

Gilts (cross of Yorkshire, Landrace, Duroc and Hampshire from the university herd) were exposed to boars once a day (in the morning) to determine the onset of oestrus (day 0 of the oestrous cycle). Gilts that underwent at least one oestrous cycle were used in the experiments. Testosterone or DHT (Sigma Chemical Co., St Louis, MO) was dissolved in ether and mixed with an appropriate volume of corn oil to make stock solutions. The ether was allowed to evaporate

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at room temperature before the stocks were used for preparation of the doses used in the different experiments. Steroids were administered daily (in the morning) by i.m. (rear leg) injections according to the experimental protocols. Gilts were mated twice to different boars at 8 and 24 h after the onset of oestrus. All procedures applied to the animals were approved by the College of Agriculture Animal Care and Use Committee.

Experiment 1: response to dose of DHT

Gilts ($n = 8$ per group) received daily injections of 0 (corn oil), 6, 60 or 600 $\mu\text{g DHT kg}^{-1}$ body weight from day 13 of the oestrous cycle to oestrus to determine the effects of dose of DHT on numbers of ovulations and day 11 blastocysts. The doses of 6, 60 or 600 $\mu\text{g DHT}$ were dissolved in 2, 2 and 8 ml of corn oil, respectively. Two millilitres of oil was used with smaller doses of DHT to decrease the amount of oil injected into the animals. Gilts from the control group received daily injections of 8 ml oil. Gilts were mated twice to different boars and ovariectomized on day 11. Corpora lutea were dissected, weighed and counted. The uterus was flushed with 0.9% (w/v) NaCl to recover the blastocysts, which were counted and examined to assess their developmental stage. Percentage blastocyst recovery was calculated as the proportion of blastocysts relative to the number of corpora lutea.

Experiment 2: effects of period of DHT treatment

The aim of this experiment was to determine whether DHT treatment during three different stages of the oestrous cycle would affect the ovulation rate in gilts. The intermediate dose of 60 $\mu\text{g DHT kg}^{-1}$, which had a highly significant effect on ovulation rate in Expt 1, was used in Expt 2. Although a lower dose of DHT (6 $\mu\text{g kg}^{-1}$ body weight) increased ovulation rate after 8 days of administration in Expt 1, its influence on ovulation rate might have been reduced by the shorter duration of treatment (4 days) applied in Expt 2, thereby decreasing our ability to detect the effect of periods of treatment. Gilts ($n = 8$ per group) received daily injections of DHT during the following stages of the oestrous cycle: day 13 to oestrus, day 13 to day 16, or day 17 to oestrus. Control gilts ($n = 8$) received vehicle (corn oil) from day 13 to oestrus. Gilts were mated and then ovariectomized on day 3. The number of corpora lutea was counted and the oviducts and tips (approximately 15 cm) of the uterine horns were flushed to recover the embryos for verification of ovulations and ova pick-up. Embryos were recovered on day 3 instead of day 11 (Expt 1) to minimize the possible influence of the uterus on conceptus survival that was observed in Expt 1.

Experiment 3: effects of androgens on amounts of androgen receptor and FSH receptor mRNAs

The effects of exogenous androgens on the relative amounts of FSH receptor and androgen receptor mRNAs in

preovulatory follicles were determined using semiquantitative RT-PCR. Vehicle (corn oil), 6 $\mu\text{g DHT}$ or 6 mg testosterone kg^{-1} body weight was administered to gilts from day 13 to oestrus ($n = 4$). This dose of androgens was used because it increased ovulation rate and was comparable (based on total body weight) to 1 mg testosterone, which was the best characterized dosage used from day 13 to oestrus in previous experiments (Cárdenas and Pope, 1997). Ovaries were removed surgically approximately 8 h after the last treatment given on the day of oestrus. The pre-ovulatory LH surge in pigs occurs at about the onset of oestrus (Van de Wiel *et al.*, 1981) and, although not verified, it was assumed that gilts had already had an LH surge before the ovaries were removed. A representative sample of tissue from preovulatory follicles was obtained by carefully dissecting pieces of follicular walls protruding from the surface of the ovary of 5–6 follicles ≥ 8 mm in diameter, which were then pooled, frozen in liquid nitrogen and stored at -80°C . Total cellular RNA was isolated from follicular tissue using the RNeasy kit (Qiagen, Valencia, CA). The purity and integrity of the RNA were verified by calculating the ratio of absorbance at 260 and 280 nm and by estimating the ratio of the 28S and 18S rRNA bands, respectively. Concentration of RNA was determined using the absorbance at 260 nm.

RT-PCR was performed using the GeneAmp RNA PCR kit (Roche Molecular Systems, Inc., Branchburg, NJ). Primer pairs spanning at least two exons for androgen receptor were obtained from Butler *et al.* (1998) and those for FSH receptor and glyceraldehyde-3 phosphate dehydrogenase (GPDH, used as loading control) were constructed based on pig sequences (GenBank accessions: AF025377 for FSH receptor and AF017079 for GPDH; Table 1). Initially, RT-PCR for each of FSH receptor, androgen receptor and GPDH was optimized independently for cycling temperatures and concentrations of primers and magnesium chloride. Additional assays were performed to establish the exponential regions of the amplification curves by sampling reactions every other cycle. Common assay conditions that would work for all three genes were used for performing RT-PCR for FSH receptor, androgen receptor and GPDH simultaneously in a multiplex setting. Authenticity of FSH receptor and GPDH fragments was determined by sequencing the PCR products. The androgen receptor product was cloned into the pCR2.1 vector (Invitrogen, San Diego, CA) and sequenced. Results were compared with sequences at the GenBank using the BLAST software from the National Center for Biotechnology Information web page. The putative androgen receptor fragment had 92% sequence identities with the androgen receptors of sheep, dogs, humans and other mammals, and 99% with a recently released pig androgen receptor sequence (GenBank accession AF202775).

First strand cDNA was synthesized using 0.5 μg total RNA and Maloney murine leukaemia virus reverse transcriptase primed with random hexamers. Tubes were incubated for 10 min at room temperature, for 30 min at 42°C , for 5 min at 99°C and then cooled to 5°C . Duplicate

Table 1. Primers used for RT-PCR of the pig androgen receptor, FSH receptor and glyceraldehyde-3 phosphate dehydrogenase (GPDH) and sizes of amplified fragments

Gene	Primer type and sequence (5' to 3')	Fragment size (bp)
Androgen receptor	Sense: CACATTGAAGGCTATGAGTG Antisense: CCCATCCAGGAGTACTGAAT	242
FSH receptor	Sense: AGCCTCTGGACCAGTCATTC Antisense: CACCATCTTCTGCCAGAGAC	308
GPDH	Sense: CCTGGCCAAGGTCATCCATG Antisense: CCACCACCCTGTTGCTGTAG	500

Table 2. Effects of dose of 5 α -dihydrotestosterone (DHT) on number of corpora lutea, number of day 11 blastocysts, duration of the oestrous cycle and corpora lutea mass in gilts

DHT ($\mu\text{g kg}^{-1}$ body weight)*	Number of corpora lutea	Number of blastocysts [†]	Blastocyst recovery (%) [†]	Oestrous cycle (days)	Corpora lutea mass (mg)
0	17.4 $\pm$ 0.3 ^a	15.9 $\pm$ 0.5	91.3 $\pm$ 2.4	19.5 $\pm$ 0.4 ^a	400 $\pm$ 29 ^a
6	20.3 $\pm$ 0.9 ^b	9.4 $\pm$ 3.2	46.5 $\pm$ 14.7	19.6 $\pm$ 0.4 ^a	412 $\pm$ 27 ^a
60	27.9 $\pm$ 1.3 ^c	0.3 $\pm$ 0.7	1.1 $\pm$ 1.1	20.6 $\pm$ 0.4 ^a	360 $\pm$ 23 ^a
600	34.6 $\pm$ 1.8 ^d	0 $\pm$ 0	0 $\pm$ 0	20.3 $\pm$ 0.4 ^a	345 $\pm$ 33 ^a

* $n = 8$ per group except for gilts treated with 600 $\mu\text{g DHT kg}^{-1}$ body weight, $n = 6$ for oestrous cycle data (onset of oestrus was not clear in two gilts) and $n = 3$ for corpora lutea mass (five gilts had fluid-filled corpora lutea).

[†]No ANOVA was performed because no embryos were recovered from any gilt treated with 600 $\mu\text{g DHT kg}^{-1}$ body weight.

^{a-d}Means within a column lacking a common superscript are significantly different ($P < 0.05$).

PCR assays were performed using aliquots (equivalent to 0.25 μg total RNA) of the reverse transcription mixtures, Taq DNA polymerase, 1.5 mmol $\text{MgCl}_2 \text{ l}^{-1}$ and 0.3 $\mu\text{mol l}^{-1}$ of each primer. Concentrations of other components were those given by the kit manufacturer. Tubes were incubated for 1 min at 95°C and subjected to cycles of 1 min at 95°C, 45 s at 55°C and 2 min at 72°C. The number of cycles was 25 for FSH receptor and androgen receptor, and 20 for GPDH. As fewer cycles were used for GPDH amplification, primers for GPDH were added during the denaturation step of cycle number 6. Reaction mixtures were covered with mineral oil to avoid evaporation during this step. Sample incubation finished with a 7 min extension at 72°C followed by cooling to 5°C. A set of negative controls was included in which no reverse transcriptase was used. Products were analysed by electrophoresis on a 1.5% (w/v) agarose gel, visualized by ethidium bromide staining and photographed using Polaroid film T57. Pictures were scanned and densitometric analysis of cDNA bands was performed using ImageJ (National Institute of Health; <http://rsb.info.nih.gov/ij/>). No bands were detected in negative controls. Staining intensity of GPDH was not influenced by treatment. The relative amount of FSH receptor or androgen receptor mRNA was defined as the ratio of densitometric values of FSH receptor or androgen receptor to GPDH. The FSH receptor bands in this study represent the full-length FSH receptor and isoform FSH receptor 2 but not FSH receptor 3, as described by Touyz *et al.* (2000). The average coefficients of variation of androgen receptor and FSH receptor

determinations in two separate PCR assays were 11.6 and 11.2%, respectively.

Statistical analyses

Data were analysed by one-way ANOVA for a completely randomized design using a computer program (SPSS, 1999). Variance of number of corpora lutea in Expt 1 was heterogeneous, so data were transformed to logarithms for use in ANOVA. Post-hoc comparisons of means were performed using protected LSD tests.

Results

Experiment 1

Daily administration of DHT, from day 13 of the oestrous cycle to oestrus, influenced ($P < 0.001$) ovulation rate in cyclic gilts. The number of corpora lutea increased by approximately 3, 10 and 17 in gilts receiving 6, 60 or 600 $\mu\text{g DHT kg}^{-1}$ body weight, respectively, compared with gilts receiving vehicle (Table 2).

Most of the corpora lutea in five of the eight gilts that received the highest dose of DHT had fluid-filled cavities. The other three gilts in this group and gilts that received lower doses of DHT or vehicle had corpora lutea that looked normal and that did not differ in mass (Table 2). Number and rate of recovery of day 11 blastocysts were decreased markedly in gilts that received DHT (Table 2). Low rates of blastocyst recovery were associated with

Table 3. Effects of 5 α -dihydrotestosterone (DHT) treatment during different periods of the oestrous cycle on numbers of corpora lutea and characteristics of day 3 embryos in gilts

DHT treatment (day of cycle)*	Number of corpora lutea	Number of embryos [†]	Embryo recovery (%) [†]	Embryos with ≥ 2 blastomeres (%) [†]
Vehicle	19.5 $\pm$ 1.2 ^a	15.5 $\pm$ 1.7 ^a	79.3 $\pm$ 5.7 ^a	81.1 $\pm$ 10.2 ^a
Day 13 to oestrus	26.6 $\pm$ 1.2 ^b	15.6 $\pm$ 2.0 ^{ab}	60.8 $\pm$ 6.5 ^b	57.3 $\pm$ 11.8 ^a
Day 13 to day 16	26.1 $\pm$ 1.2 ^b	20.4 $\pm$ 1.7 ^{bc}	77.8 $\pm$ 5.7 ^{ab}	94.9 $\pm$ 10.2 ^a
Day 17 to oestrus	23.9 $\pm$ 1.2 ^b	21.7 $\pm$ 1.9 ^c	89.4 $\pm$ 6.1 ^a	83.9 $\pm$ 10.9 ^a

*Dose was 60 $\mu\text{g kg}^{-1}$ body weight per day. Control gilts received vehicle from day 13 to oestrus.

[†]Only gilts from which embryos were recovered were included ($n = 8$ for vehicle and day 13 to day 16, $n = 7$ for day 17 to oestrus and $n = 6$ for day 13 to oestrus).

^{a-c}Means within a column lacking a common superscript are significantly different ($P < 0.05$).

Fig. 1. (a) Multiplex RT-PCR of androgen receptor, FSH receptor and glyceraldehyde phosphate dehydrogenase (GPDH) in pre-ovulatory follicles of gilts ($n = 4$ per group) treated daily with 6 μg testosterone (T) or 5 α -dihydrotestosterone (DHT) kg^{-1} body weight, or vehicle (control) from day 13 of the oestrous cycle to oestrus. The RT-PCR products were stained with ethidium bromide (negative image) in representative samples. The left lane is a 100 bp DNA ladder. (b) Relative amounts of androgen receptor (□) and FSH receptor (■) mRNAs obtained by densitometric analysis of RT-PCR products. Values for androgen receptor and FSH receptor were corrected for loading using GPDH. *Asterisk indicates significant difference from control and testosterone value for FSH receptor mRNA ($P < 0.05$).

opaque uterine flushings and were observed in gilts that received 600 $\mu\text{g DHT kg}^{-1}$ body weight and in 50% of the gilts that received 60 $\mu\text{g DHT kg}^{-1}$ body weight. All of the blastocysts recovered were spherical and > 1 mm in diameter, which is the stage expected by day 11 of gestation in pigs. Administration of DHT did not influence the duration of the oestrous cycle. Mean duration of the oestrous cycle was 20 days (Table 2) and, therefore, treatments were given for an average of 8 days.

Experiment 2

The number of corpora lutea in gilts treated with daily doses of 60 $\mu\text{g DHT kg}^{-1}$ body weight from day 13 of the oestrous cycle to oestrus, from day 13 to day 16, or from day 17 to oestrus was higher ($P < 0.05$) than in gilts that received vehicle. However, the number of corpora lutea was not different among gilts that received DHT from day 13 to oestrus, from day 13 to day 16, or from day 17 to oestrus (Table 3). The total number of embryos in gilts treated with DHT from day 13 to oestrus was not different from the total number in gilts treated from day 13 to day 16 or in vehicle-treated gilts, but was lower ($P < 0.05$) compared with gilts treated with DHT from day 17 to oestrus (Table 3). The percentage recovery of day 3 embryos was lower ($P < 0.05$) in gilts treated from day 13 of the oestrous cycle to oestrus than in gilts that received vehicle or in gilts treated from day 17 to oestrus (Table 3). Treatment with DHT did not affect the proportion of embryos with two or more blastomeres, although a numerical decrease of 23.7% was observed in gilts treated from day 13 to oestrus compared with controls (Table 3).

Experiment 3

The relative amounts of androgen receptor mRNA in preovulatory follicles were not influenced by daily treatment with 6 $\mu\text{g kg}^{-1}$ body weight of testosterone or DHT from day 13 to oestrus (Fig. 1). Gilts treated with DHT had higher ($P < 0.05$) amounts of FSH receptor mRNA compared with those treated with testosterone or vehicle. Amounts of FSH receptor in preovulatory follicles of gilts

treated with testosterone or vehicle were not significantly different (Fig. 1).

Discussion

This is thought to be the first report of an increase in ovulation rate produced by administration of DHT in any species. Although Cardenas and Pope (1994, 1997) reported that ovulation rate increased in gilts treated with testosterone, the effects of DHT in the present study indicate that androgen receptor might mediate, at least in part, these effects. DHT is an androgen receptor ligand that cannot be aromatized into oestrogens (Wilson, 1975) and is synthesized from testosterone in tissues containing 5 α -reductase, predominantly in males. The increases in ovulation rate induced by DHT were highly dependent on dose. The responses of ovulation rate to DHT were greater than those obtained previously using similar doses of testosterone (Cárdenas and Pope, 1997), which might be attributed to the higher androgenic activity of DHT relative to testosterone (Grino *et al.*, 1990).

The effects of DHT on follicular development *in vivo* have been examined in other species. Daily injections of 1 mg DHT kg⁻¹ body weight given to rats after eCG treatment decreased ovulation rate; however, smaller doses had no effects (Conway *et al.*, 1990). In rhesus monkeys, administration of 145 μ g DHT kg⁻¹ per day increased the number of small follicles but did not alter the number of preovulatory follicles (Vendola *et al.*, 1998). It has been proposed that androgens are regulators of early follicular development but serve mainly as substrates for oestrogens during late development (Tetsuka and Hillier, 1997). However, there appear to be differences between species, as our results with pigs indicate that androgens might affect late follicular development.

The possibility that the effects of DHT on ovulation rate could be due to actions occurring during follicular recruitment or after recruitment was examined in Expt 2. Treatment during all three periods (from day 13 of the oestrous cycle to oestrus (entire follicular phase), from day 13 to day 16 (recruitment), or from day 17 to oestrus (after recruitment)) increased ovulation rate. These results indicate that androgens might regulate at least one process associated with ovulation rate that is functional both during and after recruitment. In gilts, most recruited follicles become atretic before reaching 6 mm in diameter (Grant *et al.*, 1989), indicating that atresia is a relatively frequent event during the early follicular phase and decreases during oestrus. DHT might have inhibited atresia among recruited follicles; however, it is not known whether DHT also increased the total number of recruited follicles when administered from day 13 to oestrus or from day 13 to day 17. These hypotheses need to be tested in subsequent experiments.

Factors that allow androgens to influence ovulation rate are difficult to define. It is possible that DHT could have influenced certain ovarian factors or altered gonadotrophin secretion in such a way as to increase ovulation rate. Induc-

tion of superovulation by FSH or eCG significantly decreased when given after day 16 relative to day 15 or day 16 (Phillippo, 1968). However, ovulation rate was increased in gilts receiving androgen treatment after day 16 (DHT from day 17 to oestrus in the present study, or testosterone on days 17 and 18 in the study of Cárdenas and Pope, 1994). These relationships indicated a possible role for androgens in the ovary. Although experiments in female rats demonstrated that DHT had no effect on plasma concentrations of LH or FSH (Conway *et al.*, 1990), the effects of androgens on gonadotrophin secretion in pigs cannot be excluded and also require further study.

FSH is a major regulator of follicular development and inhibits apoptosis in pig granulosa cells (Guthrie *et al.*, 1998). As a result of these effects of FSH, we examined whether androgen treatments that increased ovulation rate also altered the relative amounts of FSH receptor mRNA in preovulatory follicles. The results of Expt 3 demonstrate that gilts treated with DHT have almost double the amount of FSH receptor mRNA in preovulatory follicles compared with control gilts. The less active androgen, testosterone, did not significantly alter FSH receptor mRNA content, perhaps because FSH receptor mRNA is significantly down-regulated in preovulatory follicles at oestrus (Yuan *et al.*, 1996; Liu *et al.*, 1998). Although it remains to be confirmed whether the increase in the amount of FSH receptor mRNA induced by DHT results in similar changes in FSH receptor protein, this finding indicates that androgens might be able to upregulate FSH receptor and perhaps enhance the ability of follicles to respond to FSH. Positive actions of testosterone on the amounts of FSH receptor mRNA in primary follicles of primates have also been observed (Weil *et al.*, 1999).

Direct regulation of the FSH receptor gene by androgen receptor might not be plausible because an androgen response element on the FSH receptor gene promoter has not been identified. It is not known whether androgen receptor influences FSH receptor mRNA stability. Homologous regulation of FSH receptor by FSH has been reported in pig granulosa cells *in vitro* and the results depended on the dose of FSH used: small doses of FSH increased FSH receptor mRNA content, whereas larger doses had the opposite effect (Sites *et al.*, 1994; Murphy and Dobias, 1999). In the present study, relatively high amounts of androgen receptor mRNA were detected by RT-PCR in preovulatory follicles. Little is known about the regulation of androgen receptor in the ovary and the results vary depending on the species or system used. In one experiment conducted in primates, testosterone increased androgen receptor mRNA in granulosa cells of periantral and small antral follicles (Weil *et al.*, 1998). When cultured rat granulosa cells were treated with DHT, androgen receptor mRNA content decreased, and this effect was reversed by treatment with FSH (Tetsuka and Hillier, 1996). In the present experiment, neither DHT nor testosterone influenced the amount of androgen receptor mRNA in pig preovulatory follicles.

Relatively high doses or long duration of DHT treatment

were detrimental to conceptus survival and, apparently, to uterine function. In a previous experiment, daily treatment with 1 mg testosterone from day 13 to oestrus increased ovulation rate and survival of day 11.5 blastocysts (Cárdenas and Pope, 1997). Using 60 µg DHT kg⁻¹ body weight and reducing the duration of treatment from 8 to 3–4 days in the present study (Expt 2) resulted in an increased ovulation rate without altering embryonic recovery or cleavage stage by day 3. Although these results indicate that conceptus survival by day 11 may be influenced by attenuation of uterine function after overexposure to DHT, more studies are needed to identify the factors that influence conceptus survival in DHT- and testosterone-treated gilts. It is possible that increased oestradiol content after testosterone administration (Cárdenas and Pope, 1994) might contribute to maintenance of a hormonal balance necessary for normal uterine function, which might not occur with DHT administration.

In conclusion, the results of the present study indicate that androgens might have regulatory functions during late follicular development in pigs that are related to ovulation rate and regulation of FSH receptor mRNA.

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