Decreased follicular steroids and insulin-like growth factor-I and increased atresia in diabetic gilts during follicular growth stimulated with PMSG

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Summary. Four streptozotocin-diabetic gilts (maintained on exogenous insulin for 3 months) and 4 normoglycaemic gilts were treated with 600 i.u. PMSG. Diabetic gilts had insulin therapy removed at the time of PMSG administration. Plasma glucose averaged 463 ± 5 mg/100 ml for diabetic gilts and 82 ± 4 mg/100 ml for control gilts over the 72-h sampling period. Serum insulin was lower in diabetic than in normoglycaemic gilts (glycaemic state by time interaction; $P < 0.0001$). At ovary removal 75 h after PMSG, numbers and percentages of large (≥ 7 mm) and medium (3–6 mm) non-atretic follicles were similar for diabetic and control gilts. The total percentage of non-atretic follicles < 3 mm was lower in diabetic than in control gilts (31 vs 68%; s.e.m. = 7; $P < 0.05$). Diabetic gilts had a greater percentage of atretic follicles over all size classes (50 vs 21%; s.e.m. = 7; $P < 0.03$). After PMSG, LH was suppressed within 12 h in control gilts and remained similar to values in diabetic gilts until 72 h, when LH was elevated in 2 diabetic gilts (glycaemic state by time interaction; $P < 0.001$). Pulsatile LH patterns during 52–55 h after PMSG were not affected by glycaemic state. Serum concentrations of IGF-I tended ($P < 0.1$) to be lower in diabetic gilts. Concentrations of oestradiol and FSH in serum were similar in diabetic and control gilts. Follicular fluid concentrations of oestradiol in follicles ≥ 7 mm were lower in diabetic than normoglycaemic gilts (341 vs 873 ng/ml; s.e.m. = 86; $P < 0.05$). Testosterone was higher in follicles 3–6 mm in diameter in diabetic than in normoglycaemic gilts (142 vs 80 ng/ml; s.e.m. = 26; $P < 0.05$). Progesterone concentrations in follicular fluid were not affected by glycaemic state. Concentrations of IGF-I in follicles ≥ 7 mm were lower in diabetic than control gilts (150 vs 200 ng/ml; s.e.m. = 13; $P < 0.05$). We conclude that follicles of diabetic gilts respond to external gonadotrophic stimulation with decreased hormone production and increased ovarian follicular atresia, despite an absence of effects on circulating gonadotrophin and oestradiol concentrations.

Keywords: pig; diabetes; ovarian follicles; steroids; LH; IGF-I

Introduction

Insulin has been implicated in control of ovarian function *in vivo* and *in vitro*. In pigs, ovulation rate was increased by daily administration of insulin beginning 6 days before oestrus (Cox *et al.*, 1987a). In studies to determine the effect of exogenous insulin on preovulatory follicular growth, we found that insulin administration reduced follicular atresia in cyclic (Matamoros *et al.*, 1990) and PMSG-treated prepubertal gilts (Matamoros *et al.*, 1991). Insulin administration also increased follicular fluid insulin-like growth factor-I (IGF-I) in PMSG-treated gilts (Matamoros *et al.*, 1991). Insulin and IGF-I share certain differentiating and stimulatory actions on granulosa cells *in vitro*, including cell proliferation, progesterone secretion, aromatase activity and morphological development

(May & Schomberg, 1981; Baranao & Hammond, 1984; Veldhuis *et al.*, 1985; Adashi *et al.*, 1985; Amsterdam *et al.*, 1988; Caubo *et al.*, 1989; Erickson *et al.*, 1989).

Diabetes mellitus impairs reproductive function in rats (Kirchick *et al.*, 1978) and humans (Djursing *et al.*, 1982; Diamond *et al.*, 1988). Administration of insulin replacement therapy to diabetics allows resumption of reproductive function (Davis *et al.*, 1945; Kirchick *et al.*, 1982). There is some evidence that diabetic pigs are fertile but observations were for 3 animals for which the severity of diabetes was not established (Ezekwe, 1986).

The present study was designed to explore further the role of insulin on reproductive function by examining the effects of diabetes mellitus during follicular growth. The objective was to characterize follicular development and concentrations of intrafollicular steroids and IGF-I as well as systemic LH, oestradiol and IGF-I in prepubertal diabetic gilts after stimulation with PMSG.

Materials and Methods

Induction of diabetes and sampling. Crossbred gilts from 3 litters were used, and littermates were assigned to either diabetic (N = 5) or control (N = 4) groups. Between 8 and 12 weeks of age (weight 17–31 kg), pigs were tranquilized with xylazine (Haver Bayet Division, Miles Laboratories Inc., Shawnee, KS, USA; 0.5 mg/kg) and acepromazine maleate (Tech America Group, Inc., Elwood, KS, USA; 0.5 mg/kg) and a polyethylene catheter was non-surgically and aseptically inserted into the anterior vena cava. Following the procedures of Gabel *et al.* (1985), 150 mg streptozotocin/kg (Lot#0297d, Upjohn Co., Kalamazoo, MI, USA) was administered via the catheter, with the exception that the streptozotocin was at a concentration of 50 mg/ml. The streptozotocin was administered within 2 min of its preparation.

A pig insulin suspension (Lente insulin, Squibb Novo, Inc., Princeton, NJ, USA) at an initial dose of 0.25 IU/kg was given once every 24 h to control hyperglycaemia. Blood glucose levels were monitored using Dextrostix (Miles Inc., Elkhart, IN, USA) and a portable meter. Adequate control was defined as blood glucose concentration between 30 and 80 mg/100 ml approximately 3 h after injection, and doses of insulin were adjusted every 2 days to meet this criterion. The pigs had free access to water and were fed a standard growing swine ration (corn and soybean meal, 18% crude protein; NRC, 1987). Pigs were weighed weekly and control pigs had feed limited so that growth rates were equivalent to that of the diabetic gilts, which were fed *ad libitum*.

Although all streptozotocin-treated gilts initially became diabetic, defined as fasting plasma glucose >200 mg/100 ml, one diabetic pig partly regained the ability to regulate glucose. This conclusion was based on a near-normal response to a glucose tolerance test and the fact that, upon necropsy at 1 year of age, the pancreas was enlarged; therefore, this pig was excluded from analyses. Another diabetic pig's cannula became nonfunctional; therefore, serum analyses are based on 4 normoglycaemic and 3 diabetic gilts. Follicle analyses represent 4 normoglycaemic and 4 diabetic gilts.

When the pigs were 151 ± 4 days of age and weighed 84 ± 2 kg, catheters were inserted as described above but without anaesthesia. On the next day, insulin therapy was removed, and 600 i.u. PMSG (Diasynth, Chicago, IL, USA) were injected intramuscularly at 06:00 h. Beginning immediately before PMSG injection, blood samples were taken at 6-h intervals for 72 h. Blood samples were also obtained for 3 h at 15-min intervals to assess pulsatile secretion of LH beginning 52 h after injection of PMSG. At 75 h after PMSG pigs were unilaterally ovariectomized. The remaining ovary was left undisturbed to assess attainment of puberty in all gilts. Ketamine hydrochloride (Veterinary Products, Bristol Laboratories, Syracuse, NY, USA; 2 mg/kg) and xylazine (0.5 mg/kg) were used as preanaesthetics and methoxyflurane (Pitman-Moore, Inc., Washington Crossing, NJ, USA) and nitrous oxide were used for general anaesthesia. Regular pig insulin (Iletin II, Eli Lilly and Co., Indianapolis, IN, USA; 0.05 IU/kg) was given intramuscularly to the diabetic gilts immediately after the ovary was removed to aid recovery from surgery.

Follicles at the surface of the ovary were measured to the nearest mm, follicular fluid was obtained, and follicles were assessed visually for macroscopic signs of atresia (opaqueness of follicle wall and viscosity of fluid immediately after removal) following the procedures of Moor *et al.* (1978) and Matamoros *et al.* (1990). Follicular fluid from individual follicles was diluted in 0.01 M-phosphate-buffered saline, containing 0.1% gelatin (PBS), pH 7.5, to dilutions ranging from 1:10 to 1:40 depending on the amount of follicular fluid available, and fluid was frozen until assayed.

Assays. Serum LH concentrations were determined by a double-antibody RIA using a rabbit anti-porcine LH serum (#566) validated previously (Niswender *et al.* 1970; Cox *et al.*, 1987a). Intra- and interassay coefficients of variation were 14 and 11%, respectively, and sensitivity of the assay was 0.3 ng/ml. Serum FSH concentrations were measured by an RIA validated in our laboratory (Cox *et al.*, 1987a). Intra- and interassay coefficients of variation were 6.4 and 11.2% respectively, and sensitivity of the assay was 0.25 ng/ml.

Stored follicular fluid was further diluted to concentrations of 1:100 or 1:200 in PBS for progesterone and testosterone RIA and to 1:1000 for medium-sized follicles, and 1:6000 for large-sized follicles for oestradiol RIA. Sample

volumes of 200 µl of the diluted fluid were assayed. Small (< 3 mm) follicles were excluded from all hormone analyses due to insufficient follicular fluid volume. Progesterone concentrations were determined by a RIA validated in our laboratory (Rainey *et al.*, 1990) with the exception that ^{125}I -labelled progesterone (Ventrex Laboratory Inc., Portland, ME, USA; No 143, sp. act. 200 µCi/µg) was the isotope used. The standard was P 0130 progesterone (Sigma Chemical Co., St. Louis, MO, USA). Recovery values for progesterone corrected for endogenous source averaged $100.6\% \pm 9$ when 0.156, 0.312, and 0.625 ng exogenous progesterone were added to a serum pool. Intra- and inter-assay coefficients of variation were 13 and 18% respectively, and the sensitivity was 0.08 ng/ml. A single extraction resulted in an 80% recovery of 20,000 c.p.m. $[1,2,6,7\text{-}^3\text{H}(\text{N})]\text{progesterone}$ (NET-381, NEN Research Products, Boston, MA, USA), and values were corrected for recovery.

Concentrations of oestradiol were assayed by procedures previously validated in our laboratory for serum (Cox *et al.*, 1987b) and follicular fluid (Matamoros *et al.*, 1990). Intra- and interassay coefficients of variation were 14 and 20%, respectively, and the sensitivity of the assay was 1.8 pg/ml in serum and 2.4 pg/ml in follicular fluid with recoveries of 95%.

Serum and follicular fluid IGF-I concentrations were assayed according to the procedure of Houseknecht *et al.* (1988). In order to disassociate IGF-I from its binding proteins, 50 µl serum or 50–200 µl follicular fluid (diluted from 1:10 to 1:40) were incubated for 36 and 24 h, respectively. The incubation solution (pH 3.2–3.5) contained 0.5–1.0 ml IGF-I assay buffer and 480 µl 0.2 M-glycyl glycine HCl. The first antibody was anti-somatomedin-C/IGF-I rabbit antiserum (UBK487, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Baltimore, MD, USA) diluted at 1:2400 in 100 µl IGF-I assay buffer containing normal rabbit serum at 1:200. The second antibody (sheep-anti-rabbit gamma globulin; MSU #24, 9-13-83) was diluted 1:10 in 50 µl assay buffer. IGF-I standard was lot #1401-3 (Amgen 141000, Amgen Biological, Thousand Oaks, CA, USA). The radiolabelled IGF-I was (3-(^{125}I) iodotyrosyl) insulin-like growth factor (Thr⁵⁹) (IGF, Amersham IM-172; Amersham Corp., Arlington Heights, IL, USA) and had a specific activity of 2000 Ci/mmol. The average recovery of 50, 100 and 200 pg IGF-I added to 50 µl serum and follicular fluid and corrected for endogenous IGF-I was 106%, and increasing amounts of serum and follicular fluid (25, 50 and 75 µl) produced a curve parallel to the standard curve. The intra- and interassay coefficients of variation were 13 and 15%, respectively, and the sensitivity was 6.0 ng/ml for serum and follicular fluid.

Concentrations of testosterone in follicular fluid were measured according to a procedure validated in our laboratory (Matamoros *et al.*, 1990). Intra- and interassay coefficients of variation were 13 and 21% respectively, and the sensitivity of the assay was 0.08 ng/ml with extraction efficiency of 80%. Concentrations of insulin in serum were measured by a procedure previously validated in our laboratory (Cox *et al.*, 1987a). Intra- and interassay coefficients of variation were 8 and 15% respectively, and sensitivity of the assay was 0.04 ng/ml. Glucose concentrations were measured in plasma by the glucose oxidase-peroxidase method as described by Cox *et al.* (1987a).

Statistical analyses. Proportions of non-atretic follicles in individual size classes were expressed as the percentages of total follicles present in each ovary. Proportions and numbers of follicles were analysed using one-way analysis of variance (Steel & Torrie, 1980) with glycaemic state as the independent variable. For follicular fluid steroid concentrations and ratios, models included effects of glycaemic state, pig within glycaemic state (error term used to test effect of glycaemic state), follicle size class (3–6 mm and ≥ 7 mm), and the interaction. For plasma and serum concentrations of hormones and glucose, models included effects of glycaemic state, pig within glycaemic state (error term), time and the interaction. When main effects and interactions were significant, means were separated by the method of least significant difference (SAS, 1988).

The methods of Goodman & Karsch (1980) were used to identify LH pulses. The criteria for LH pulses were: (1) a peak had to occur within 2 samples of the previous nadir; (2) the amplitude had to be greater than the sensitivity of the assay; and (3) the LH concentration at the peak had to exceed the 95% confidence limits of the concentrations at both the preceding and subsequent nadir. The baseline LH concentration was defined as the mean LH after peaks and values associated with the ascending phase of peaks were deleted, and the peak height was defined as the peak value minus the average baseline concentration for that animal. Variables obtained using these criteria were analysed for effects due to glycaemic state using one-way analysis of variance (SAS, 1988). Pearson correlation coefficients were calculated between all hormones measured in follicular fluid (SAS, 1988).

Results

Diabetic and normoglycaemic pigs responded similarly to PMSG with respect to numbers of follicles (Table 1). However, the percentage of non-atretic follicles < 3 mm and the percentage of macroscopically atretic follicles were significantly decreased and increased, respectively, in diabetic gilts. The influence of glycaemic state on the incidence of atresia was greatest in follicles < 3 mm. The percentages of follicles < 3 mm which were atretic were 61 and 23% for diabetic and control animals, respectively (s.e.m. = 8; $P < 0.01$; data not in table). For follicles 3–6 mm, incidence of atresia was similar for diabetic and control gilts (12.7 and 12.3%, s.e.m. = 6). There were no macroscopically identifiable atretic follicles ≥ 7 mm in diameter.

Table 1. Numbers and percentages of follicles

Follicle class	No. of follicles*			% of total follicles*		
	Control	Diabetic	s.e.m.	Control	Diabetic	s.e.m.
< 3 mm	78	24	18	68 ^a	31 ^b	7
3–6 mm	6	6	4	6	12	4
≥ 7 mm	6	4	1	6	7	2
Atretic	20	43	13	20 ^a	50 ^b	7
Total	109	78	22			

*Values are least squares means and s.e.m.

^{a,b}Within a row for percentages, means with different superscripts are different ($P < 0.05$).

Intrafollicular concentrations of IGF-I and oestradiol in follicles ≥ 7 mm in diameter were lower in diabetic than control gilts (Table 2). Diabetic gilts had higher concentrations of testosterone in follicles 3–6 mm (Table 2). There was no influence of glycaemic state on progesterone concentrations in follicular fluid. For all follicular fluid hormones, follicles ≥ 7 mm had higher concentrations than those 3–6 mm in diameter ($P < 0.05$; not indicated in table).

Table 2. Follicular fluid concentrations of IGF-I and steroids (ng/ml)

Follicle class	State	IGF-I	Oestradiol	Progesterone	Testosterone
3–6 mm	Control	118 $\pm$ 13	67 $\pm$ 81	142 $\pm$ 97	80 $\pm$ 16 ^a
	Diabetic	129 $\pm$ 22	55 $\pm$ 128	368 $\pm$ 154	142 $\pm$ 26 ^b
≥ 7 mm	Control	200 $\pm$ 11 ^a	873 $\pm$ 73 ^a	511 $\pm$ 87	97 $\pm$ 15
	Diabetic	150 $\pm$ 13 ^b	341 $\pm$ 86 ^b	853 $\pm$ 103	75 $\pm$ 17

Values are least squares means $\pm$ s.e.m.

^{a,b}Within a column and size class, means with different superscripts are different ($P < 0.05$).

In analyses relating follicular fluid hormone concentrations to diameter (regardless of size class), oestradiol was more positively correlated with diameter in normal ($r = 0.8$, $P < 0.0001$) than diabetic ($r = 0.46$, $P < 0.03$) gilts. Similarly, progesterone was positively correlated with diameter for normoglycaemic ($r = 0.56$, $P < 0.0002$) and diabetic ($r = 0.42$, $P < 0.05$) gilts. In contrast, IGF-I was correlated with diameter in normoglycaemic gilts only ($r = 0.56$; $P < 0.0002$ vs $r = 0.28$, $P < 0.19$). There were no effects of follicular diameter on testosterone in either group of animals.

Correlations among hormones in follicular fluid were significant for normoglycaemic pigs for the following pairs: oestradiol and testosterone ($r = 0.41$; $P < 0.01$), oestradiol and progesterone ($r = 0.72$; $P < 0.0001$), and oestradiol and IGF-I ($r = 0.35$; $P < 0.03$). In contrast, for diabetic gilts, the only significant correlation was between oestradiol and progesterone ($r = 0.47$; $P < 0.03$). There were no effects of glycaemic state on ratios of oestradiol to progesterone or oestradiol to testosterone in follicles 3–6 mm (Table 3). In follicles ≥ 7 mm, diabetic gilts had significantly lower ratios of oestradiol to both progesterone and testosterone than did normoglycaemic gilts. The ratios of oestradiol to both progesterone and testosterone were affected by follicular diameter ($P < 0.01$), with follicles ≥ 7 mm having higher ratios than 3–6 mm follicles ($P < 0.05$; not indicated in table).

Plasma glucose value was significantly higher and serum insulin lower in diabetic than control gilts throughout the entire 72-h sampling period (treatment by time interaction; $P < 0.0001$). Also, diabetic gilts did not exhibit fluctuations in insulin similar to those in controls, for which insulin increased approximately every 24 h, associated with morning feeding activity (Fig. 1).

Table 3. Follicular fluid steroid ratios

Follicle class	Steroids	Control	Diabetic
3–6 mm	Oestradiol:progesterone	0.40 ± 0.3	0.04 ± 0.4
	Oestradiol:testosterone	0.04 ± 1.3	0.44 ± 2.1
≥ 7 mm	Oestradiol:progesterone	2.20 ± 0.2 ^a	0.40 ± 0.3 ^b
	Oestradiol:testosterone	10.30 ± 1.2 ^b	3.90 ± 1.4 ^b

^{a,b}Within rows, means with different superscripts are different ($P < 0.0001$).

Fig. 1. Least squares means for concentrations of (a) insulin in serum and (b) glucose in plasma during 72 h after PMSG. Vertical lines represent the positive portion of the standard error of the mean.

Serum concentrations of IGF-I tended to be lower in diabetic gilts (effect of glycaemic state; $P < 0.1$). Concentrations of IGF-I averaged 68 ± 3.9 in diabetic and 104 ± 2.6 ng/ml in normoglycaemic gilts over all sampling times.

Immediately before PMSG, serum LH was 0.04 ± 0.02 ng/ml for diabetic gilts and 0.09 ± 0.03 ng/ml for control gilts ($P < 0.05$; Fig. 2). There was a treatment by time interaction for LH ($P < 0.0001$), and suppression of LH was evident within 12 h after PMSG. At 72 h after PMSG, LH in diabetic gilts was elevated ($P < 0.01$). This increase was due to the beginning of an apparent preovulatory LH surge in one diabetic gilt with values of 3 and 7 ng/ml at 66 and 72 h and another diabetic gilt with a value of 2 ng/ml at 72 h after PMSG. Concentrations of serum FSH were similar in diabetic and control gilts, respectively, averaging 0.60 ± 0.04 and 0.58 ± 0.03 ng/ml over the sampling period.

There was no influence of diabetes on serum oestradiol concentrations (Fig. 2). Oestradiol concentrations increased during the 72 h after PMSG and ranged from 2.5 to 20 pg/ml in normoglycaemic gilts (average 15 ± 1.3 pg/ml) and from 3 to 23 pg/ml in diabetic gilts (average 19 ± 1.9 pg/ml).

Fig. 2. Least squares means of concentrations of (a) LH and (b) oestradiol in serum during 72 h after PMSG. Vertical lines represent the positive portion of the standard error of the mean.

From 52–55 h after PMSG, there were no effects of glycaemic state on number of LH peaks (1.30 vs 1.25 , s.e.m. = 0.45), amplitude (0.17 vs 0.22 ng/ml, s.e.m. = 0.07), baseline (0.24 vs 0.33 ng/ml, s.e.m. = 0.04) or overall average LH (0.32 vs 0.41 ng/ml, s.e.m. = 0.06) in diabetic and control gilts, respectively.

Discussion

One of the major effects of diabetes in this study was an increase in atresia of small follicles in diabetic compared with normal gilts despite similar numbers of larger follicles which were not visibly atretic. In earlier studies we reported that insulin administration to normoglycaemic cyclic gilts caused a dramatic reduction in atresia of preovulatory-sized follicles (Matamoros *et al.*, 1990). However, in normoglycaemic PMSG-treated prepubertal gilts, the converse of the present study was observed; insulin reduced atresia in small (<3 mm) follicles only (Matamoros *et al.*, 1991). Together these observations suggest a role for insulin in prevention of follicular atresia.

Since ovaries were not examined during the period of insulin therapy before this experiment, conclusions cannot be made about possible differences in atresia which may have existed before PMSG administration. However, when insulin therapy was resumed for these diabetic gilts after this experiment, function of the remaining ovary appeared normal, since there was no influence

of glycaemic state on age at puberty (183 ± 3 days for normoglycaemic and 187 ± 4 days for diabetic gilts) and subsequent cyclicity (Meurer *et al.*, 1990). There is therefore no evidence that hypothalamo-pituitary maturation or ovarian function was delayed by diabetes.

The diabetic gilts initially responded to PMSG in the same way as did the control gilts, as shown by equivalent numbers of follicles ≥ 3 mm. Similar ovulatory responses to exogenous gonadotrophins were evident in normal and diabetic mice (Diamond *et al.*, 1989); similar numbers of oocytes were recovered from normal and diabetic mice, but maturation of oocytes from diabetic mice was delayed. In contrast, diabetic rats did not ovulate in response to PMSG (Kirchick *et al.*, 1982). The present study did not address whether diabetic pigs would ovulate after PMSG but, based on the number of follicles of preovulatory size, PMSG recruitment of follicles probably began normally in the diabetic gilts.

Despite the absence of macroscopic morphological differences in follicles ≥ 3 mm in diameter, diabetes was associated with lowered concentrations of several intrafollicular hormones. Oestradiol concentration was decreased by diabetes in large non-macroscopically atretic follicles (≥ 7 mm), which are of the size most likely to be preovulatory (Foxcroft & Hunter, 1985). Ainsworth *et al.* (1980) and Hammond *et al.* (1988) reported that oestradiol concentrations increased steadily in follicles of increasing size for up to 72 h after PMSG. Measurement of oestradiol in serum alone would not have reflected the decreased follicular oestradiol caused by diabetes in this study. Follicles of diabetics produced oestradiol sufficient to trigger an LH surge, but later oestradiol secretion appeared to lessen, as indicated by lowered oestradiol values in large follicles at 75 h after PMSG. It is likely that serum oestradiol would have triggered a preovulatory LH surge in the other diabetic gilts, but it is not known how follicles would respond to the ovulatory stimulus.

In diabetic gilts elevated testosterone concentrations in medium-sized follicles as well as lowered oestradiol in large-sized follicles may indicate decreased aromatization to oestradiol as follicles increase in size. The ratios of oestradiol to other steroids also support the concept that steroidogenic pathways are altered by the diabetic condition, since the ratios of oestradiol to progesterone and oestradiol to testosterone in follicles ≥ 7 mm in diameter were significantly reduced in diabetic animals. Reduced ratios of oestrogen to total follicular androgens precede macroscopically visible atresia in all sizes of follicles and the lack of oestrogen reflects a limitation in the aromatizing system (Moor *et al.*, 1978; McNatty *et al.*, 1979; Carson *et al.*, 1981). These findings support the conclusion that preovulatory-sized follicles of diabetic gilts in the present study were in early stages of atresia, although there were no macroscopic indications of atresia detected.

The indication of a preovulatory LH surge in 2 diabetic gilts is in contrast to the results of Kirchick *et al.* (1982) for diabetic rats treated with PMSG. Exposure to the preovulatory LH surge could be expected to alter intrafollicular steroid concentrations. Decreased steroid concentrations in pig follicles were not evident by 4 h but were evident at 18 h after an ovulatory injection of hCG (Ainsworth *et al.*, 1980). In our study, elevation of LH in 2 diabetic pigs was first evident in the samples obtained 3 h and 9 h before ovary removal, respectively. Therefore it is likely that one diabetic animal could have experienced significant exposure to LH. However, when data were examined individually, concentrations of follicular fluid steroids and IGF-I as well as ratios of oestradiol to testosterone and progesterone and serum oestradiol values of these diabetic gilts were not distinguishable from those of the other diabetic gilts. The results of this study are supported by a comparison of diabetic and normoglycaemic gilts on Day 18 of the oestrous cycle, in which none of the gilts had experienced an LH surge and yet follicular hormone differences were similar to those in the present study (Meurer *et al.*, 1990).

Lack of insulin in diabetic gilts was associated with lowered IGF-I values in large follicles and an absence of correlation between IGF-I and follicular diameter. We know of no other reports of effects of diabetes on intrafollicular IGF-I in pigs. However, Matamoros *et al.* (1991) observed that exogenous insulin administration doubled IGF-I concentrations in follicles of PMSG-treated normoglycaemic gilts. *In vivo* IGF-I increased in follicular fluid in response to PMSG, suggesting

that intrafollicular IGF-I content is regulated by gonadotrophins (Hammond *et al.*, 1988). Also, intrafollicular IGF-I concentrations were correlated with size of pig follicles, in contrast to intrafollicular concentrations of insulin (Hammond *et al.*, 1985). Our data indicate that the lack of insulin prevented the PMSG-induced increase in intrafollicular IGF-I as follicles developed.

In the present study concentrations of IGF-I and oestradiol were positively correlated in normoglycaemic but not in diabetic gilts. Eden *et al.* (1988) also reported a positive correlation between IGF-I and oestradiol concentration in normal follicles. In diabetic pigs the lack of correlation between oestradiol and IGF-I provides further evidence for a disruption in follicular hormone production. There were positive correlations in normoglycaemic animals between oestradiol and testosterone, progesterone and IGF-I. However, in diabetic gilts the only positive correlation was between oestradiol and progesterone. Assuming that the correlations in control gilts are representative of normal preovulatory follicles, this is further support for the argument that aromatization does not progress normally in the absence of insulin. Aromatase activity of granulosa cells *in vitro* was increased by both insulin (Garzo & Dorrington, 1984) and IGF-I (Erickson *et al.*, 1989). The impairment of steroidogenesis in this study may be primarily related to the lack of insulin and secondarily related to lowered intrafollicular IGF-I.

In contrast to results with follicular fluid, significant effects of diabetes were not observed for serum IGF-I concentrations. Inhibitory effects of diabetes on systemic IGF-I have been observed by others. Bornfeldt *et al.* (1989) reported that messenger RNA levels for IGF-I in body organs are lowered in diabetes. An additional mechanism by which diabetes decreases systemic IGF-I may be by causing higher concentrations of its binding protein (Holly & Wass, 1989). Hammond *et al.* (1988) concluded that serum and follicular fluid IGF-I concentrations are not necessarily correlated in pigs. However, Eden *et al.* (1988) observed that IGF-I concentrations in follicles were correlated with those in the circulation except for the dominant follicle of humans, in which IGF-I was elevated.

Circulating gonadotrophins were not decreased by diabetes. Kirchick *et al.* (1978) observed that basal LH was similar in diabetic and control rats. In contrast to the present study, in rats diabetes abolished the oestradiol-induced LH surge, which was restored by insulin (Kirchick *et al.*, 1978, 1982; Katayama *et al.*, 1984). Also, results from our laboratory indicate that ovariectomized diabetic pigs have a preovulatory LH surge in response to oestradiol benzoate (Carlton *et al.*, 1990). Serum FSH was similar in diabetic and control gilts in this study and there was no increase in FSH after PMSG administration, in agreement with the results of Flowers *et al.* (1989). Peripheral FSH concentration was not affected by insulin administration to normoglycaemic PMSG-treated gilts (Matamoros *et al.*, 1991).

The failure of diabetes to affect circulating gonadotrophin values argues against a role for the hypothalamo-pituitary complex in the impairment of steroidogenesis observed in this study. In our earlier studies, we observed that effects of insulin on ovulation rate were not necessarily associated with changes in gonadotrophins (Cox *et al.*, 1987a; Matamoros *et al.*, 1991). Although not tested directly in the present study, lack of insulin may have interfered with the action of gonadotrophins on granulosa cells. A synergism of both insulin and IGF-I with gonadotrophins to enhance granulosa cell function is well-established (Adashi *et al.*, 1985; Poretsky & Kalin, 1987).

In summary, the numbers of presumed preovulatory follicles at 75 h after PMSG were not affected by diabetes, although atresia of small follicles was increased. However, although numbers and gross morphology of follicles ≥ 3 mm in diameter were similar, the ability of follicles to produce oestradiol, testosterone and IGF-I was impaired in diabetic gilts. This impairment was in spite of apparently adequate concentrations of LH in serum and progesterone and testosterone in large follicles. We therefore conclude that the diabetic condition, the subsequent lack of insulin, and possibly the lack of IGF-I, impair steroidogenesis at some point subsequent to progesterone production.

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