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Effect of in-vitro heat stress on prostaglandin and protein secretion by endometrium from pregnant and cyclic gilts at Day 14 after oestrus*

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Summary. Endometrium from cyclic (N = 4) and pregnant (N = 4) gilts at Day 14 after oestrus was placed into three bilateral perifusion devices which allow separate perifusion of luminal and myometrial sides. Perifused endometrium was subjected to 39 or 42°C. Incorporation of [^{3}H]leucine into secreted and tissue proteins by endometrial explants following incubation at 39 or 42°C was examined using trichloroacetic acid (TCA) precipitation and one-dimensional SDS polyacrylamide gel electrophoresis. Secretion of PGF was greater from the myometrial side for cyclic gilts (endocrine orientation), but greater from the luminal side for pregnant gilts (exocrine orientation). Regardless of reproductive status or endometrial side, heat stress induced a rapid increase ($P < 0{\cdot}01$) in PGF secretion rates. However, PGF secretion in response to heat stress was greater ($P < 0{\cdot}01$) from the myometrial side and greater ($P < 0{\cdot}01$) for pregnant gilts. PGF secretion rates increased by 63% and 42% from the luminal side, and 40% and 156% from the myometrial side in response to heat stress for cyclic and pregnant gilts, respectively (status × treatment × side interaction; $P < 0{\cdot}01$). Heat stress did not alter incorporation of [^{3}H]leucine into secreted proteins regardless of reproductive status, while incorporation into tissue proteins was decreased ($P < 0{\cdot}05$) by heat stress for pregnant gilts, but not altered for cyclic gilts. Heat stress, *in vitro*, redirects PGF secretion for endometria of pregnant gilts from an exocrine to an endocrine orientation where it would be available to effect luteolysis and compromise the establishment of pregnancy.

Keywords: pig; heat stress; endometrium; prostaglandins; perifusion; proteins

Introduction

Transient infertility often occurs during periods of environmental heat stress in cattle (Thatcher, 1974), sheep (Dutt, 1963) and pigs (Trujano & Wrathall, 1985). Embryos are extremely sensitive to the harmful effects of maternal hyperthermia, which is reflected by an increase in the frequency of nonviable, abnormal and retarded embryos (Trujano & Wrathall, 1985; Putney *et al.*, 1988a). In-vivo heat stress of gilts between Days 8 and 16 of pregnancy increased concentrations of 13,14-dihydro-15-keto PGF-2α (PGFM) in plasma (Wettemann *et al.*, 1988). This may account for the reduced concentrations of progesterone in plasma during Days 13–19 (Hoagland & Wettemann, 1984). Increased concentrations of systemic PGFM may reflect increased uterine secretion of PGF in response to heat stress.

The establishment of pregnancy, involves maintenance of luteal function due to suppression or alteration of uterine luteolytic mechanisms. In cattle (Gross *et al.*, 1988c) and sheep (Bazer *et al.*,

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1989) the anti-luteolytic mechanisms, during early pregnancy, involves an attenuation of endometrial responsiveness to stimulators of prostaglandin synthesis (i.e. oxytocin). However, pig endometrium is capable of responding to oxytocin with increased PG secretion regardless of reproductive status (Gross *et al.*, 1988a). Bazer & Thatcher (1977) proposed that a reorientation of endometrial prostaglandin (PG) F release into the uterine lumen (exocrine mode) might occur at the onset of pregnancy in pigs. Gross *et al.* (1988a) demonstrated *in vitro* that PG secretion for perifused endometrial tissue was primarily in an endocrine direction, towards the myometrium and the uteroovarian vasculature, during the oestrous cycle. However, during early pregnancy, PG secretion was primarily exocrine, towards the uterine lumen. Such a reorientation in secretion might prevent release of PGF into the uterine vasculature and allow CL maintenance. Heat stress might alter uterine and embryonic function in a manner that would attenuate the antiluteolytic processes that occur during early pregnancy.

Endometrial protein secretions provide nutrients essential for conceptus development during early pregnancy (Bazer, 1975). Therefore, heat stress might also alter endometrial protein synthesis and secretion in a manner that would contribute to pregnancy failure and embryonic mortality.

The present study examined whether in-vitro heat stress alters endometrial protein synthesis or secretion and the orientation of secretion of PGF by endometrial tissue from pregnant and cyclic gilts at Day 14 after oestrus.

Materials and Methods

Materials. Radioisotopes of L-[4,5-^{3}H]leucine (sp. act. 150 Ci/mmol) and [5,6,8,11,12,14,15-^{3}H]PGF-2α (sp. act. 160–180 Ci/mmol) were purchased from Amersham Corporation (Arlington Heights, IL, USA). Oxytocin was purchased from Sigma Chemical Company (St Louis, MO, USA). Antiserum to PGF was provided by T. G. Kennedy. Dialysis tubing was purchased from Spectrum Medical (Los Angeles, CA, USA). Supplies for polyacrylamide gel electrophoresis (PAGE) were as follows: Tris and *N,N,N',N'*-tetramethyl ethylenediamine (TEMED) were purchased from Sigma Chemical Co.; sodium salicylate, 2-mercaptoethanol, glycine and ammonium peroxydisulphate were purchased from Fisher Scientific (Orlando, FL, USA); acrylamide, urea, dithiothreitol and sodium dodecyl sulphate (SDS) were purchased from Research Organics (Cleveland, OH, USA); and *bis*-acrylamide, gelatin and Tween-20 were purchased from Bio-Rad (Richmond, CA, USA).

A modified minimum essential medium (MEM; custom formula No. 87-5007) and other medium ingredients were purchased from Gibco (Grand Island, NY, USA). Medium was prepared as described by Basha *et al.* (1980) and supplemented with 1% (v/v) Gibco MEM vitamin solution. For cultures in the presence of [^{3}H]leucine, medium was prepared at a reduced leucine content (10% of the normal concentration).

Tissue collection. Sexually mature crossbred (Duroc × Yorkshire × Hampshire) gilts were observed for oestrous behaviour in the presence of a boar between 07:30 and 08:00 h each day. Day of onset of oestrus was designated Day 0. Gilts were either mated by natural service when detected in oestrus and at 12 h and 24 h after onset of oestrus (pregnant, N = 4) or not mated (cyclic, N = 4). All gilts were hysterectomized at Day 14 after oestrus. Reproductive tracts were removed rapidly and flushed with a modified Minimal Essential Medium (MEM) to collect conceptuses and confirm reproductive status. Endometrium was dissected from myometrial tissue over a side area of approximately 8–10 cm^2.

Perifusions. Endometrium was placed into sterile Krebs–Ringer–bicarbonate solution (KRB; Sigma Chemical Co., with 0·1 M-$CaCl_2$ and 0·2 M-$NaHCO_3$) which had been gassed with O_2:CO_2 (95:5), washed twice with fresh KRB and dissected into three pieces of approximately 2 cm^2. Each endometrial sample was placed into a bilateral perifusion device which allowed separation of myometrial and luminal sides so that each was perifused separately in a 0·5 ml hemi-chamber (Lacroix & Kann, 1983; Gross *et al.*, 1988a). Tissue was anchored into each device with the aid of 4 needles. Each chamber was placed into a 39°C water bath, attached to dual 60 ml syringes containing freshly gassed KRB, and perifused at a rate of 3 ml/10 min for 5 h using a Harvard infusion pump system. Fractions (3 ml) were collected every 10 min for a total of 30 fractions.

Each of the three endometrial perifusion preparations within each gilt were subjected to a different temperature and oxytocin (1 i.u./ml KRB) treatment sequence: (1) control–oxytocin: 1 h at 39°C, 2 h at 39°C, 0·5 h at 39°C with oxytocin, 0·5 h at 39°C and 1 h at 39°C; (2) heat–oxytocin; 1 h at 39°C, 2 h at 42°C, 0·5 h at 42°C with oxytocin, 0·5 h at 42°C and 1 h at 39°C; and (3) heat–saline: 1 h at 39°C, 2 h at 42°C, 0·5 h at 42°C with saline, 0·5 h at 42°C and 1 h at 39°C. Temperature treatments were imposed on endometrium by placing perifusion devices in water baths maintained at 39 or 42°C and perifusion of tissues with KRB maintained at 39 or 42°C.

RIA procedures. Fractions were analysed for PGF using a direct radioimmunoassay (RIA) procedure (Gross *et al.*, 1988b) and an antibody characterized by Kennedy (1985). Cross-reactivities of the PGF-2α antiserum were; 94% for

PGF-1α, 2·4% for PGE-2, and <0·1% for PGFM and arachidonic acid. Because of the high cross-reactivity with PGF-1α, the assay results are listed as concentrations of PGF. Inter- and intra-assay coefficients of variation were 9·8% and 11·6%, respectively.

Incorporation of [^{3}H]leucine into proteins. Endometrial tissue was dissected into approximately 1 mm^3 pieces and placed (500 mg) into a Petri dish containing 0·1 mCi L-[4,5-^{3}H]leucine and 15 ml MEM. Tissues were incubated for 8 h at 39 or 42°C on a rocker platform under an atmosphere of 47·5% O_2, 50% N_2 and 2·5% CO_2. At the conclusion of incubation, tissue and medium were separated by centrifugation (3500 ***g***, 4°C, 30 min) and stored at −70°C until analysed.

Radiolabelled protein determination. Incorporation of radiolabelled leucine into secreted (medium) and intracellular (tissue) proteins was determined by trichloroacetic acid (TCA) precipitation. Tissue from incubations with [^{3}H]leucine was solubilized in 50 mM-Tris–acetate buffer (2 ml buffer/500 mg tissue) containing 1 mM-phenylmethylsulphonyl-fluoride, 1 mM-ethylenediamine-tetraacetic acid and 2% (v/v) Nonidet P-40. Aliquants (0·05 ml) of medium and solubilized tissue were each placed and dried onto Whatman 3MM paper that had been saturated previously with 20% TCA (w/v). Precipitation of proteins onto the filter paper and removal of non-proteinaceous compounds was accomplished by serial washing of filter paper with 20% and 5% TCA followed by 95% ethanol as described by Mans & Novelli (1961). Radioactivity of precipitated protein was determined by scintillation spectrometry.

Electrophoresis. Medium from endometrial explants containing [^{3}H]leucine was dialysed extensively (3 changes of 4 l) against distilled water using dialysis tubing with an M_r exclusion limit of 6000–8000 to remove low M_r compounds and unincorporated radiolabelled precursors. Dialysed samples of medium and samples of solubilized tissue were each examined qualitatively using sodium dodecyl sulphate (SDS), polyacrylamide gel electrophoresis (SDS-PAGE). One-dimensional SDS-PAGE was performed using the buffer system of Laemmli (1970) with 12·5% (w/v) polyacrylamide gels. Samples containing 100 000 d.p.m. were lyophilized, dissolved in 0·1 ml of dissociating buffer containing 1% (w/v) SDS and 5% (v/v) 2-mercaptoethanol, and boiled (3 min; boiling point approximately 96°C) before being loaded onto gels. Proteins were localized by staining with Coomassie Blue R-250 and fluorography as described by Roberts *et al.* (1984). Fluorographs were prepared with Kodak XAR film using sodium salicylate as a fluor (Chamberlain, 1979).

Statistical analysis. Data were analysed by Least Squares Analysis of Variance using the General Linear Models procedure of the Statistical Analysis System (SAS, 1985). Secretion rates of PGF for luminal, myometrial or luminal+myometrial sides of endometrium were analysed for the total perifusion sequence and for the following perifusion treatment periods (thermoneutral [39°C], heat stress [42°C], oxytocin, saline): (1) 1 h at 39°C, (2) 2 h at 39 or 42°C, (3) 0·5 h at 39°C with oxytocin or at 42°C with saline or oxytocin, (4) 0·5 h at 39 or 42°C, and (5) 1 h at 39°C. Secretion rates were also analysed for combinations of these various perifusion treatment periods: periods 1+2, periods 2+3, periods 3+4, and periods 4+5. The statistical model components for analyses of PGF secretion rates were: status (pregnant or cyclic), status (gilt), treatment (temperature and oxytocin), status × treatment, status (gilt) × treatment, side, status × side, status (gilt) × side, treatment × side, status × treatment × side, status (gilt) × treatment × side, time, status × time, treatment × time, side × time, status × treatment × time, status × side × time, treatment × side × time, status × treatment × side × time, and error. Data for incorporation of [^{3}H]leucine into proteins were analysed using the model components of status, status (gilt), treatment status × treatment, status(gilt) × treatment, and error.

Results

PGF secretion

Endometrial tissues remained viable throughout the 5 h of perifusion as indicated by sustained PGF secretion (Fig. 1). Mean rates of endometrial PGF secretion, before heat stress, were higher ($P < 0·01$) from the myometrial than luminal side for Day 14 cyclic gilts (Fig. 1). In contrast, PGF secretion rates, before heat stress, were higher ($P < 0·01$) from the luminal than myometrial side for Day 14 pregnant gilts (Fig. 1). This resulted in a status × side interaction ($P < 0·01$) (Period 1).

Before the initiation of heat stress (Period 1), PGF secretion rates by endometria within each reproductive status and endometrial side were similar between treatment groups (Table 1). Elevation of perifusion temperature from 39 to 42°C (heat stress) induced a rapid and sustained increase in PGF secretion rates that varied depending upon reproductive status and endometrial side (Period 2; Fig. 1; Table 1; $P < 0·01$; status × treatment × side interaction). Secretion of PGF in response to heat stress was greater from the myometrial side of endometrium from pregnant gilts ($P < 0·01$). Secretion rates of PGF during the first 2 h of heat stress (Period 2; Table 1) were increased ($P < 0·01$) 63% and 42% from the luminal side and 40% and 156% from the myometrial

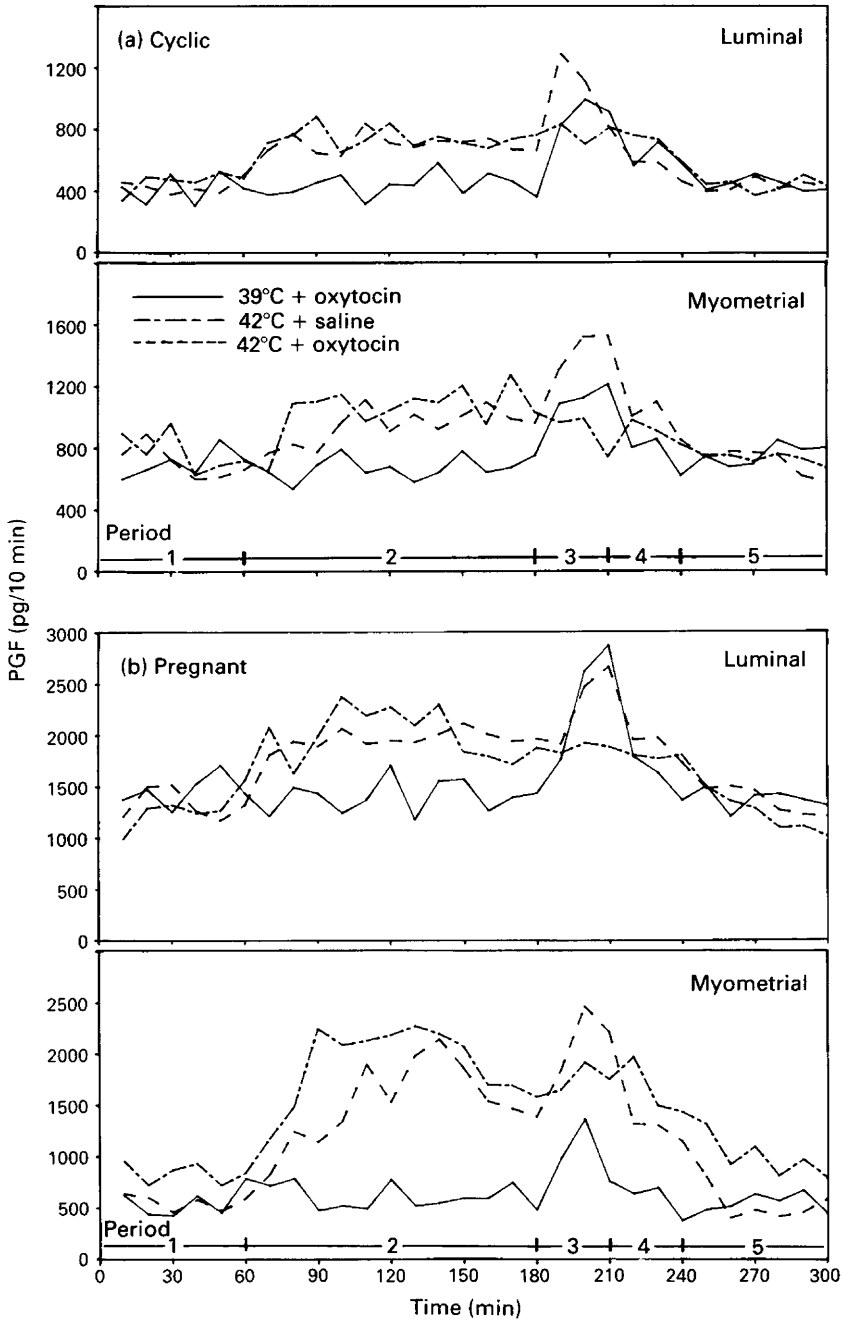

Fig. 1. Endometrial secretion rates of PGF from luminal and myometrial sides for (a) cyclic and (b) pregnant gilts at Day 14 after oestrus. Treatments were: **(1)** Control–oxytocin, 1 h at 39°C, 2 h at 39°C, 0·5 h at 39°C with oxytocin, 0·5 h at 39°C and 1 h at 39°C; **(2)** heat–oxytocin, 1 h at 39°C, 2 h at 42°C, 0·5 h at 42°C with oxytocin, 0·5 h at 42°C and 1 h at 39°C; and **(3)** heat–saline, 1 h at 39°C, 2 h at 42°C, 0·5 h at 42°C, 0·5 h at 42°C and 1 h at 39°C.

side for cyclic and pregnant gilts, respectively (Table 1). This resulted in a disproportionate increase in PGF secretion from the myometrial side of endometrium from pregnant gilts in response to heat stress. During heat stress (Period 2), PGF secretion no longer differed between luminal and myometrial sides for endometrial tissue from pregnant gilts. However, PGF secretion remained higher from the myometrial than the luminal side for endometrium from cyclic gilts regardless of treatment (temperature).

Table 1. Endometrial secretion rates of PGF (pg/10 min mean ± s.e.m.) by luminal and myometrial sides for pregnant and cyclic gilts at Day 14 after oestrus: treatment periods were: (1) 1 h at 39°C; (2) 2 h at 39 or 42°C; (3) 0·5 h at 39°C with oxytocin or at 42°C with saline or oxytocin; (4) 0·5 h at 39 or 42°C; and (5) 1 h at 39°C

	Perifusion treatment period				
Treatment	1[a]	2[b]	3[c]	4[c]	5[a]
Pregnant (N = 4)					
Luminal					
39°C–oxytocin	1449 ± 66	1433 ± 80	2421 ± 136	1468 ± 75	1372 ± 63
42°C–oxytocin	1329 ± 81	1781 ± 89	2003 ± 105	1767 ± 111	1353 ± 73
42°C–saline	1276 ± 65	1901 ± 117	1642 ± 96	1591 ± 90	1289 ± 70
Myometrial					
39°C–oxytocin	582 ± 87	598 ± 51	1029 ± 142	681 ± 90	542 ± 48
42°C–oxytocin	645 ± 61	1506 ± 124	2016 ± 93	1242 ± 101	636 ± 54
42°C–saline	703 ± 95	1944 ± 180	1736 ± 77	1527 ± 138	716 ± 97
Cyclic (N = 5)					
Luminal					
39°C–oxytocin	415 ± 35	536 ± 33	915 ± 79	522 ± 37	443 ± 26
42°C–oxytocin	427 ± 50	731 ± 50	1075 ± 79	645 ± 42	436 ± 19
42°C–saline	458 ± 37	714 ± 47	636 ± 52	695 ± 58	458 ± 33
Myometrial					
39°C–oxytocin	782 ± 37	715 ± 67	1169 ± 122	798 ± 59	726 ± 59
42°C–oxytocin	755 ± 79	1035 ± 68	1455 ± 98	985 ± 66	802 ± 29
42°C–saline	807 ± 53	1156 ± 58	975 ± 51	852 ± 36	797 ± 27

[a] $P < 0.01$ for status × side.
[b] $P < 0.01$ for status × treatment × side.
[c] $P < 0.05$ for status × treatment × side.

Oxytocin (Period 3) induced a rapid increase in the secretion of PGF that varied depending upon reproductive status, endometrial side, and treatment (temperature) (Fig. 1; Table 1; $P < 0.05$; status × treatment × side interaction). Endometrium (luminal and myometrial sides) from cyclic and pregnant gilts responded to oxytocin at either 39 or 42°C. However, PGF secretion at 39°C from the luminal side remained higher than that from the myometrial side for endometrium from pregnant gilts, and the luminal response to oxytocin was greater than that from the myometrial side. In contrast, PGF secretion at 42°C did not differ between luminal and myometrial sides for endometrial tissue from pregnant gilts, and the response to oxytocin did not differ with endometrial side. For cyclic gilts, myometrial responses to oxytocin were greater than luminal responses when perifused at either 39 or 42°C, and PGF secretion was greater from the myometrial than luminal side regardless of perifusion temperature.

After oxytocin treatment (Period 4), secretion rates of PGF declined rapidly to pre-oxytocin rates of secretion (Period 2, Table 1). Heat stress (42°C) resulted in increased secretion rates of PGF that varied depending upon reproductive status, endometrial side and treatment (temperature) (status × treatment × side interaction; $P < 0.01$; Table 1). Secretion of PGF remained higher from the myometrial side of endometrium from cyclic gilts regardless of perifusion temperature or previous oxytocin treatment during Period 3. In contrast, PGF secretion for endometrium from

pregnant gilts, perifused at 39°C, remained higher from the luminal side. However, PGF secretion for endometrium from pregnant gilts, perifused at 42°C, did not differ between luminal and myometrial sides when previously treated with saline during Period 3, whereas luminal secretion was greater when previously treated with oxytocin during Period 3. Indeed, the myometrial side of endometrium from pregnant gilts was less able to respond to additional heat stress following oxytocin treatment during the previous period (Period 3) than was the saline-treated myometrial side (Table 1; Fig. 1).

When perifusion temperatures were reduced from 42 to 39°C (Period 5), PGF secretion rates declined ($P < 0{\cdot}01$) to pre-heat stress rates (Period 1) within 30 min of the temperature change (Fig. 1). Mean secretion rates of PGF did not differ during Period 5 between treatments for each status and endometrial side (Table 1). Secretion rates of PGF during period 5 were similar to those observed during Period 1.

Incorporation of [^{3}H]leucine into endometrial proteins

Incorporation of [^{3}H]leucine into secreted and tissue proteins was examined quantitatively by TCA precipitation. Incorporations into secreted (2942 and 1258 d.p.m./mg tissue, respectively) and tissue (27 222 and 9702 d.p.m./mg tissue, respectively) proteins were greater ($P < 0{\cdot}01$) for endometrium from pregnant than cyclic gilts at 39°C. Heat stress (42°C) did not alter incorporation into secreted proteins regardless of reproductive status (pregnant, 3233 d.p.m./mg; cyclic, 1307 d.p.m./mg). However, heat stress (42°C) decreased incorporation into tissue proteins for endometrium from pregnant gilts (19 807 d.p.m./mg tissue), but did not alter incorporation into tissue proteins for cyclic gilts (10 406 d.p.m./mg tissue) (treatment × status; $P < 0{\cdot}01$).

Polyacrylamide gel electrophoresis was utilized to examine qualitative differences in incorporation of [^{3}H]leucine into secreted and tissue proteins (equal d.p.m. utilized for each electrophoretogram). The array of [^{3}H]leucine labelled endometrial tissue proteins (Fig. 2) present on one-dimensional fluorographs did not differ qualitatively between pregnant and cyclic gilts. However, heat stress enhanced the relative radiolabelling of tissue proteins of M_r 70 000 and 90 000 (possibly heat shock proteins) regardless of reproductive status (Fig. 2). The major [^{3}H]leucine-labelled secreted proteins were in an M_r 30 000–95 000 range, and pig serum albumin (PSA, M_r 69 000) was present as a major unlabelled protein component regardless of reproductive status (Fig. 3). The presence of PSA probably reflects leaching of serum components into the medium. Unlabelled PSA appeared to be more prevalent in media from cyclic gilts, but this may have been due to the larger volumes of medium required to yield equivalent radiolabel activity to that utilized for pregnant gilts. Increased levels of unlabelled PSA, present in medium from cyclic gilts, are indicated by the increased distortion of protein patterns on the fluorographs (Fig. 3). Radiolabelled secreted proteins of M_r 70 000 may be enhanced in medium for endometrial explants from pregnant gilts. However, the PSA-induced distortion in protein patterns for cyclic gilts does not enable a clear comparison. Heat stress did not alter the array of [^{3}H]leucine-labelled secretory proteins regardless of reproductive status (Fig. 3). However, there was a decreased intensity of radiolabelled proteins of M_r 35 000 for endometrium from pregnant compared to cyclic gilts (see arrows Fig. 3).

Discussion

Previous results (Gross *et al.*, 1988a) demonstrated that secretion of PGF during early pregnancy in gilts is redirected from the uterine vasculature (towards the myometrium; endocrine secretion) towards the uterine lumen (exocrine secretion). This reorientation of PG secretion in pregnant gilts results in decreased maternal levels of PGF and, therefore, a reduction in the luteolytic signal. Data from the present study reconfirm this reorientation in the direction of endometrial PGF secretion in pregnant gilts.

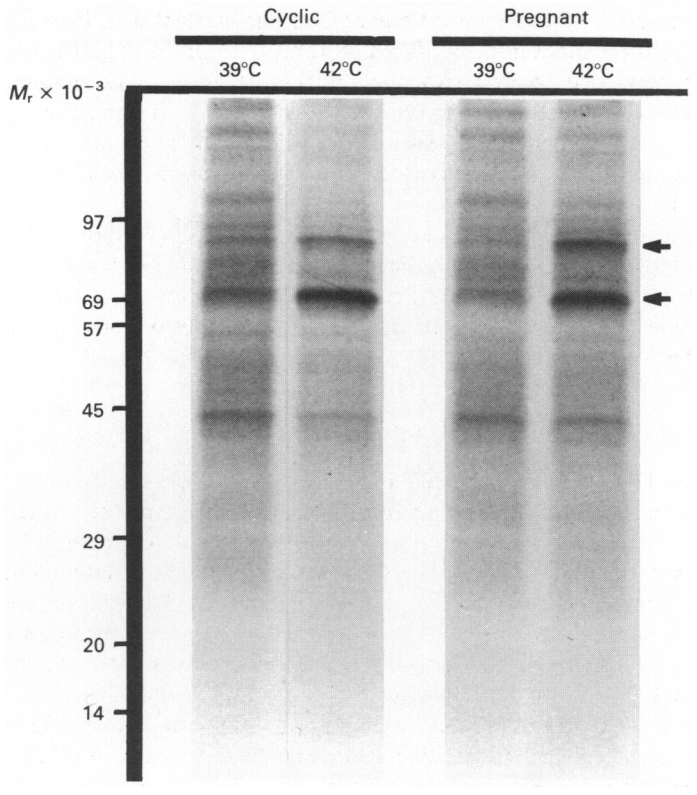

Fig. 2. Fluorograph of a one-dimensional SDS-polyacrylamide gel electrophoretogram of solubilized tissue from endometrium of a cyclic and a pregnant gilt at Day 14 after oestrus cultured in the presence of [^{3}H]leucine for 8 h at thermoneutral (39°C) or heat stress (42°C) temperatures. An aliquant of each sample of solubilized tissue (100 000 d.p.m.) was lyophilized, dissolved in dissociating buffer and separated on a 12·5% (w/v) polyacrylamide gel in the presence of reducing agent. Note that the array of radiolabelled proteins in tissue did not differ for endometrium from cyclic and pregnant pigs. Note the increased intensity of proteins of M_r 70 000 and 90 000 (see arrows) in response to heat stress (possibly heat shock proteins) regardless of reproductive status.

Increased secretion of PGF in response to heat stress *in vitro* by endometrial explants from cattle at Day 17 of pregnancy has been reported (Putney *et al.*, 1988b). In-vivo heat stress of gilts between Days 8 and 16 of pregnancy increased concentrations of PGFM in plasma and subsequently induced luteolysis (Hoagland & Wettemann, 1984; Wettemann *et al.*, 1988). In the present study, heat stress induced a rapid and sustained increase in endometrial secretion rates of PGF by luminal and myometrial sides both for pregnant and cyclic gilts. However, heat stress (42°C) induced a disproportionate increase in PGF secretion from the myometrial side of endometrium from pregnant gilts. Likewise, the myometrial side of endometrium from pregnant gilts becomes more responsive to oxytocin when heat stressed. An endocrine orientation (towards the myometrium) of PGF secretion is normally present for cyclic gilts and is critical for the availability of PGF for luteolysis (Bazer & Thatcher, 1977; Gross *et al.*, 1988a). However, secretion of PGF is normally greater from the luminal surface (exocrine orientation), and the luminal surface is normally more responsive to oxytocin during early pregnancy in pigs (Gross *et al.*, 1988a). Therefore, a disproportionate increase in PGF secretion from the myometrial side of endometrium from pregnant gilts in response to heat stress might redirect PGF secretion from the normal exocrine orientation to an

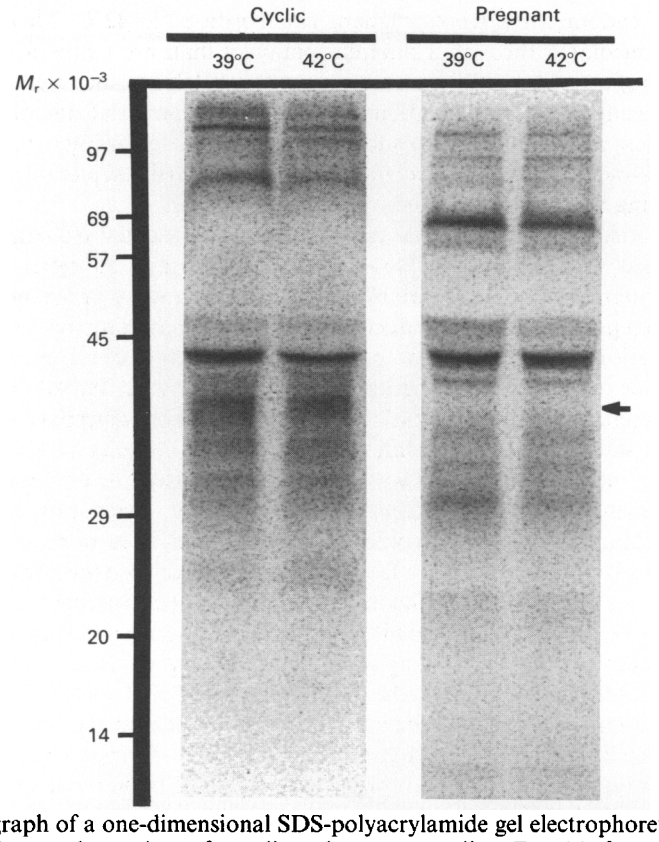

Fig. 3. Fluorograph of a one-dimensional SDS-polyacrylamide gel electrophoretogram of dialysed medium from endometrium of a cyclic and a pregnant gilt at Day 14 after oestrus cultured in the presence of [^{3}H]leucine for 8 h at thermoneutral (39°C) or heat stress (42°C) temperatures. An aliquant of each sample of culture medium (100 000 d.p.m.) was lyophilized, dissolved in dissociating buffer and separated on a 12·5% (w/v) polyacrylamide gel in the presence of reducing agent. Note the decreased intensity of proteins of M_r 35 000 for pregnant compared to cyclic gilts (see arrow). Heat stress did not alter the secretion of radiolabelled proteins regardless of reproductive status.

endocrine orientation. Increased secretion from the myometrial side, in response to heat stress, might allow PGF to reach the CL and compromise the establishment of pregnancy.

Increased PGF secretion by endometrium exposed to heat stress may be due to alterations in cellular membranes which result in increased mobilization of substrate for prostaglandin biosynthesis. The primary cellular site for the action of heat stress is cellular membranes (Bowler *et al.*, 1973; Hahn, 1982). Increased ambient temperature increases membrane fluidity, phospholipase activity and phosphoinositide turnover (Calderwood *et al.*, 1987), which would increase the release of arachidonic acid (Flint *et al.*, 1986). Arachidonic acid is the principal substrate for PGF synthesis in endometrium before initiation of endometrial PGF synthesis and luteolysis (Hansel *et al.*, 1975; Shemesh & Hansel, 1975). Exogenous arachidonic acid also stimulates PG synthesis by endometrial tissues *in vitro* (Thatcher *et al.*, 1984). Therefore, increased PGF secretion in response to heat stress may be due to increased availability of substrate for PG synthesis.

Oxytocin also increases the availability of substrate for endometrial PG synthesis (Flint *et al.*, 1986). Indeed, the present study demonstrates increased PGF secretion in response to oxytocin regardless of reproductive status. Oxytocin also augmented the increased secretion of PGF observed during heat stress. In addition, this increase in response to heat stress was greatest for the

myometrial side of endometrium from pregnant gilts perifused at 42°C. Therefore, the effects of oxytocin might be mediated through a different pathway than are temperature effects. Alternatively, heat stress may not maximally increase substrate availability such that oxytocin would still be capable of increasing substrate availability. Indeed, the myometrial side of endometrium from pregnant gilts was less able to respond to additional heat stress following oxytocin treatment than following saline treatment. These data may indicate at least a partial depletion of substrate for PGF biosynthesis following both heat stress and oxytocin treatments.

Endometrial protein secretions provide nutrients that are essential for conceptus development (Bazer, 1975). Indeed, results from the present study demonstrate an increase in the amount of radiolabelled endometrial tissue and secretory proteins during early pregnancy. Therefore, alterations in endometrial protein synthesis induced by heat stress may contribute to pregnancy failure. A differential regulation between secreted and tissue incorporation of [^{3}H]leucine has been shown for endometrial tissue during early pregnancy for cows (Gross *et al.*, 1988d) and sheep (Godkin *et al.*, 1984). In the present study, a differential regulation between endometrial tissue from animals of cyclic and pregnant status appears to occur, as measured by TCA-precipitable protein. Incorporation of [^{3}H]leucine into tissue proteins was selectively increased for endometrial explants from pregnant gilts. Likewise, heat stress differentially reduced label incorporation into tissue proteins for endometrial explants from pregnant compared to cyclic gilts. Incorporation of [^{3}H]leucine into intracellular proteins is much higher than for incorporation into secreted proteins (approximately 10% of tissue proteins). Furthermore, incorporation into tissue proteins for endometrium from pregnant gilts is 280% greater than for endometrium from cyclic gilts. This higher incorporation for endometrium from pregnant gilts may increase the ability to detect alterations due to heat stress. None the less, the present results indicate a decrease in the intensity of proteins of M_r 35 000 for pregnant compared to cyclic gilts. Heat stress (42°C) increased the intensity of [^{3}H]leucine-labelled tissue proteins of M_r 70 000 and 90 000 (possibly heat shock proteins) detected on one-dimensional fluorographs regardless of reproductive status. Endometrial explants incubated at 39°C also produced radiolabelled tissue proteins of M_r 70 000 and 90 000, but at reduced intensities compared to 42°C. Similar proteins, heat shock proteins, have been described for other heat-stressed mammalian tissues (Nover, 1984; Putney *et al.*, 1988b). Heat shock proteins are believed to play a role in cellular homeostasis and thermotolerance during periods of stress. Their involvement, if any, in prostaglandin synthesis is not known. However, heat shock proteins may increase endometrial prostaglandin secretion and/or alter the direction of this secretion in gilts exposed to heat stress conditions.

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