Production of platelet-activating factor by the pre-implantation sheep embryo

K. M. Battye¹, A. J. Ammit¹, C. O'Neill¹ and G. Evans²

¹Human Reproduction Unit, Royal North Shore Hospital, St Leonards, NSW 2065, Australia; and

²Department of Animal Science, University of Sydney, Sydney, NSW 2006, Australia

Summary. Embryos were collected from superovulated ewes on Day 2 (2–8 cell), Day 4 (8–16 cell) and Day 6 (morula/early blastocyst). Two embryos were cultured in 1 ml of one of four media: (i) Ham's F10 + 4 mg bovine serum albumin (BSA)/ml, (ii) synthetic oviduct fluid medium + 20% human serum, (iii) Quinn's human tubal fluid medium (HTF) + 3 mg BSA/ml or (iv) HTF + 10% acid-treated fetal calf serum for 24 h. They were transferred to fresh media of the same type and their further development was monitored. A quantitative bioassay and radioimmunoassay was used to measure the concentration of platelet-activating factor (PAF, 1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phosphocholine) produced. Following extraction and partial purification, 21/95 (22.1%) of the embryo-conditioned media samples had PAF concentrations greater than that measured in corresponding control media. This was designated as embryo-derived PAF and the corresponding cultures were termed 'PAF-positive'. PAF was produced by embryos at all three developmental stages examined and in each of the four media used, and the average amount of PAF produced was 60.9 ± 9.8 pmol/embryo/24 h. However, neither the developmental stage of the embryo, nor the type of media affected the proportion of PAF-positive cultures nor the amount of PAF produced during culture. Thus, it is demonstrated for the first time that early ovine embryos can secrete PAF *in vitro*, and that there is considerable variability in their capacity for PAF secretion.

Keywords: platelet-activating factor; embryo; *in vitro*; sheep

Introduction

Pre-implantation mice (O'Neill, 1985; Collier *et al.*, 1988; Ryan *et al.*, 1989) and human (Collier *et al.*, 1988, 1990) embryos secrete an ether phospholipid which is homologous with platelet-activating factor (PAF, 1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phosphocholine). The amount of PAF produced by human embryos was positively correlated with their pregnancy potential after transfer (O'Neill, 1987). Embryo-derived PAF appears to have an autocrine role, since (i) there is a direct, specific influence of exogenous PAF *in vitro* on the oxidative metabolism of energy substrates (glucose and lactate) by mouse embryos (Ryan *et al.*, 1989, 1990a), (ii) supplementation of culture media with PAF in which 2-cell mouse embryos were cultured for 72 h increased the number of cells in the expanded blastocysts, (iii) similar supplementation of media significantly increased the implantation rate and pregnancy potential of both mouse (Ryan *et al.*, 1990b) and human (O'Neill *et al.*, 1989) embryos and (iv) treatment of mouse embryos with PAF inhibitors prevented blastocyst outgrowth *in vitro* (Spinks *et al.*, 1990) and the ability of embryos to implant *in vivo* (Spinks & O'Neill, 1988; Spinks *et al.*, 1990). PAF may also exert effects on maternal physiology as evidenced by mild, but significant, thrombocytopenia in some species (mouse, O'Neill, 1985; cow, Hansel, 1988), the attenuation of oxytocin-induced prostaglandin (PG) F-2 α release from the ovine

uterus *in vivo* (Battye *et al.*, 1989), and the modification of prostaglandin secretion by human (Smith & Kelly, 1988) and bovine (Gross *et al.*, 1990) endometrium *in vitro*.

To date, the production of PAF by the ovine embryo has not been investigated. This experiment was conducted to determine whether PAF is secreted at various developmental stages by sheep embryos.

Materials and Methods

Superovulation. Thirty Merino ewes (6 years old) underwent superovulation and embryo recovery twice, with a 6-week interval between surgical embryo collection. The oestrous cycle was synchronized by treatment with intravaginal sponges (60 mg medroxyprogesterone; Repromap, Upjohn, Sydney, NSW, Australia) for 12–13 days. The ewes were superovulated by an intramuscular injection of 1200 iu pregnant mares' serum gonadotrophin (PMSG; Folligon: Intervet, Boxmeer, Netherlands) 2 days before sponge removal. Oestrus was detected using harnessed entire rams. Laparoscopic insemination was conducted 48–52 h after sponge removal. The ewes were inseminated with fresh semen, diluted 1:2 with Dulbecco's phosphate-buffered saline (PBS) with antibiotics.

Embryo recovery. Embryos were recovered by midventral laparotomy as described by Moore (1980) on Days 2, 4 and 6 after oestrus. Embryo collections were conducted under general anaesthesia (60 mg sodium pentobarbitone/ml: Cava Chemicals, NSW). Embryos were flushed from the uterus in PBS supplemented with 5% heat-inactivated fetal calf serum (FCS). Embryos were rinsed twice in PBS + 20% FCS, transferred to sterile 5 ml tubes (Flow Laboratories, Sydney, NSW, Australia) containing 1 ml of PBS + 20% FCS. Embryos were held in the incubator at 30°C until surgery was completed and then transferred to the culture laboratory.

Embryo culture. Since optimal culture conditions for ovine embryos are not well defined, embryos were cultured in either (i) Ham's F10 (Flow Laboratories) supplemented with 4 mg bovine serum albumin/ml (BSA, Pentex, Miles, Inc., Karakee, IL, USA; w/v), (Ham's + BSA), (ii) synthetic oviduct fluid medium (Walker *et al.*, 1988) supplemented with 20% (v/v) heat-inactivated human serum (HS) (SOFM + HS), (iii) Quinn's human tubal fluid medium (HTF; Quinn *et al.*, 1985) supplemented with 3 mg BSA/ml (w/v), (HTF + BSA) or (iv) HTF supplemented with 10% (v/v) acid-treated FCS (O'Neill *et al.*, 1989) (HTF + FCS). Acidified FCS was used as a protein source, as acidification destroys PAF:acetylhydrolase activity (Blank *et al.*, 1983), an enzyme that deactivates PAF. Where possible, embryos from each sheep were allocated across the four media treatments. Embryos were rinsed twice in the specified medium before transfer to the culture tube. Generally, two embryos were cultured in 1 ml of media in sterile plastic tubes (Flow Laboratories) in 5% CO₂ in air at 37°C. After 24 h, embryos were removed from the culture tube, assessed for development, transferred to a 10 µl drop of the same medium under oil and placed back into culture. Culture was continued in 5% CO₂ in air at 37°C for another 144, 96 or 48 h for embryos collected on Days 2, 4 and 6, respectively. The culture tubes were gassed with 5% CO₂ in air and stored at –20°C until extraction.

Extraction of embryo-derived PAF from culture media. Embryo-derived PAF was extracted from the 24 h culture media using the method described by Collier *et al.* (1988). All solvents used were high-performance liquid chromatography grade (BDH, Poole, UK). The culture medium (1 volume) was deproteinized by dropwise addition to 19 volumes of methanol. After 30 min of incubation at room temperature, the protein precipitate was removed by centrifugation (2500 g for 20 min at 4°C). The supernatant was passed through a Sep-pak C18 cartridge (Millipore, Milford, MA, USA) to remove nonpolar lipids, and to 1 volume of eluate 0.8 volumes of water and 0.95 volumes of chloroform were added to effect phase separation. The chloroform phase was reduced to 0.5–1.0 ml in a rotary evaporator and further concentrated to 200 µl under a stream of N₂. This concentrate was applied to a 0.25 mm silica gel thin-layer chromatography (TLC) plate (Merck, Darmstadt, Germany) and developed in chloroform:methanol:water (65:35:6, v/v). The migration of synthetic PAF (5 µg), run as a reference, was visualized with Phospray (Supelco, Bellefonte, PA, USA).

Embryo-derived PAF was removed from the TLC plate by scraping the area corresponding to the R_f of synthetic PAF ($R_f = 0.22 \pm 0.01$, $n = 32$) and eluted with 2 ml of chloroform:methanol:water (1:2:0.8, v/v). Following centrifugation (1500 g for 10 min), the solvent was removed to a clean tube. Chloroform (0.5 ml) and water (0.5 ml) were added to effect phase separation. The lower chloroform phase was washed once with 1 ml of methanol:water (10:9, v/v). The lower phase was removed to a clean tube and evaporated to dryness under N₂. The residue was resuspended in 250 µl in PBS free of Ca²⁺ and Mg²⁺ + 2.5 mg BSA/ml (w/v) (CSL, Melbourne, Vict, Australia), for quantification in the bioassay.

The efficiency of PAF extraction was followed by monitoring the recovery of [³H]PAF (NET-668 ~200 000 c.p.m., 56.7 Ci/mmol, NEN Du Pont) added to the culture media prior to extraction. The recovery of [³H]PAF was used to correct for losses of embryo-derived PAF during extraction by multiplying the observed activity in the PAF assays by the reciprocal of the [³H]PAF recovery.

For all extractions of embryo culture medium, a control blank was also extracted. The control blank was the same medium, but had not been exposed to embryos. The PAF concentrations measured in the control blanks were subtracted from the unknown sample PAF determinations after both had been corrected for [³H]PAF recovery.

PAF bioassay. The embryo-derived PAF was measured using a sensitive rabbit platelet bioassay (Collier *et al.*, 1988). The concentration of PAF was related directly to the degree of platelet aggregation induced in a sample of whole blood in the presence of citrate anticoagulant, an ADP scavenger (phospho(enol) pyruvate and pyruvate kinase), and the cyclo-oxygenase inhibitor acetylsalicylic acid (all Sigma Chemical Co., St Louis, MO, USA). The number of single nonaggregated platelets was counted on a Baker Series 810 platelet analyser (Baker Instruments, Pennsylvania, USA). Each positive sample was tested with the PAF-specific receptor antagonist SRI 63-441 (Sandoz, NJ, USA) (Handley *et al.*, 1986) to confirm that the platelet aggregation induced by embryo culture media extract was due to PAF. The detection limit of the bioassay was 5.6 nM (3 ng/ml) (Collier *et al.*, 1990).

A culture was defined as PAF positive if the PAF concentration was greater than the mean + 1.96 standard deviations of the specific control blanks.

Comparison of PAF bioassay and PAF-specific radioimmunoassay. There was insufficient sample left after bioassay to test each sample individually with the radioimmunoassay (RIA). Therefore, samples of embryo-derived PAF in PBS-BSA which showed similar concentration in the bioassay were pooled. Very low (below the sensitivity of the bioassay), low (11–37 nM), medium (43–112 nM) and high (141–366 nM) extracted samples of embryo-derived PAF were pooled and assayed using a radioimmunoassay for PAF (NEN Du Pont, NEK-062). The PAF-RIA was performed according to the manufacturer's instructions. The PAF-RIA kit uses PAF-specific antibodies raised in sheep. The labelled PAF-antibody complex was precipitated by a second antibody and the radioactivity in the precipitate was measured. The amount of labelled PAF in the precipitate is inversely proportional to the amount of PAF in the solution being assayed. The sheep anti-PAF antibody used in the RIA is highly specific for PAF (Smal *et al.*, 1990). The sensitivity of the RIA was 0.56 nM (0.30 ng/ml) and the interassay coefficient of variation was 5.3%.

As the PAF-RIA was not commercially available until towards the completion of this study, all samples were initially measured using the quantitative bioassay. Therefore, the concentrations of PAF reported in the Results are derived from the bioassay.

Enzymatic analysis. Analysis of embryo-derived PAF was performed using phospholipase A₂ (PLA₂) (EC 3.1.1.4, bee venom, Sigma, USA). The enzyme was dissolved in Tris-buffered saline, pH 8, at 180 iu/ml and dilutions in the same buffer were made from a stock solution. Samples of synthetic PAF or embryo-derived PAF (75 µl) with the same bioactivity, were incubated with PBS-BSA or various concentrations of PLA₂ in 15 µl of buffer for 15 min at 37°C. Samples (50 µl) were added to 50 µl of rabbit blood in the standard bioassay.

Statistical analysis. Embryo development and the proportion of PAF-positive cultures were analysed by logistic regression. The concentration of PAF produced by cultures was analysed by one-way analysis of variance with respect to media treatment and developmental stage of the embryo. The concentration of PAF in control culture medium was analysed by one-way analysis of variance. Differences between means were compared using Duncan's multiple range test (Steel & Torrie, 1981). Data are presented as mean ± s.e.m.

Results

Embryo development

When embryos were recovered on Day 6 (morula/early blastocyst), the proportion developing through to expanded/hatching blastocyst by Day 8 (after 48 h in culture) was significantly higher ($P < 0.001$) than when embryos were recovered on Day 2 (2–8 cell) and cultured for 144 h or Day 4 (8–16 cell) and cultured for 96 h (Table 1). SOFM + HS was the only medium in which 2–8 cell embryos (recovered on Day 2) developed to the expanded/hatching blastocyst stage by Day 8. Over the three developmental stages examined, more embryos cultured in Ham's + BSA or SOFM + HS tended to develop to expanded/hatching blastocyst ($P = 0.07$) than embryos cultured in HTF + BSA or HTF + FCS.

PAF concentration in culture media

Low concentrations of PAF were measured consistently in culture media not exposed to embryos (control blanks), and were higher in SOFM + HS than in the three other media ($P < 0.01$, Table 2). The PAF activity was inhibited when tested in the presence of the PAF-receptor antagonist, SRI 63-441, suggesting that it was PAF, but not PAF derived from embryos.

There was wide variation in the concentration of PAF measured in culture medium in which sheep embryos had been grown for 24 h (0–411 nM, i.e. 0–205 pmol/embryo/24 h). Overall, 21/95 (22.1%) cultures were PAF-positive (Table 3). There was no significant effect of culture medium used, or the developmental stage of the embryo, on the proportion of cultures that were positive for

Table 1. Proportion of ovine embryos which developed to expanded blastocysts or further by Day 8; percentages in parentheses

Time in culture (h)	Developmental stage of embryos at collection			
	Day 2 144	Day 4 96	Day 6 48	Total
Media*				
Ham's + BSA	0/4	1/4 (25.0)	25/31 (80.6)	26/39 (66.7)
SOFM + HS	5/11 (45.5)	3/6 (50.0)	25/31 (80.6)	33/48 (68.8)
HTF + BSA	0/4	0/5	22/31 (71.0)	22/40 (55.0)
HTF + FCS	0/10	1/6 (16.7)	26/33 (78.8)	27/49 (55.1)
	5/29 (17.2) ^b	5/21 (23.8) ^b	98/126 (77.8) ^a	108/176 (61.4)

*BSA, bovine serum albumin; SOFM, synthetic oviduct fluid medium; HS, human serum; HTF, human tubal fluid medium; FCS, fetal calf serum.
Values with different superscripts are significantly different at ($P < 0.05$).

Table 2. Concentration of platelet-activating factor (PAF, nmol/l) in culture medium not exposed to ovine embryos, measured using the quantitative bioassay after extraction of culture media and partial purification by thin-layer chromatography

Media*	<i>n</i>	PAF (nmol/l) mean $\pm$ s.e.m.
Ham's + BSA	16	18.28 $\pm$ 2.09 ^b
SOFM + HS	13	32.42 $\pm$ 3.86 ^a
HTF + BSA	20	19.03 $\pm$ 2.32 ^b
HTF + FCS	20	19.68 $\pm$ 1.71 ^b

*See Table 1 for key to abbreviations.
Values with different superscripts are significantly different at $P < 0.05$.

PAF. The media $\times$ day interaction was significant ($P = 0.03$) and may be explained by the observation that PAF-positive cultures were detected in Ham's + BSA and SOFM + HS across the 3 embryo collection days, but not in HTF + BSA Day 2 or Day 4 cultures, and HTF + FCS Day 2 and Day 6 culture media.

PAF was produced by embryos in the four media tested, the amount of PAF secreted by embryos not differing between media ($P > 0.05$), although there was a trend towards higher amounts being produced in SOFM + HS when PAF-positive cultures only were considered (Table 4). The amount of PAF produced by embryos was not affected by the stage of development (Table 4). Therefore, when the data were pooled, the average amount of PAF produced by embryos in the PAF-positive cultures was 60.9 ± 9.8 pmol/embryo/24 h.

Overall, 19/23 (82.6%) of the embryos in Day 6 PAF-positive cultures developed during culture, so that in all of these cultures at least one of the two embryos had cleaved. Of the Day 2 and Day 4 PAF-positive cultures, 14/18 (77.8%) embryos went through one mitotic division in the first 24 h in culture. There was one Day 2 PAF-positive culture in which the embryos did not develop. In the 'PAF-negative' cultures, 67/80 (83.8%) Day 6 embryos developed, and 43/60 (71.7%) Day 2 and Day 4 embryos went through at least one mitotic division in the first 24 h period.

Table 3. Proportion of ovine embryo cultures where the concentration of platelet-activating factor was greater than the mean $\pm$ 1.96 s.d. of that measured in culture media not exposed to embryos; percentages in parentheses

Media*	Developmental stage of embryos at collection			
	Day 2	Day 4	Day 6	Total
Ham's + BSA	1/5 (20)	2/6 (33)	5/16 (31)	8/27 (30)
SOFM $\pm$ HS	2/4 (50)	2/7 (29)	2/10 (20)	6/21 (29)
HTF + BSA	0/5	0/5	5/14 (36)	5/24 (21)
HTF + FCS	0/5	2/5 (40)	0/13	2/23 (9)
	3/19 (16)	6/23 (26)	12/53 (23)	21/95 (22)

*See Table 1 for key to abbreviations.

Table 4. Concentration of platelet-activating factor (PAF, nmol/l) in PAF-positive cultures, measured using the quantitative bioassay after extraction of culture media and partial purification by thin-layer chromatography

Media*	Developmental stage of ovine embryos at collection			
	Day 2	Day 4	Day 6	Mean
Ham's + BSA	37.0	42.0	164.2 $\pm$ 27.9	117.7 $\pm$ 28.2
SOFM + HS	155.4	265.5	131.0	184.0 $\pm$ 48.7
HTF + BSA	—	—	70.6 $\pm$ 9.9	70.6 $\pm$ 9.9
HTF + FCS	—	73.6	—	73.6
	115.9 $\pm$ 45.9	127.0 $\pm$ 45.5	119.7 $\pm$ 18.4	121.2 $\pm$ 19.5

*See Table 1 for key to abbreviations.

Comparison of bioassay and radioimmunoassay

Pooled samples of embryo-derived PAF (previously bioassayed) were measured by the PAF-RIA. There was agreement between the two assay systems in ranking of concentrations of the samples, i.e. low, medium and high, the RIA having greater sensitivity than the bioassay and giving 1.5–3.5 times higher values. Because of the limited amount of material available for this retrospective analysis, a direct comparison of the bioassay and the RIA was not possible.

Structural study

The acetyl group from the C₂ position is essential for biological activity of PAF, and is removed by PLA₂ (Benveniste *et al.*, 1977). Pretreatment of synthetic and embryo-derived PAF (41 nm) with 30 iu/ml PLA₂ (measured in duplicate), reduced the platelet-aggregating activity of both to a similar extent (Fig. 1). Only single measurements were made at PLA₂ concentrations of 7.5 and 15 iu/ml, because of a lack of sample, but, at each of these concentrations, the bioactivities of synthetic PAF and embryo-derived PAF were similar.

Discussion

This is the first report showing that some early pre-implantation sheep embryos secrete the ether phospholipid, PAF, in culture. Biologically active, sheep embryo-derived PAF co-migrated with

Fig. 1. Deactivation of platelet-activating factor (PAF) by phospholipase A₂ (PLA₂). Samples of phosphate-buffered saline and bovine serum albumin (▲), synthetic PAF (●) or ovine embryo-derived PAF (■) of equivalent activity to 41 nmol/l were pre-incubated for 15 min at 37°C with 7.5, 15 or 30 iu PLA₂/ml prior to bioassay. A 50 µl sample was then added to 50 µl of rabbit blood and the number of single platelets remaining after 15 min was expressed as a percentage of those present initially. Data points at 0 and 30 iu/ml are the mean of two determinations, the remainder are single determinations because of a lack of embryo-derived PAF.

both synthetic PAF and [³H]PAF on silica TLC. While this study was being conducted, a radioimmunoassay for PAF (NEN Du Pont, NEK-062) became available for the quantitative detection of PAF. The RIA was more sensitive than the bioassay (Ammit & O'Neill, 1991) while both methods for measuring PAF ranked samples in the same way. Substantial support exists for the specificity of the antisera for 1-*o*-alkyl-2-acetyl-sn-glycerol-3-phosphocholine (Smal *et al.*, 1989, 1990; Cooney *et al.*, 1990). The detection of putative embryo-derived PAF by the radioimmunoassay offers further support for structural homology between ovine embryo-derived PAF and 1-*o*-alkyl-2-acetyl-sn-glycerol-3-phosphocholine. The deactivation of sheep embryo-derived PAF by PLA₂ confirms that the PAF produced by sheep embryos is homologous with that produced by mouse and human embryos (Collier *et al.*, 1988).

PAF was produced by sheep embryos at the three developmental stages examined (2–8 cell, 8–16 cell, morula/early blastocyst) (Tables 3 and 4). PAF production by mouse embryos at these stages has been reported (Collier *et al.*, 1988; Ryan, 1989), and by human embryos at the 1–4 cell stage (Collier *et al.*, 1988, 1990). In this study, no difference was observed between the three developmental stages on the proportion of cultures which were PAF-positive. However, the numbers of positive cultures on Days 2 and 4 were low.

It has been suggested that PAF production by embryos may serve as a marker for embryo viability and pregnancy potential (O'Neill, 1987). Collier *et al.* (1990) showed that, while there was a significant correlation between PAF production by human embryos and pregnancy potential, it was not an absolute marker since 13% of patients who became pregnant received embryos which did not produce PAF *in vitro*. In this study it has been shown that, for the sheep embryo, there was no correlation between the secretion of PAF and their development *in vitro*. Since embryo transfers were not performed, it is not possible to determine if it was a marker of pregnancy potential, as in the human.

Collier *et al.* (1988) reported the measurement of nonspecific PAF activity in protein-free media, media supplemented with 3 mg/ml BSA and HTF plus 10% maternal human serum (Collier *et al.*, 1990). PAF activity was measured consistently in control media blanks in this study, but was

highest in SOFM + HS. The apparent low 'PAF-like' activity was observed in both the bioassay and RIA. The extraction procedure and/or addition of [^3H]PAF to culture media may have contributed to the low levels measured. The presence of PAF in human and rat serum has been reported (Caramelo *et al.*, 1984); this may explain the significantly higher concentrations detected in SOFM + HS. However, similar concentrations of PAF were not measured in HTF supplemented with acid-treated FCS. The reason for this is unclear and may be a result of acidification, or of a species difference.

The detection of embryo-derived PAF in media containing serum suggests that embryo-derived PAF is not susceptible to serum acetylhydrolase and may be protected in some way, perhaps by a binding protein or carrier molecule (Collier *et al.*, 1990).

It has been shown that early stage sheep embryos produce the ether phospholipid PAF. The mean amount of PAF produced by sheep embryos (60.9 ± 9.8 pmol/embryo/24 h) was comparable to that reported for human (range 9–717 pmol/embryo/24 h) and mouse (1.5–31.0 pmol/embryo/24 h) embryos (Collier *et al.*, 1988). The four culture media used in this study supported the production of PAF by some sheep embryos to a similar extent. It was shown previously that culture *in vitro* compromised the capacity of mouse embryos to secrete PAF (Ryan *et al.*, 1989), but that the addition of exogenous PAF could apparently compensate for this, resulting in enhanced metabolism and viability for both human (O'Neill *et al.*, 1989) and mouse embryos (Ryan *et al.*, 1990a, b). This evidence, with the current demonstration of variable ovine embryo-derived PAF secretion, suggests that PAF might also be beneficial as a supplement to ovine embryo culture media.

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References

- Ammit, A.J. & O'Neill, C. (1991) Comparison of a radioimmunoassay and bioassay for embryo-derived platelet activating factor. *Hum. Reprod.* **6**, 872–878.
- Battye, K.M., Evans, G. & O'Neill, C. (1989) A role for platelet activating factor in maintenance of the ovine corpus luteum. *Proc. Aust. Soc. Reprod. Biol.* **21**, abst 32.
- Benveniste, J., LeCouedic, J.P., Polonsky, J. & Tence, M. (1977) Structural analysis of purified platelet activating factor by lipases. *Nature (Lond.)* **269**, 170.
- Blank, M.L., Hall, M.N., Cress, E.A. & Snyder, F. (1983) Inactivation of 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine by a plasma acetylhydrolase: higher activities in hypertensive rats. *Biochem. biophys. Res. Commun.* **113**, 666–671.
- Caramelo, C., Fernandez-Gallardo, S., Marin-Cao, D., Inarrea, P., Santos, J.C., Lopez-Novoa, J.M. & Sanchez Crespo, M. (1984) Presence of platelet activating factor in blood from human and experimental animals. Its absence in anephric individuals. *Biochem. biophys. Res. Commun.* **120**, 789–796.
- Cooney, S.J., Smal, M.A., Harle, D.G. & Baldo, B. (1990) Combining site specificities of rabbit antibodies to platelet-activating factor (PAF). *Molec. Immunol.* **27**, 405–412.
- Collier, M., O'Neill, C., Ammit, A.J. & Saunders, D.M. (1988) Biochemical and pharmacological characterization of human embryo-derived platelet activating factor. *Hum. Reprod.* **3**, 993–998.
- Collier, M., O'Neill, C., Ammit, A.J. & Saunders, D.M. (1990) Measurement of human embryo-derived platelet-activating factor (PAF) using a quantitative bioassay of platelet aggregation. *Hum. Reprod.* **5**, 323–328.
- Gross, T.S., Thatcher, W.W., O'Neill, C. & Danet-Desnoyers, G. (1990) Platelet-activating factor alters the dynamics of prostaglandin and protein synthesis by endometrial explants from pregnant and cyclic cows at Day 17 following oestrus. *Theriogenology* **34**, 205–218.
- Handley, D.A., Tomesch, J.C. & Saunders, R.N. (1986) Inhibition of PAF-induced systemic responses in the rat, guinea pig, dog and primate by the receptor antagonist SRI 63-441. *Thromb. Haemostasis* **56**, 40–44.
- Hansel, W. (1988) Establishment of pregnancy: regulation of the corpus luteum. *Proc. 11th Int. Congr. Anim. Reprod. Artificial Insem.* pp. 61–68. Dublin.
- Moore, N.W. (1980) Embryo transfer: procedures and results obtainable in sheep and goats. In *Current Therapy in Theriogenology*, pp. 89–94. Ed. D. A. Morrow. W. B. Saunders Co., Philadelphia.
- O'Neill, C. (1985) Partial characterization of the embryo-derived platelet-activating factor in mice. *J. Reprod. Fert.* **73**, 567–577.

- O'Neill, C. (1987) Embryo-derived platelet activating factor: a preimplantation embryo mediator of maternal recognition of pregnancy. *Domestic Animal Endocr.* **4**, 69–85.
- O'Neill, C., Ryan, J.P., Collier, M., Saunders, D.M., Ammit, A.J. & Pike, I.L. (1989) Supplementation of *in-vitro* fertilisation culture medium with platelet activating factor. *Lancet* **ii**, 769–772.
- Quinn, P., Warner, G.M., Kerin, J.R. & Kirby, C. (1985) Culture factors affecting the rate of *in vitro* fertilization and embryo transfer. *Ann. NY Acad. Sci.* **442**, 195–203.
- Ryan, J.P., Spinks, N.R., O'Neill, C., Ammit, A.J. & Wales, R.G. (1989) Platelet activating factor (PAF) production by mouse embryos *in vitro* and its effect on embryonic metabolism. *J. Cell. Biochem.* **40**, 387–395.
- Ryan, J.P., O'Neill, C. & Wales, R.G. (1990a) Oxidative metabolism of energy substrates by preimplantation mouse embryos in the presence of platelet-activating factor. *J. Reprod. Fert.* **89**, 301–307.
- Ryan, J.P., Spinks, N.R., O'Neill, C. & Wales, R.G. (1990b) Implantation potential and fetal viability of mouse embryos cultured in media supplemented with platelet-activating factor. *J. Reprod. Fert.* **89**, 309–315.
- Smal, M.A., Baldo, B.A. & Redmond, J.W. (1989) Production of antibodies to platelet activating factor. *Molec. Immunol.* **26**, 711–719.
- Smal, M.A., Baldo, B.A. & Harle, D.G. (1990) The specificity of the binding of platelet activating factor (PAF) to anti-PAF antibodies. *J. molec. Recognition* **3**, 168–172.
- Smith, S.K. & Kelly, R.W. (1988) Effect of platelet-activating factor on the release of PGF-2 α and PGE-2 by separated cells of human endometrium. *J. Reprod. Fert.* **82**, 271–276.
- Spinks, N.R. & O'Neill, C. (1988) Antagonists of embryo-derived platelet-activating factor prevent implantation of mouse embryos. *J. Reprod. Fert.* **84**, 89–98.
- Spinks, N.R., Ryan, J.P. & O'Neill, C. (1990) Antagonists of embryo-derived platelet-activating factor act by inhibiting the ability of the mouse embryo to implant. *J. Reprod. Fert.* **88**, 241–248.
- Steel, G.D. & Torrie, J.H. (1981) *Principles and Procedures of Statistics. A Biometrical Approach*. McGraw-Hill International Book Co., New York.
- Walker, S.K., Seamark, R.F., Quinn, P., Warnes, G.M., Ashman, R.J., Smith, D.H. & Ancell, P. (1988) Culture of pronuclear embryos of sheep in a simple medium. *Proc. 11th Int. Congr. Anim. Reprod. Artificial Insem.*, abstr 483, Dublin.

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