

Sphingosine 1-Phosphate Protects Bovine Oocytes from Heat Shock During Maturation¹

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ABSTRACT

Sphingosine 1-phosphate (S1P) is a sphingolipid metabolite that can block apoptosis by counteracting the proapoptotic effects of ceramide. Experiments were performed to evaluate whether S1P blocks the disruption in oocyte developmental competence caused by heat shock. Cumulus-oocyte complexes (COCs) were placed in maturation medium and cultured at 38.5 or 41°C for the first 12 h of maturation. Incubation during the last 10 h of maturation, fertilization, and embryonic development were performed at 38.5°C. Heat shock during the first 12 h of maturation reduced cleavage rate, the number of oocytes developing to the blastocyst stage, and the percentage of cleaved embryo that subsequently developed to blastocysts. Addition of 50 nM S1P to maturation medium had no effect on oocytes matured at 38.5°C but blocked effects of thermal stress on cleavage and subsequent development. The blastocysts formed at Day 8 did not differ between S1P and control groups in caspase activity, total cell number, or percentage of cells that were apoptotic. Blocking endogenous generation of S1P by addition of 50 nM *N*₁*N*-dimethylsphingosine, a sphingosine kinase inhibitor, reduced or tended to reduce cleavage rate and blastocyst development regardless of whether maturation of COCs was at 38.5 or 41°C. Results demonstrate that S1P protects oocytes from a physiologically relevant heat shock and affects oocyte maturation even in the absence of heat shock. The S1P-treated oocytes that survived heat shock and became blastocysts had a normal developmental potential as determined by caspase activity, total cell number, and percentage of apoptotic cells. Thus, modulation of developmental competence of oocytes using S1P may be a useful approach for enhancing fertility in situations where developmental competence of oocytes is compromised.

early development, embryo, environment, gamete biology, heat shock, oocyte, oocyte development, sphingosine 1-phosphate

INTRODUCTION

Disruption in oocyte competence to undergo maturation, fertilization, and subsequent development is an important factor in infertility [1, 2]. This concept is well developed in the dairy cow [3, 4]. Among the factors that affect oocyte developmental competence in that species are age [5], parity, genetic ability for milk yield, body condition [6], level of protein feeding [7], and environmental pollutants [8].

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There is abundant evidence that bovine oocytes are susceptible to thermal stress at various stages of follicular development. Developmental competence of oocytes is reduced in the summer [9, 10] and remains compromised for a period of 2–3 estrous cycles after hot weather [11], suggesting that oocytes can be damaged by heat stress during early stages of follicular growth. The preovulatory oocyte and maturing oocyte is also susceptible to thermal stress, as shown in vivo [12] and in vitro [13–15]. Recently, it has been demonstrated that induction of apoptosis of the oocyte or investing cumulus is one of the mechanisms underlying disruption of oocyte competence caused by heat shock in cattle [15]. In that study, exposure of oocytes to elevated temperature during the first 12 h of maturation increased the percentage of TUNEL-positive nuclei in parallel with reduced cleavage rate and embryonic development. Maturation in the presence of z-DEVD-fmk, an inhibitor of group II caspases, reduced the deleterious effect of heat shock on oocytes.

In many cells, heat shock induces apoptosis via activation of the sphingomyelin pathway involving hydrolysis of membrane sphingomyelin by sphingomyelinases to generate the second messenger, ceramide [16–18]. Ceramide can also be metabolized by ceramidase to sphingosine and then to sphingosine 1-phosphate (S1P) through phosphorylation by sphingosine kinase [19]. While ceramide is associated with growth arrest and apoptosis [20, 21], S1P is associated with proliferation and cell survival [19]. The balance between amounts of ceramide and S1P has been proposed to be an important determinant for sphingolipid regulation of proliferation and apoptosis [22, 23]. Pretreatment with S1P preserved ovarian follicular dynamics and oocyte competence in mice exposed to radiation [24]. Moreover, S1P was reported to preserve fertility in irradiated female mice without propagating genomic damage [25]. Whether S1P provides a similar protection to oocytes exposed to physiologically relevant heat shock is not known.

Accordingly, objectives of the present study were to determine whether S1P plays a role in survival of bovine oocytes exposed to thermal stress. The study examined whether 1) external S1P protects bovine oocytes from heat shock, 2) blocking the intracellular generation of S1P impairs oocyte competence, and 3) the quality of embryos developed from oocytes that survived heat shock is normal or compromised.

MATERIALS AND METHODS

Materials

The *N*₁*N*-dimethylsphingosine (DMS), sphingosine 1-phosphate and Hoechst 33342 dye were from Sigma (St. Louis, MO). The PhiPhiLux-G₁D₂ assay was obtained from OncoImmunit (College Park, MD). The in situ cell-death detection kit (fluorescein) was obtained from Roche Diagnostics (Indianapolis, IN). Polyvinyl pyrrolidone (PVP) was purchased from Eastman Kodak (Rochester, NY). The Prolong Antifade Kit was ob-

tained from Molecular Probes (Eugene, OR) and RQ1 RNA-free DNase was from Promega (Madison, WI). HEPES-tyrode lactate (HEPES-TL), in vitro fertilization-Tyrode lactate (IVF-TL), and sperm-TL (SP-TL), obtained from Cell and Molecular Technologies (Lavallete, NJ) were used to prepare IVF-Tyrode albumin lactate pyruvate (TALP) and SP-TALP [26]. Essentially fatty acid-free bovine serum albumin and bovine serum albumin (BSA) were purchased from Sigma. Oocyte collection medium (OCM) was Tissue Culture Medium 199 (TCM-199) with Hanks salts without phenol red (Atlanta Biologicals, Norcross, GA) supplemented with 2% (v/v) bovine steer serum (Pel-Freez Roger, AR) containing 0.04 U/ml heparin, 100 U/ml penicillin-G, 0.1 mg/ml streptomycin, and 1 mM glutamine. Oocyte maturation medium (OMM) was TCM-199 with Earle salts (Cell and Molecular Technologies, Lavallete, NJ) supplemented with 10% (v/v) steer serum, 22 µg/ml sodium pyruvate, 20 µg/ml FSH (Follotropin-V; Vetrepharm Canada, London, ON), 2 µg/ml estradiol 17-β, 50 µg/ml gentamicin, and 1 mM glutamine. Embryo culture medium was potassium simplex optimized medium (KSOM) from Cell and Molecular Technologies modified for bovine embryos (KSOM-BE2) as described elsewhere [27]. Frozen semen from various bulls was donated by Southeastern Breeders Service (Wellborn, FL). Percoll was from Amersham Pharmacia Biotech (Uppsala, Sweden).

In Vitro Production of Embryos

Embryos were produced using procedures described earlier [27, 28] with oocytes harvested from ovaries from a local slaughterhouse. After slicing the ovary, cumulus-oocyte complexes (COCs) with several layers of compact cumulus cells and an evenly granulated cytoplasm were collected by swirling the ovary into a beaker containing OCM. COCs were washed and matured in groups of 10 in 50-µl drops of OMM overlaid with mineral oil for 22 h at 38.5°C in atmosphere of 5% (v/v) CO₂ in humidified air. Groups of 30 COCs were then transferred to four-well plates containing 600 µl IVF-TALP per well and fertilized with 25 µl (~1 × 10⁶) Percoll-purified spermatozoa supplemented with 25 µl PHE [0.5 mM penicillamine, 0.25 mM hypotaurine, and 25 µM epinephrine in 0.9% (w/v) NaCl]. After 18 h, putative zygotes were removed from fertilization wells, denuded of cumulus cells by vortexing COCs with 1000 U/ml hyaluronidase in 1 ml HEPES-TALP for 5 min, washed two to three times in HEPES-TALP and placed in groups of 25–30 in 50-µl drops of KSOM-BE2 overlaid with mineral oil at 38.5°C and 5% (v/v) CO₂ in humidified air. The number of cleaved oocytes and blastocysts were examined on Days 3 and 8 postinsemination, respectively. Matured oocytes were harvested after 22 h of maturation and developing blastocysts on Day 8 after fertilization.

Caspase Activity

Group II caspase activity of blastocysts was measured using the fluoroprobe PhiPhiLux-G₁D₂ that incorporates the group II caspase-recognition sequence DEVD into a bifluorophore-derived peptide that mimics the structural loop conformation present in native protease cleavage sites. Blastocysts were washed three times in 50-µl drops of HEPES-TALP (prewarmed at 38.5°C) and incubated in 25-µl microdrops of HEPES-TALP containing 5 µM PhiPhiLux-G₁D₂ at 38.5°C for 40 min in the dark. The negative control was incubated in HEPES-TALP only. Following incubation, blastocysts were washed twice in 50-µl drops of HEPES-TALP and placed on two-well slides (catalog number 12-560B; Fisher) containing 100 µl of prewarmed HEPES-TALP. Caspase activity was determined using a Zeiss Axioplan microscope and fluorescein isothiocyanate filters. Images were acquired using AxioCam MRm digital camera and subjected to analysis using the AxioVision software program to determine intensity of fluorescence. Blastocysts were classified as having low (<15 intensity units/area), medium (15–25 intensity units/area), or high caspase activity (>25 intensity units/area).

Determination of DNA Fragmentation by TUNEL Labeling

Blastocysts were removed from culture medium, washed three times in 100-µl drops of phosphate buffered saline (10 mM KPO₄, pH 7.4, containing 0.15 M NaCl; PBS) supplemented with 1 mg/ml polyvinyl pyrrolidone (PBS+PVP). Then samples were fixed in 4% (w/v) paraformaldehyde in PBS for 1 h at room temperature and stored in PBS-PVP at 4°C for up to 2 wk before assay. The TUNEL assay was initiated by permeabilizing blastocysts in 100-µl drops of 0.1% (v/v) Triton X-100 containing 0.1% (w/v) sodium citrate for 30 min at room temperature. Blastocysts were then washed three times in PBS+PVP and incubated in 50-µl drops of TUNEL reaction mixture (containing fluorescein isothio-

cyane-conjugated dUTP and terminal deoxynucleotidyl transferase) for 1 h at 37°C in the dark. Blastocysts were washed in PBS-PVP, transferred to 50-µl drops of 1 µg/ml Hoechst 33342 in PBS-PVP for 30 min at room temperature, washed three times in PBS-PVP, placed on 10% (w/v) polylysine-coated slides, and prepared with cover slips using 5 µl mounting medium containing Antifade (Molecular Probes). Each TUNEL procedure contained blastocysts treated with RQ1 RNase-free DNase (50 U/ml) at 37°C for 1 h as a positive control and blastocysts incubated in the absence of the terminal deoxynucleotidyl transferase as a negative control. TUNEL labeling was observed using a Zeiss Axioplan fluorescence microscope (Carl Zeiss, Inc., Göttingen, Germany). Images were acquired using AxioVision software and an AxioCam MRm digital camera (Zeiss). Each blastocyst was analyzed for total number of nuclei and number of TUNEL-labeled nuclei.

Experiments

Heat shock during maturation and oocyte competence. The experiment was performed to examine the effect of heat shock on developmental competence of oocytes by comparing heat shock at temperatures similar to those experienced by heat-stressed cows (41°C) with a severe heat shock (43°C). COCs were matured at 38.5°C for 22 h, 41°C for 12 h followed by 38.5°C for 10 h, or 43°C for 12 h followed by 10 h of maturation at 38.5°C. Oocytes were then fertilized and cultured at 38.5°C and 5% (v/v) CO₂ in humidified air as described above. The gaseous carbon dioxide concentration was adjusted to maintain a constant pH at all temperatures. Cleavage rate and percentage of oocytes and cleaved embryos developing to blastocysts were recorded on Days 3 and 8 after insemination, respectively. The experiment was replicated four times using 219–252 oocytes/treatment.

Inhibition of heat shock effects on oocyte competence by S1P. The experiment was designed with a 2 × 2 factorial arrangement of treatments. Maturation was performed at either 38.5°C for 22 h or at 41°C for 12 h followed by 38.5°C for 10 h and in the presence of either 50 nM S1P reconstituted in 0.1% (v/v) methanol in maturation medium or 0.1% (v/v) methanol alone in medium (vehicle). Fertilization and culture were at 38.5°C as described above. Cleavage rate and the number of blastocysts were recorded on Days 3 and 8 after insemination, respectively. The experiment was replicated nine times using 368–392 oocytes per treatment. Blastocysts were recovered on Day 8 (55–90 per treatment) and subjected to either TUNEL analysis (n = 78) or determination of caspase activity (n = 108).

Effect of N₁N-dimethylsphingosine on oocyte competence. The experiment had a 2 × 2 factorial design, where maturation was performed at either 38.5°C for 22 h or at 41°C for 12 h followed by 10 h of maturation at 38.5°C and in the presence of either 0.1% (v/v) ethanol (vehicle) in maturation medium or in maturation medium containing 50 nM N₁N-dimethylsphingosine, a sphingosine kinase inhibitor [29] reconstituted in 0.1% (v/v) ethanol. Fertilization and culture were at 38.5°C, as described above. Cleavage rate and the number of blastocysts were recorded on Days 3 and 8 after insemination, respectively. The experiment was replicated five times using 185–235 oocytes/treatment.

Statistical Analysis

Data were analyzed by least-squares analysis of variance using the General Linear Models procedure of SAS (SAS for windows, Version 8, 1999–2001, Cary, NC). Dependent variables were percentage of oocytes that cleaved; percentage of oocytes that became blastocysts; percentage of cleaved oocytes becoming blastocysts; total cell number of blastocysts; percentage of blastomeres that were TUNEL positive; and percent of blastocysts expressing low, medium, and high caspase activity. Percentage data were transformed using the arcsine transformation before analysis. Independent variables varied according to the experimental design—the mathematical model included main effects and all interactions. Replicate was considered random and other main effects were considered fixed. A means separation procedure of SAS called pdiff was performed when appropriate to determine differences between levels of individual treatments. Probability values for main effects and interactions are reported in the text while individual means that differ based on pdiff are represented in the figures through the use of superscripts. For all analyses, probability values of *P* < 0.05 or less are considered significant and *P* values between 0.05 and 0.10 are also reported as trends that may be real and are worthy of note.

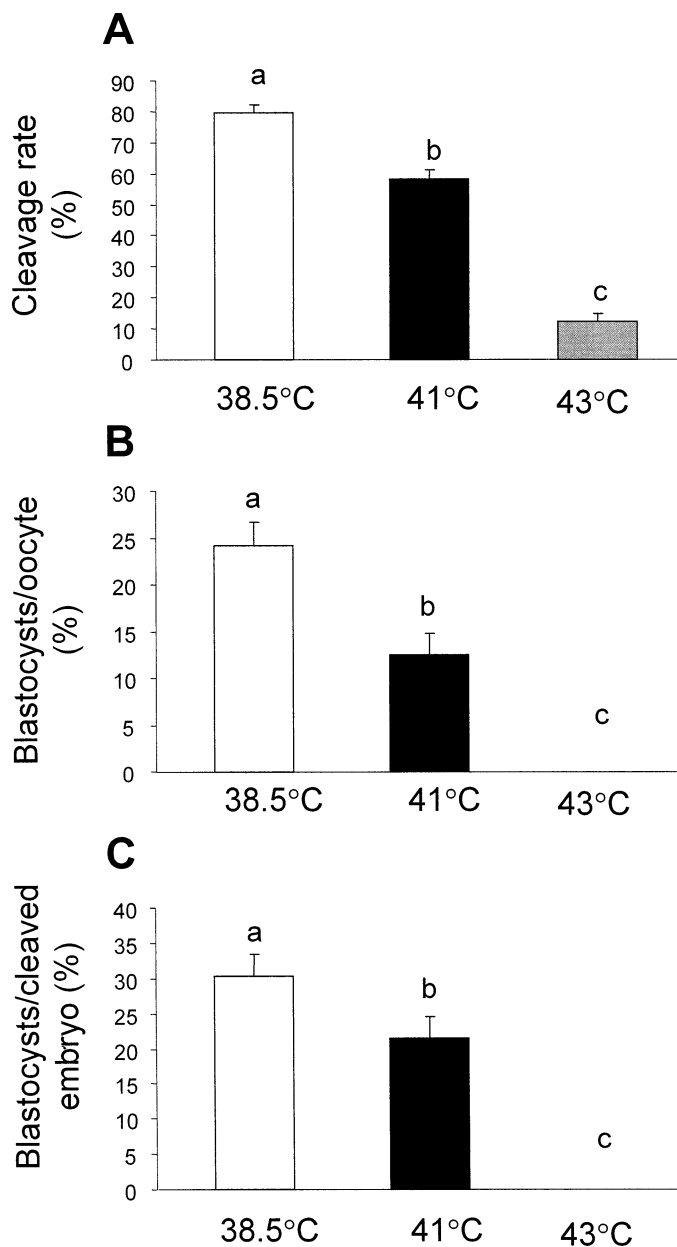


FIG. 1. Cleavage rate (A) and the percentage of oocytes (B) and cleaved embryos (C) that became blastocysts at Day 8 after insemination as affected by incubation temperature during the first 12 h of maturation. Data represent least-squares means \pm SEM. Superscripts above each bar that are different represent means that differ at $P < 0.05$ or less.

RESULTS

Heat Shock During the First 12 h of Maturation Disrupts Oocyte Competence

Heat shock at both 41 and 43°C reduced ($P < 0.05$) the percentage of oocytes that cleaved by Day 3 (Fig. 1A). Heat shock at both 41 and 43°C also reduced ($P < 0.01$) the proportion of oocytes that became blastocysts at Day 8 after insemination (Fig. 1B) and the percent of cleaved embryos that developed to blastocysts ($P < 0.05$; Figure 1C).

S1P Blocks Disruption in Oocyte Competence Caused by Heat Shock

There was a main effect of heat shock ($P < 0.01$) and S1P ($P < 0.01$) and a temperature \times S1P interaction ($P <$

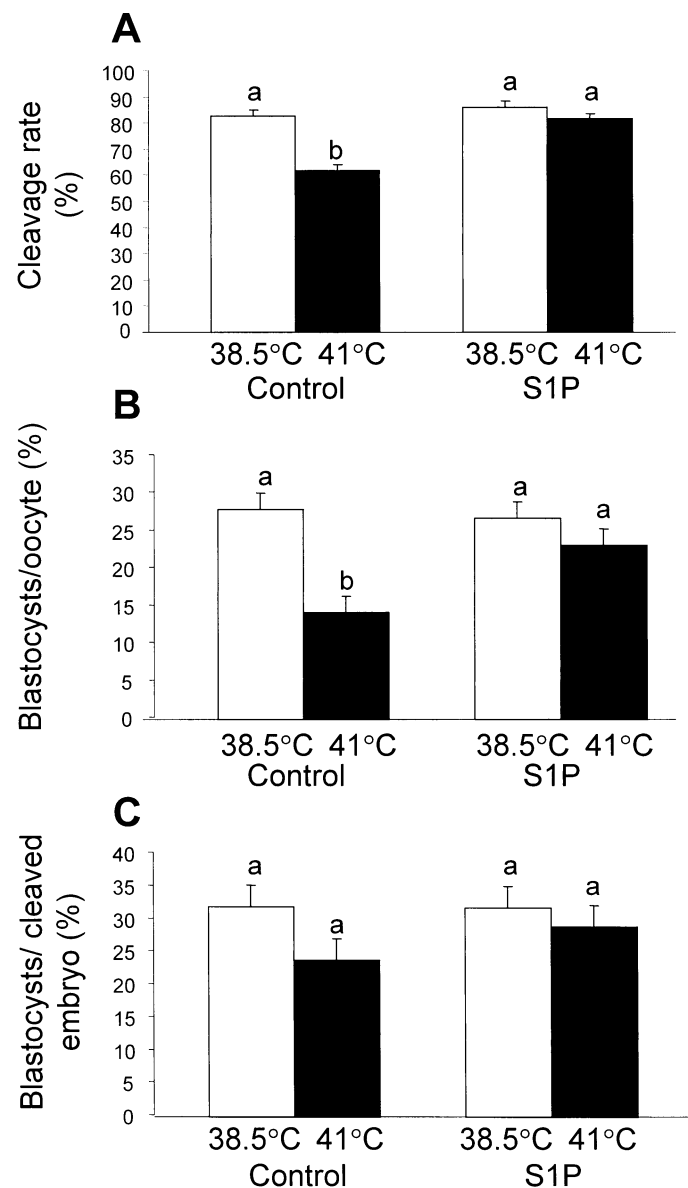


FIG. 2. Sphingosine 1-phosphate (S1P) inhibits deleterious effect of heat shock during oocyte maturation. Shown are cleavage rate (A) and the percentage of oocytes (B) and cleaved embryos (C) that became blastocysts at Day 8 after insemination. Open bars represent oocytes cultured at 38.5°C while solid bars represent oocytes cultured at 41°C. Data represent least-squares means \pm SEM. Superscripts above each bar that are different represent means that differ at $P < 0.05$ or less.

0.01) for cleavage rate. The interaction reflects the result that S1P had no effect on cleavage rate for oocytes matured at 38.5°C but blocked effects of thermal stress on cleavage rate (Fig. 2A). A similar effect of temperature ($P < 0.01$) and temperature \times S1P interaction ($P < 0.04$) was observed for the percentage of oocytes that became blastocysts. Again, the interaction occurred because S1P reduced the effect of heat shock while having no effect on development for oocytes matured at 38.5°C (Fig. 2B). The percentage of cleaved oocytes that became blastocysts tended to be reduced when oocytes were matured at 41°C ($P < 0.08$) and, while not significant, this effect of heat shock tended to be lower for S1P-treated oocytes than for control oocytes (Fig. 2C).

To assess whether blastocysts that formed from heat-shocked oocytes were of normal developmental potential,

TABLE 1. Effect of maturation temperature and sphingosine 1-phosphate (S1P) treatment during the first 12 h of maturation on distribution of blastocysts into low (<15 intensity units/area), medium (15–25 intensity units/area), and high (>25 intensity units/area) categories based on group II caspase activity.^a

Temperature (°C)	Medium	n	Low (%)	Medium (%)	High (%)
38.5	Vehicle	28	34 ± 8	40 ± 9	26 ± 5
38.5	S1P	28	41 ± 8	36 ± 9	23 ± 5
41.0	Vehicle	24	32 ± 8	57 ± 9	11 ± 5
41.0	S1P	28	61 ± 8	34 ± 9	5 ± 5

^a Data represents least-squares means ± SEM.

blastocyst cell number and apoptosis status were determined. Representative images of blastocysts classified according to caspase activity are shown in Figure 3, a–d, while representative images of TUNEL labeling in blastocysts are shown in Figure 3, e and f. There was no effect of incubation temperature, S1P treatment, or the interaction on the proportion of blastocysts with low (<15 intensity units), medium (15–25 intensity units), or high (>25 intensity units) caspase activity (Table 1), total blastocyst cell number (Table 2), or percentage of blastomeres that were TUNEL positive (Table 2).

N₁-Dimethylsphingosine Disrupts Oocyte Competence

There were main effects of heat shock ($P < 0.05$) and tendencies for a main effect of DMS ($P < 0.06$) and a temperature × DMS interaction ($P < 0.07$) for the percentage of oocytes that cleaved. In particular, DMS reduced cleavage rate at both 38.5 and 41°C and the inhibitory effect of DMS tended to be greater at 38.5°C (Fig. 4A). Using the pdiff means separation procedure, the difference between control and DMS-treated oocytes was significant ($P < 0.05$) at both 38.5 and 41°C.

There was also a main effect ($P < 0.01$) of heat shock and a tendency for a temperature × DMS interaction ($P < 0.06$) for the proportion of oocytes becoming blastocysts; DMS reduced the percentage of oocytes developed to blastocysts and the inhibitory action of DMS was greater at 38.5°C (Fig. 4B). Using the pdiff means separation procedure, the difference between control and DMS-treated oocytes was significant ($P < 0.05$) at both 38.5 and 41°C.

While not significant, heat shock reduced the proportion of cleaved embryos that developed to blastocysts (Fig. 4C) and DMS reduced this proportion further for embryos formed from oocytes matured at 38.5°C but not for embryos from oocytes matured at 41°C. Note that, while the effect of heat shock on percent cleaved embryos developing to the blastocyst stage was not significant, analysis of the combined data set from the two experiments where oocytes were cultured at 41°C resulted in a significant ($P < 0.05$) reduction by heat shock in the percent of the blastocysts that developed from cleaved embryos (21.0% ± 2.7% for 41°C vs. 29.8% ± 2.5% for 38.5°C).

DISCUSSION

S1P is a sphingomyelin metabolite involved in regulation of cell growth, proliferation, and apoptosis [18, 19]. The present study demonstrates that S1P plays an important role in protection of oocytes from a physiologically relevant heat shock. In particular, oocytes cultured with S1P under conditions of heat shock did not experience the reduction in cleavage rate and subsequent development to the blastocyst stage as oocytes cultured without S1P. Moreover, inhibition of S1P synthesis caused by addition of DMS reduced or tended to reduce the proportion of oocytes un-

dergoing cleavage and subsequent development even in the absence of heat shock, suggesting that S1P is directly or indirectly involved in processes leading to oocyte maturation. It is likely that the thermoprotective effect of S1P seen in the current study is the result of an antiapoptotic action of S1P. Caspase-mediated apoptosis is a key pathway for the disruption of developmental competence of bovine oocytes caused by heat shock [15] and S1P blocked apoptosis of mouse oocytes induced by doxorubicin [30, 31] and radiation [24]. In Jurkat T cells, S1P inhibits activation of executioner caspases [e.g., caspase-3, -6, and -7] during Fas- and ceramide-mediated apoptosis [32]. Also, S1P reduced caspase-3 activation induced by tumor necrosis factor- α in human endothelial C11 cells [33]. Other studies have demonstrated that S1P inhibits the apoptotic cascade upstream of the release of the mitochondrial apoptogenic factors, cytochrome c and Smac/DIABLO, in acute leukemia cells, Jurkat, U937, and HL-60 cells [34].

Using the limited number of markers of developmental competence available, it appears that blastocysts formed from heat-shocked oocytes and treated with S1P had a developmental potential similar to that of blastocysts from control oocytes because there was no difference in blastocyst cell number, percentage of apoptotic cells, or caspase activity. Consistent with this idea are results in mice that pretreatment with S1P preserved ovarian follicular dynamics and oocyte competence after radiation exposure [24] without propagating genomic damage [25]. Further studies in which posttransfer survival rates of blastocysts and fetal development are compared will be needed to obtain conclusive evidence regarding effects of heat shock and S1P on embryo quality.

The fact that, even in the absence of heat shock, oocyte competence to cleave and develop was reduced or tended to be reduced by treatment with DMS, a sphingosine kinase inhibitor [29], suggests that S1P plays a direct or indirect role in oocyte function in the absence of stress. Similar results were recently reported in the pig, where DMS inhibited nuclear maturation in oocytes [35]. Studies with cell lines have provided evidence that sphingosine kinase plays a prominent role in proliferation and apoptosis. For example, overexpression of sphingosine kinase in NIH 3T3 fibroblasts increased the proportion of cells in the S phase of the cell cycle by promoting G₁-S transition and reduced the doubling time of these cells [36]. Overexpression of sphingosine kinase also protected against apoptosis induced by serum deprivation or by ceramide in PC12 cells and was associated with inhibition of caspase-2, -3, and -7 and of the stress-activated protein kinase, JNK [37]. Some cancer therapies are based on inhibition of sphingosine-kinase activity to amplify ceramide-mediated apoptosis in tumor cells [38].

Whether heat shock inhibits intracellular synthesis of S1P and whether such impairment is involved in disruption of developmental competence of oocytes by heat shock is

FIG. 3. Representative images of caspase activity and TUNEL labeling in blastocysts. Shown are blastocysts exhibiting low (a, b), medium (c), and high (d) caspase activity as well as blastocysts with a low (e) and high (f) frequency of apoptosis. Note that green nuclei are TUNEL positive while blue represents staining of nuclei with Hoescht 33258.

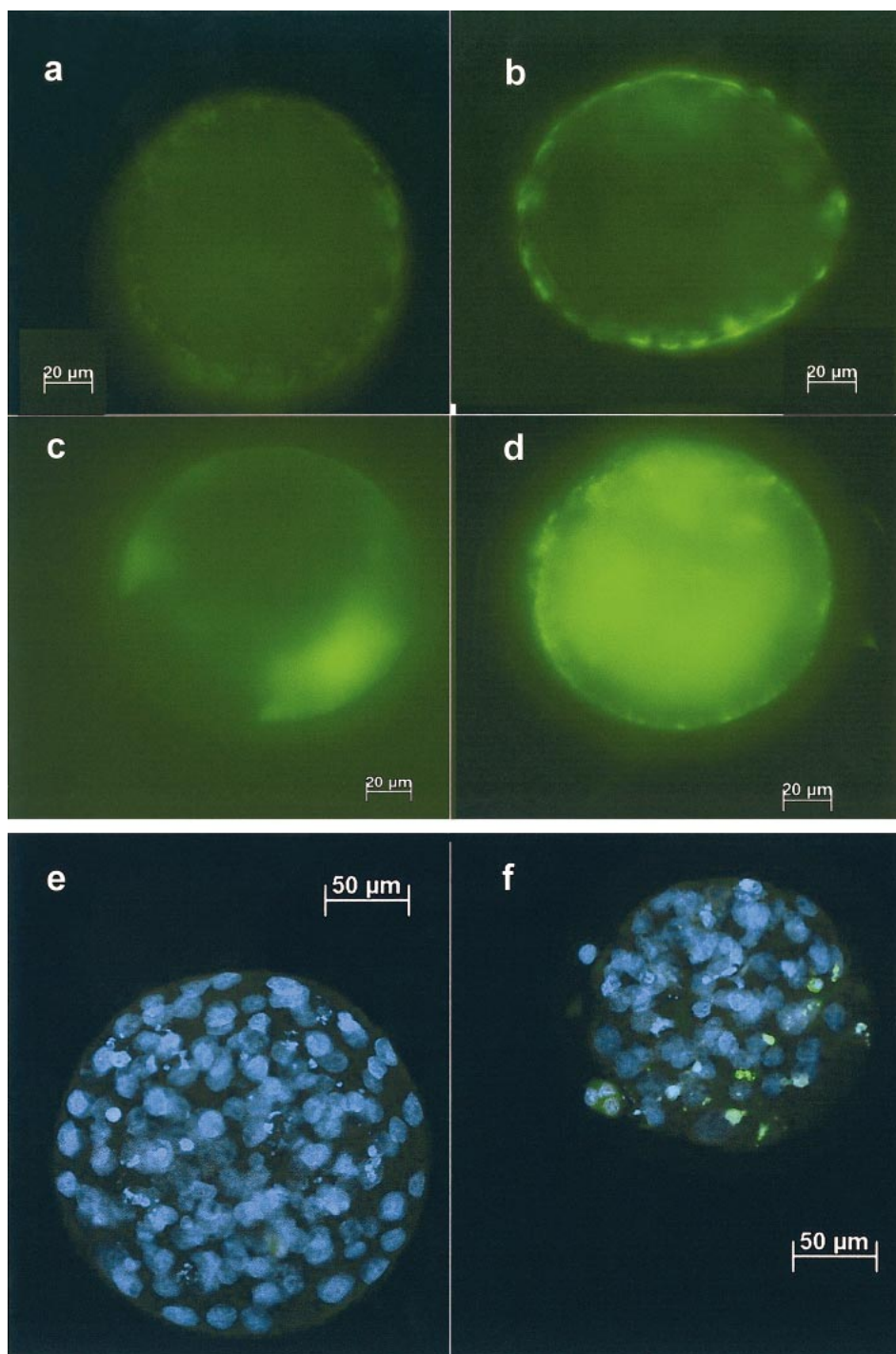


TABLE 2. Total cell number and percentage of blastomeres that were TUNEL positive in blastocysts developed from oocytes matured at 38.5 or 41°C during the first 12 h of maturation and in the presence or absence of sphingosine 1-phosphate (S1P).^a

Temperature (°C)	Medium	n	Total cell number	Percentage of blastomeres that were TUNEL positive
38.5	Vehicle	14	129 ± 14	6.6 ± 2.8
38.5	S1P	23	131 ± 14	8.5 ± 2.8
41.0	Vehicle	11	122 ± 14	4.4 ± 2.8
41.0	S1P	25	109 ± 14	4.1 ± 2.8

^a Data represent least-squares means ± SEM.

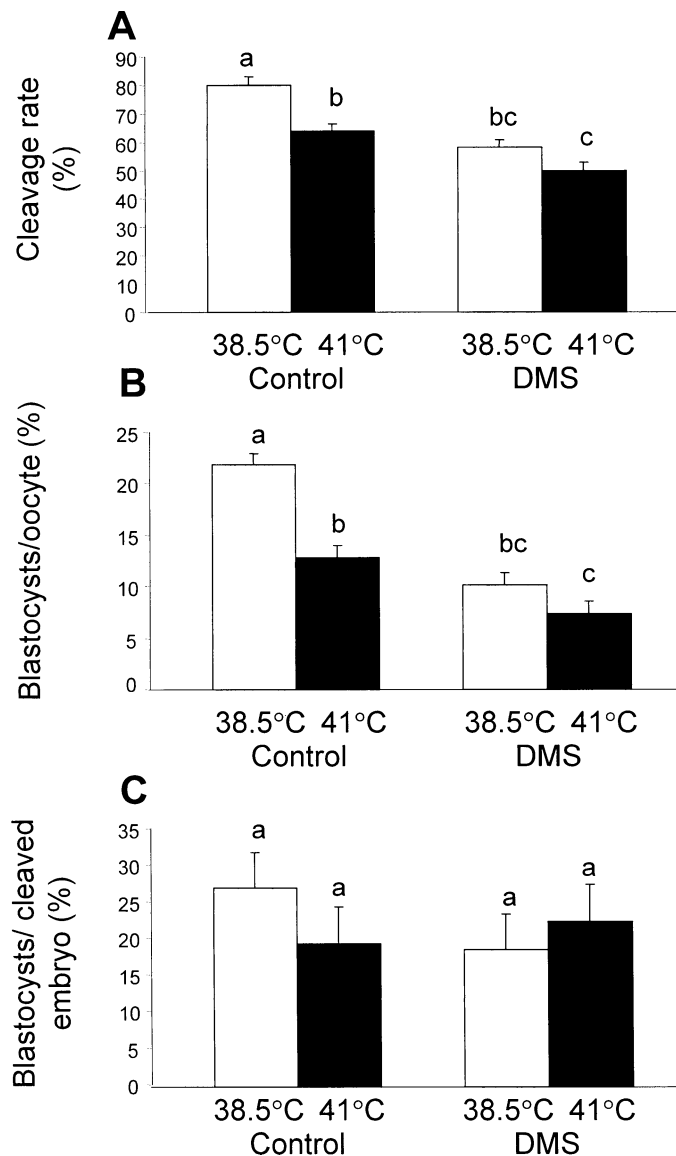


FIG. 4. Effects of inhibition of intracellular generation of S1P during oocyte maturation by *N,N*-dimethylsphingosine (DMS) on cleavage rate (A) and the percentage of oocytes (B) and cleaved embryos (C) that became blastocysts at Day 8 after insemination. Open bars represent oocytes cultured at 38.5°C while solid bars represent oocytes cultured at 41°C. Data are least-squares means \pm SEM. Superscripts above each bar that are different represent means that differ at $P < 0.05$ or less.

not certain, although there is indirect evidence that this is the case. Indeed, there was a tendency for actions of DMS on cleavage rate to be reduced for heat-shocked oocytes. This result is interpreted to indicate that one action of heat shock was to reduce S1P concentrations in the oocyte (through reduced synthesis or increased metabolism). Thus, DMS was less effective in heat-shocked oocytes because S1P concentrations were already reduced. Because the intracellular concentration of S1P is mainly regulated by the balance between its synthesis catalyzed by sphingosine kinase and its degeneration by S1P lyase and S1P phosphatase [20], it is possible that heat shock affects the activity of one or more of these enzymes.

Ceramide concentrations in the cell increase on exposure to environmental stresses such as heat shock [16, 17], radiation [39], and oxidative stress [40]. While ceramide is associated with growth arrest and apoptosis, S1P is asso-

ciated with proliferation and cell survival [23]. In fact, the dynamic balance between intracellular concentration of ceramide and S1P has been proposed to form a biological sphingolipid rheostat, which determines whether the cell survives or dies [22, 23]. Manipulation of the ceramide-S1P rheostat may allow development of methods to protect oocytes from thermal stress. For example, in vivo administration of S1P before ionizing radiation protected the pool (primordial, primary, preantral) of follicles and preserved competence of oocytes of the pretreated mice [24]. Conversely, disruption of ceramide generation by knocking out the gene for acid sphingomyelinase, which hydrolyzes sphingomyelin to ceramide, blocked oocyte apoptosis in mice treated with doxorubicin [24].

The complexity of sphingomyelin metabolism offers multiple opportunities for potentially modifying oocyte responses to heat shock to enhance fertility of females exposed to heat stress. Upon heat shock, ceramide can be produced by activation of sphingomyelin hydrolysis or de novo from condensation of serine with palmitoyl-CoA [17, 18]. Two saturated free fatty acids (palmitate [C16:0] and stearate [C18:0]), which are precursors of de novo ceramide synthesis, can induce apoptosis in the murine hematopoietic cell line, LyD9 WEHI-231 [41], and in rat Leydig cells [42]. In contrast, the monounsaturated fatty acids palmitoleate and oleate and linoleate were found to prevent the proapoptotic effect of palmitate in rat and human β cells [43]. Arachidonic acid was found to stimulate cell proliferation and to block the apoptotic effect of palmitate [42]. Other polyunsaturated fatty acids (α -linolenic and linoleic acid) were found to suppress apoptosis in W256 carcinosarcoma cells [44]. It may be possible, therefore, to affect the composition of the membrane fatty acids by dietary manipulations to reduce ceramide-induced apoptosis and protect oocytes from thermal stress.

In conclusion, the findings show that S1P can reduce or block effects of heat shock during maturation on the developmental competence of oocytes. Moreover, evidence was found to indicate that S1P plays a direct or indirect role in oocyte function even in the absence of stress. Future studies should be conducted to evaluate whether regulation of the ceramide/S1P rheostat, by administration of S1P, controlling of sphingomyelin metabolism by regulation of enzymes, or dietary manipulation can provide thermoprotection to the oocytes of heat-stressed females.

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