Heat Stress Induced Alterations in Prostaglandins, Ionic and Metabolic Contents of Sheep Endometrial Epithelial Cells In Vitro

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Abstract

Background: Heat stress is one of the major factors responsible for reduced fertility in livestock including sheep. The present study was aimed to elucidate the effect of heat stress on prostaglandins, ionic and metabolic contents of sheep endometrial epithelial cell in vitro. Sheep whole genitalia containing ovaries were collected immediately after slaughter and transported to the laboratory. Following washing the healthy and non pregnant uterine lumen with HBSS with gentamycin and 0.1% BSA, HBSS containing 0.3% trypsin was then infused and epithelial cells were isolated by incubation at 37°C for 60 min. After cell counting and viability determination, the epithelial cells were cultured in at 38.5°C for 24 hr (control). Heat stressed cultures were acclimated at 38.5°C for 6 hr and then placed at 40.5°C for 18 hr.

Results: In vitro heat shock significantly (P<0.05) increased protein, phosphorous, urea and PGF$_{2\alpha}$ contents in culture medium whereas significantly (P<0.05) decreased SOD content as compared to control. Elevated temperature did not significantly (P>0.05) increase calcium contents but non-significantly (P>0.05) increase glucose, chloride and PGE$_2$ levels in culture medium. It was concluded that heat stress altered prostaglandins, ionic and metabolic contents of endometrial epithelial cells in vitro.

Keywords: Heat stress; Prostaglandins; Endometrial epithelial cells; sheep

Abbreviations: HS: Heat stress; PGs: Prostaglandins; SOD: Superoxide Dismutase Activity

Introduction

Early embryonic mortality is one of the major intriguing factors of reproductive failure that causes considerable challenge to the mammalian cell biologists. Majority of the losses occurs due to failure of cellular and molecular dialogues at embryo uterine interface. Higher environmental temperature is one of the major factors responsible for reduced fertility in farm animals [1]. Heat stress (HS) can compromise reproductive events by decreasing the expression of estrous behavior, altering ovarian follicular development, compromising oocyte competence, and inhibiting embryonic development. Heat stress also increases the production of PGF$_{2\alpha}$ in the endometrium, leading to the early regression of CL or the death of embryos [2]. It was observed that heat stress from 8 to 16 days after insemination modulated the uterine environment [3], reduced the weight of corpora lutea and impaired conceptus growth [4].

Prostaglandins (PGs) produced by endometrium serves as a crucial mediators in maternal recognition of pregnancy, implantation and parturition [5]. The endometrial epithelial and stromal cells have specific morphological and functional properties. Epithelial cells preferentially produce PGF$_{2\alpha}$ whereas stromal cells produce mainly PGE$_2$ [6]. PGF$_{2\alpha}$ acts as the luteolytic agent [7] to control the estrous cycle in ruminants. Endometrial secretion of PGF$_{2\alpha}$ by pregnant uterus has been found to increase in response to heat stress and decrease the embryonic survivability by altering the signals required for maintenance of corpus luteum function during early pregnancy. Increased PGF$_{2\alpha}$ synthetic capacity of endometrium exposed to heat stress may be due to heat-induced alterations in endometrial cellular membranes resulting in increased mobilization of substrate for prostaglandin biosynthesis. Similar increases in uterine PGF$_{2\alpha}$ secretion in
response to heat stress in vitro by endometrial expiants from cows at Day 17 of pregnancy have been reported [2]. Furthermore, in-vivo heat stress of gilts between Days 8 and 16 of pregnancy increased concentrations of PGFM in the peripheral circulation and compromised luteal function, as indicated by reduced plasma concentrations of progesterone during Days 13 through 19 [8]. The present study was undertaken to investigate the effect of heat stress on prostaglandins, ionic and metabolic contents of sheep endometrial epithelial cells in vitro.

Materials and Methods

Isolation of endometrial epithelial cells

Sheep uteri were collected from the local abattoir immediately after slaughter and transported to the laboratory on ice. The epithelial cells from the sheep endometrium were separated by the method [9] with slight modification. Briefly, the uterine lumen was initially washed with HBBS supplemented with gentamycin and 0.1% BSA. Sterile HBSS containing 0.3% trypsin was then infused into the uterine lumen. Epithelial cells were isolated by incubation at 37°C for 60 min. The cell suspension obtained from the digestion was filtered to remove undissociated tissue fragments. The filtrate was washed with HBBS supplemented with gentamycin and 0.1% BSA by centrifugation at 600 x g for 10 min. The number of viable cells that excluded Trypan blue was counted using a hemocytometer.

Exposure of Heat Stress to Epithelial Cells in vitro

Following isolation of epithelial cells, the cells were cultured in RPMI 1640 medium at 38.5°C in presence of 5% CO2. Control cultures were maintained at 38.5°C for 24 hr. Control cultures were incubated under conditions representing normal body temperature of normal sheep in a thermo neutral environment. Heat stressed cultures were acclimated at 38.5°C for 6 hr and then placed at 40.5°C for 18 hr. Control cultures were washed with HBBS supplemented with gentamycin and 0.1% BSA by centrifugation at 600 x g for 10 min. The cell suspension obtained from the digestion was filtered to remove undissociated tissue fragments. The filtrate was washed with HBBS supplemented with gentamycin and 0.1% BSA by centrifugation at 600 x g for 10 min. The number of viable cells that excluded Trypan blue was counted using a hemocytometer.

Biochemical analysis

The concentrations of metabolites (Glucose, Total Protein and Urea) and ions (Calcium, Chloride and Phosphorus) were analyzed by using Biochrom Libra S32 UV/Vis Spectrophotometer. Commercial kits used for estimations except SOD were from Span Diagnostics Ltd, Surat, India. Superoxide dismutase activity (SOD) of the samples was measured spectrophotometrically using Ransod kit (Randox Laboratories Ltd., UK). All measurements were carried out according to the manufacturer’s instructions. The intra and inter assay coefficients of variation for all analyses were below 7%.

Determination of PGF2α and PGE2

The concentrations of PGF2α and PGE2 were determined in 50µl aliquots of culture medium after 10 fold dilution with extraction buffer using ELISA kits supplied by Neogen, USA. The sensitivity of the PGF2α and PGE2 assays were 0.002 and 0.1 ng/ml respectively. The intra and inter assay coefficients of variation of PGF2α assay were less than 19%. The intra and inter assay coefficients of variation of PGE2 assay were less than 14%. The cross reactivity of the antisera against 6-keto prostaglandin E1, 13, 14 dihydro-15 keto-prostaglandin F1α prostaglandin D2 and prostaglandin E2 were 3.05%, 0.05%, 0.05% and <0.01%, respectively. The cross reactivity of the antisera against 6-keto prostaglandin E1, 13, 14 dihydro-15 keto-prostaglandin F1α, and prostaglandin D2 were 0.91%, 0.01%, 0.01%, respectively.

Statistical Analysis

The experiment was replicated 6 times on different days. Results are expressed as mean ± SEM. Concentrations of each factor in control and heat stressed culture medium were analyzed by using Graph Pad Prism 5 (Graph Pad Software Inc., San Diego, CA, USA).

Results

Figure 1a: Protein content in culture medium following heat stress in vitro (CTR, Control; HS, Heat Stress). Different superscript letters indicate a significant difference (P<0.05; ANOVA).

Figure 1b: Phosphorus content in culture medium following heat stress in vitro (CTR, Control; HS, Heat Stress). Different superscript letters indicate a significant difference (P<0.05; ANOVA).

The effect of heat stress on endometrial ionic, metabolic contents and prostaglandin profiles (Figure 1a-1i). The elevated temperature significantly (P<0.05) increased protein, phosphorus, urea and PGF2α contents but significantly (P<0.05) decreased SOD content in culture medium as compared to control. Exposure of epithelial cells to 40.5°C decreased (P<0.05) calcium but increased (P>0.05) glucose, chloride and PGE2 levels in culture medium.
Discussion

Higher environmental temperature is one of the major intriguing factors responsible for infertility in farm animals. Suppression of reproductive function by heat stress is marked by abnormal endometrial function [10], compromised follicular growth [11], alteration in hormonal secretion [12] and embryonic function [13]. The present study was undertaken to delineate the effect of in vitro heat stress on prostaglandin production in sheep. In vitro heat stress mediated alterations in ionic and metabolic contents of endometrial epithelial cells were also analyzed. In the present, in vitro elevation of incubation temperature of sheep endometrial epithelial cells from 38.5°C to 40.5°C increased the production of protein. Our results agree with earlier reports [2] in terms of increase in protein secretion by conceptus and endometrium during early pregnancy in bovines. This may be due to enhanced ability of endometrium to regulate the rate of metabolic activity at
40.5°C. In vivo thermal stress has been found to enhance in vitro protein production by conceptus in pig[14].

This may be due to fact that high environmental temperature may severely alter metabolic activity and lead to failure of conceptus to produce biochemical signals in adequate amount required for preventing CL regression. Heat shock results in specific changes in the patterns of protein synthesis by mammalian cells, characterized by the synthesis of a small number of intracellular proteins referred to as heat-shock or stress proteins that may provide a degree of tolerance to stress [15]. In the current study, in vitro heat stress increased phosphorous and urea contents in culture medium. Our results are in agreement with the report of Daniel and Korsmeyer [16] who suggested that any stress conditions induces the influx of ions and metabolites particularly calcium, magnesium, phosphorus, chloride as well as glucose and glucose regulated proteins [17].

Significant increase in PGF$_{2\alpha}$ contents was observed in epithelial cells exposed to 40.5°C as compared to those incubated at 38.50°C (control). Our results agree with the report of Putney [2] and Malayer [10] wherein they suggested that secretion of both PGF$_{2\alpha}$ and PGE$_{2}$ in endometrium increased in response to heat stress.

Exposure of heat shock in the present study resulted in marked increase in the release of PGF$_{2\alpha}$ into culture medium due to alterations in membranes resulting in increased mobilization of substrates for prostaglandin biosynthesis. Heat induced increase in the turnover of membrane phospholipids and the release of arachidonic acid may provide substrates for prostaglandin synthesis [18]. As maintenance of luteal function is associated with alterations in endometrial prostaglandin production [19], increased prostaglandin secretion following heat stress may compromise CL function and initiate luteal regression. Elevated temperature decreased the activity of the endometrial inhibitor of PG synthesis present at Day 17 of pregnancy. Putney [20] found similar results when the endometrium was subjected to elevated temperature and the isolated inhibitor was tested for activity. Reduced function of this proteinaceous inhibitor of the cyclooxygenase-endoperoxidase enzyme complex might account partially for increased PGF$_{2\alpha}$ and PGE$_{2}$ production in pregnant animals but cannot account for heat-induced PGF$_{2\alpha}$ release from endometrium of cyclic animals.

The temperature induced increase of PGF$_{2\alpha}$ secretion by the endometrium was also not due to a direct effect of temperature on reaction rate of cyclooxygenase activity, because the production of PGF$_{2\alpha}$ from a cell-free preparation of cyclooxygenase-endoperoxidase enzyme complex from periparturient cotyledon was depressed by elevated temperature. In the present study, the levels of PGE$_{2}$ did not significantly (P>0.05) increase in epithelial cells exposed to 40.5°C as compared to control group. Our results are in agreement with the reports of [2,20] wherein elevated temperature did not increase PGE$_{2}$ secretion, indicating that elevated temperature affects PG secretion in some manner specific for PGF$_{2\alpha}$. At Day 17, most PGF$_{2\alpha}$ is released from the endometrial epithelium, while most of the PGE$_{2}$ originates in the stroma [6]. Perhaps high temperature affects these two cell types differently or preferentially enhances the activity of endoperoxide F reductase.

Similarly, Kobayashi [21] suggested that elevated temperature did not affect PGE$_{2}$ production in isthmic epithelial cells of cattle oviduct.

They suggested that the synthesis of PGE$_{2}$ was not affected by elevated temperatures in the isthmus due to the presence of different cell type populations (ciliated and secretary). Our results differed from the report of [21] wherein they suggested that PGE$_{2}$ production was increased by elevated temperature in ampullary epithelial cells of cattle oviduct and the increased PGE$_{2}$ production could be due to either increases in the expressions of PGESs or increases in the PGES activity. It is concluded that in vitro heat stress altered prostaglandins, ionic and metabolic contents of sheep endometrial epithelial cells. These in vitro results suggest that exposure of uterine epithelial cells to high environmental temperature may disrupt the endometrial factors responsible for maintenance of pregnancy.

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