Impact of *in vitro* Heat Shock (42.5°c) on Prostaglandins, Ionic and Metabolic Contents in Sheep Endometrial Epithelial Cells

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**Abstract**

Elevated temperature is one of the major factors responsible for reduced fertility in domestic ruminants including sheep. The aim of the study was to delineate the effect of heat shock on prostaglandins, ionic and metabolic contents of sheep endometrial epithelial cells in *vitro*. Endometrial epithelial cells were cultured in RPMI 1640 medium at 38.5 °C in CO₂ incubator for 24hr (5% CO₂ in air, 90-95% relative humidity; Control culture; n=6). Heat stressed cultures (n=6) were acclimated at 38.5°C for 6 hr and then placed at 42.5 °C for 18 hr. *In vitro* heat stress (42.5 °C) significantly (P<0.05) increased calcium, glucose and PGE2 levels and increased (P>0.05) protein, phosphorus, urea, SOD, PGF2α levels in epithelial cells as compared to those exposed to 38.5 °C. It is concluded that heat shock altered prostaglandins, ionic and metabolic contents of endometrial epithelial cells *in vitro*.

**Keywords**: Heat stress; Endometrial epithelial cells; Ionic content; Metabolic content; Sheep

**Introduction**

Higher environmental temperature is one of the major factors responsible for reduced fertility in farm animals. Heat stress causes a great suppression in endometrial function [1] besides compromised follicular growth [2], embryonic function [3] and oocyte developmental potential [4]. Heat stress also increases the production of PGF2α in the endometrium, leading to the early regression of CL or the death of embryos [5]. Heat stress-induced hyperthermia has been found to decrease the pregnancy rate in cattle during the summer in regions associated with elevated ambient temperatures [6]. Prostaglandins (PGs) produced by endometrium serves as a crucial mediators in maternal recognition of pregnancy, implantation and parturition [7,8]. The endometrial epithelial cells preferentially produce PGF2α whereas stromal cells produce mainly PGE2. PGF2α acts as the luteolytic agent to control the estrous cycle in ruminants. Endometrial secretion of PGF2α by pregnant uterus has been found to increase in response to heat stress and decrease the embryonic survivality by altering the signals required for maintenance of corpus luteum function during early pregnancy. Increased PGF2α 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 PGF2α secretion in response to heat stress *in vitro* by endometrial expiants from cows at Day 17 of pregnancy have been reported [5]. No reports are available on impact of *in vitro* heat stress on endometrial epithelial prostaglandins, ionic and metabolic contents in sheep. The present study was undertaken to investigate the effect of heat shock on prostaglandins, ionic and metabolic contents of sheep endometrial epithelial cells *in vitro*.

**Materials and Methods**

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 as previously described [7]. Following isolation of epithelial cells, the cells were cultured in RPMI 1640 medium at 38.5 °C in presence of 5% CO₂. Control cultures (n=6) were maintained at 38.5°C for 24hr. Control cultures were incubated under conditions representing normal body temperature of normal sheep in a thermo-neutral environment. Heat stressed cultures (n=6) were acclimated at 38.5 °C for 6 hr and then placed at 42.5 °C for 18hr. The culture medium was collected after 24hr and stored at-70 °C until analysis. The concentrations of metabolites (glucose, total protein and urea) and ions (calcium, chloride, phosphorus) were analyzed by using
Biochrom Libra S32 UV/Vis Spectrophotometer. The intra and inter assay coefficients of variation for all analyses were below 7%. The concentrations of PGF2α and PGE2 were determined in 50 μl 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 0.1ng/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%. Results are expressed as mean±SEM. Concentrations of each factor in control and heat stressed culture medium were analyzed by ‘t’-test using GraphPad Prism 5 (Graph Pad Software Inc., San Diego, CA, USA). Differences between mean values were considered significant when the probability values were < 0.05.

Results and Discussion

The effect of heat shock on prostaglandins, ionic and metabolic contents of sheep endometrial epithelial cells were presented in Table 1. The elevated temperature (42.5°C) significantly (P<0.05) increased calcium, glucose and PGE2 contents of epithelial cells. However, exposure of epithelial cells to 42.5°C did not significantly (P>0.05) increase protein, phosphorus, ura, PGF2α and SOD contents in epithelial cells compared to those exposed to 38.5 °C. Chloride contents decreased (P>0.05) in epithelial cells following exposure to higher temperature.

Table 1: Effect of heat shock on metabolic and ionic contents of endometrial epithelial cells.

| Parameters | Control(38.5 °C) | Heat Shock(42.5 OC) |
|------------|-----------------|------------------|
| Protein (mg/dl) | 0.50±0.12 | 0.79±0.12 |
| Glucose (mmol/L) | 36.79±1.28a | 42.96±2.76b |
| Calcium (mmol/L) | 0.65±0.02a | 0.70±0.02a |
| Phosphorus (gm/dl) | 7.46±0.42 | 8.85±0.55 |
| Urea (mg/dl) | 6.9±0.07 | 7.44±1.01 |
| Chloride (mmol/L) | 135.5±5.65 | 131.8±1.59 |
| SOD (U/ml) | 4.2±1.57 | 6.10±2.07 |
| PGE2 (pg/ml) | 2180±269a | 6360±1319b |
| PGF2α (pg/ml) | 2308±1001 | 4450±1356 |

Different values in the superscripts in the same column differ significantly (P <0.05).

In the present study in vitro incubation temperature of sheep endometrial epithelial cells from 38.5 °C to 42.5°C increased the production of protein. Our results agree with earlier report [8] 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 42.5 °C. Heat stress was found to be responsible for the secretion of proteins from mouse endometrial cells particularly the heat shock proteins thus increasing the total protein concentrations [9]. Heat shock had been found to result 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 [10]. Daniel & Korsmeyer [11] reported that any stress conditions induced the influx of ions and metabolites particularly calcium, magnesium, phosphorus, chloride as well as glucose and glucose regulated proteins.

Exposure of heat shock in the present study resulted in marked increase in the release of PGF2α 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 [12]. Elevated temperature had been found to decrease the activity of the endometrial inhibitor of PG synthesis present at Day 17 of pregnancy. Putney et al. [13] found similar results when the endometrium was subjected to elevated temperature and the isolated inhibitor was tested for activity. In the current study, the secretion of PGE2 increased following exposure to heat stress in vitro. This may be due to stress induced phospho-inositide turnover. However, our results differ from the earlier reports by Putney et al. [5,13,14] wherein they suggested that elevated temperature did not increase PGE2 secretion, indicating that elevated temperature affects PG secretion in some manner specific for PGF2α. At Day 17, most PGF2α is released from the endometrial epithelium, while most of the PGE originates in the stroma [15]. Perhaps high temperature affects these two cell types differently or preferentially enhances the activity of endoperoxide F reductase.

Conclusion

Our results indicate that in vitro heat shock 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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Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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