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Impact of *in Vitro* Heat Shock (42.50c) 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 cell *in vitro*. Endometrial epithelial cells were cultured in RPMI 1640 medium at 38.5 °C in CO_2 incubator for 24hr (5% CO_2 in air, 90-95% relative humidity; Control culture; n=6). Heat stressed cultures (n=6) were acclimated at 38.5^{0}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, PGF2a 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% CO_2. Control cultures (n=6) were maintained at 38.5^{0}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^{0}C did not significantly (P>0.05) increase protein, phosphorus, urea, 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 0C)
Protein (mg/dl)	0.50±0.12	0.79±0.12
Glucose (mmol/L)	36.79±1.28^a	42.96±2.76^b
Calcium (mmol/L)	0.65±0.02^a	0.70±0.02^b
Phosphorus (gm/dl)	7.46±0.42	8.85±0.55
Urea (mg/dl)	6.96±0.27	7.44±1.01
Chloride (mmol/L)	135.5±3.65	131.8±1.59
SOD (U/ml)	4.23±1.57	6.10±2.07
PGE_2 (pg/ml)	2180±269^a	6360±1319^b
$PGF_{2\alpha}$ (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* elevation of incubation temperature of sheep endometrial epithelial cells from 38.5 °C to 42.5˚C increased the production of protein. Our results agrees 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.

References

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1. Malayer JR, Hansen PJ, Buhi WC (1988) Effect of day of the oestrous cycle, side of the reproductive tract and heat shock on *in-vitro* protein secretion by bovine endometrium. J Reprod Fertil 84(2): 567-578.
2. Badinga L, Collier RJ, Thatcher WW, Wilcox CJ (1985) Effects of Climatic and Management Factors on Conception Rate of Dairy Cattle in Subtropical Environment. J Dairy Sci 68(1): 78-85.
3. Ealy AD, Drost M, Hansen PJ (1993) Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. J Dairy Sci 76(10): 2899-2905.
4. Al-Katanani YM, Paula-Lopes FF, Hansen PJ (2002) Effect of season and exposure to heat stress on oocyte competence in Holstein cows. J Dairy Sci 85(2): 390-396.
5. Putney DJ, Malayer JR, Gross TS, Thatcher WW, Hansen PJ, et al (1988a) Heat stress-induced alterations in the synthesis and secretion of proteins and prostaglandins by cultured bovine conceptuses and uterine endometrium. Biology of Reproduction 39(3): 717-728.
6. Stott GH, Williams RJ (1962) Causes of Low Breeding Efficiency in Dairy Cattle Associated with Seasonal High Temperatures. J Dairy Sci 45(11): 1369-1375.
7. Mondal S, Nandi S, Reddy IJ, Suresh KP (2009) Isolation, culture and characterization of endometrial epithelial cells in buffalo (Bubalus bubalis). Buffalo Bulletin 28(2): 101-106.
8. Poyser NL (1995) The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. Prostaglandins Leukot Essent Fatty Acids 53(3): 147-195.
9. Papaconstantinou AD, Benjamin R, Fisher R, Umbreit TH, Brown KM (2002) Increases in Mouse Uterine Heat Shock Protein Levels Are a Sensitive and Specific Response to Uterotrophic Agents. Environmental. Health Perspective 110(12): 1207-1212.
10. Ashburner M (1982) Heat Shock from Bacteria to Man. In: Schlesinger S, Tissieres A, Ashburner M, Cold Spring Harbor Laboratory press, USA, pp. 1-7.
11. Danial NN, Korsmeyer SJ (2004) Cell Death: Critical Control Points. Cell 116(2): 205-219.
12. Flint APF, Leat WMF, Sheldrick EL, Stewart HJ (1986) Stimulation of phosphoinositide hydrolysis by oxytocin and the mechanism by which oxytocin controls prostaglandin synthesis in the ovine endometrium. Biochemical Journal 237(3): 797-805.
13. Putney DJ, Mullins S, Thatcher WW, Drost M, Gross TS (1989) Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between the onset of estrus and insemination. Animal Reproduction Science 19(1-2): 37-51.
14. Putney DJ, Drost M, Thatcher WW (1988) Embryonic development in superovulted dairy cattle exposed to elevated temperature between days 1 to 7 post insemination. Theriogenology 30(2): 195-209.

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