ISSN: 2320-1983 Print ISSN: 2320-1991 Online Volume 2 Issue 3

Applied Cell Biology

Regular Paper

ACB, 2(3), 2013 [110-113]

Metabolic stressors in ovine and caprine sera and ovarian follicular fluid

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Received: 25th June, 2013 ; Accepted: 10th August, 2013

ABSTRACT

The metabolic changes in blood may reflect the biochemical composition of follicular fluid and this may influence oocyte and surrounding somatic cell quality. The aim of this study was to examine the metabolic stressors composition composition of follicular fluid and sera of sheep and goat. Serum and follicular fluid samples were assayed for glucose, cholesterol, triglycerides, urea, ammonia, non-esterified fatty acids and beta-hydroxyburyric acids. Result showed that the trends of metabolic stressors compositions in sera and follicular fluid were found to be similar in sheep and goats. The serum concentrations for glucose, cholesterol, triglycerides and non-esterified fatty acids were significantly higher compared to follicular fluid. The ammonia and beta-hydroxyburyric acids concentrations were significantly higher in follicular fluid. No significant difference was observed in urea concentration between sera and follicular fluid. ! 2013 Trade Science Inc. - INDIA

KEYWORDS

Follicular fluid;
Sera;
Metabolic stressors;
Sheep;
Goat.

INTRODUCTION

At the onset of production there is a massive and rapid increase in nutritional requirements, which the animal is unable to meet because of the limitation in voluntary dry matter intake[1]. There is mobilization of body reserves. These metabolic loads involved in fat mobilization lead to a stressful situation and reduced welfare[1]. Below a certain level of metabolic load the animal is not challenged; even at high intensity of metabolic load the animal remains largely unchallenged provided that the duration is short and vice versa. When the metabolic load reaches a level where it becomes challenging, the animal will attempt to cope by behavioural and physiological response[1]. Further increases in metabolic load will leave the animal unable to cope and will lead to pathological response[2]. The follicular fluid formed the biochemical environment of the oocytes[3]. Follicular fluid was in part exudates of serum and was in addition partially composed of locally produced substances, which are related to the metabolic activity of the follicular cells[4]. Most substances present in the follicular fluid could diffuse freely into and out of follicle. Follicular fluid composition was under intensive investigation to know the follicular development, oocyte maturation and follicular atresia[5]. The present study was undertaken to study the levels of metabolic stressors in sera and ovarian follicles in sheep and goat models.

MATERIAL AND METHODS

Twenty non-pregnant, cycling, parous ewes (*Ovis*

aries) and does (*Capra hircus*) in good health and with normal reproductive tracts upon macroscopical examination after slaughter were used for this study. Ovaries were transported to the laboratory in 0.9% chilled (4°C) normal saline supplemented with gentamicin (50 µg/mL) within 1 h of slaughter. The sera were collected from the same animals from which the ovaries were collected. The follicular fluid was collected by aspiration technique and was centrifuged for 5 min at 1000g. The follicular fluids were subjected to biochemical analysis (glucose, cholesterol, triglycerides, urea, ammonia, non-esterified fatty acids and beta-hydroxyburyric acids). Metabolites were analyzed as per the Association of Official Analytical Chemists (1990) guidelines and also by using a UV spectrophotometer. Reagent kits used for estimation of glucose, cholesterol, triglycerides, and urea were from Span Diagnostics (Bangalore, India). Ammonia, Beta-hydroxybutyric acid (beta –hydroxybutyrate) and non-esterified fatty acids (NEFA) kits were from Randox laboratories, UK and the estimations of stearic, palmitic and oleic acids were taken from a commercial clinic. All measurements were carried out according to the manufacturer's instructions. The intra- and inter assay coefficients of variation for all analyses were below 5%. Four samples (replicates) from separate groups of ovaries for each of the follicle size categories were formed. The composition of each sample was performed in quadruplicates, and the mean values for the quadruplicates were calculated and used for analysis.

Results are expressed as means ± S.E.M. The overall mean concentration ± S.E.M. of each metabolite for follicular fluid and for blood serum. A comparison was made for the levels in the follicular fluid and those of serum. A value of $P < 0.05$ was considered statistically significant.

RESULTS

The trends of metabolic stressors compositions in sera and follicular fluid were found to be similar in sheep and goats. The serum concentrations of glucose, and total cholesterol were significantly higher in sera than in ovarian follicles ($P < 0.05$). β-OHB was significantly inlower concentration in blood serum compared to the levels in ovarian follicles ($P < 0.05$). The serum concentration of triglycerides was significantly higher than in ovarian follicles ($P < 0.05$). Both in serum and in follicular fluid, oleic acid, palmitic acid and stearic acid were the three predominant free fatty acids. The NEFA composition differed significantly between the two compartments. The average relative importance of oleic acid, palmitic acid and stearic acid was 40%, 25% and 15% respectively. The follicular fluid concentrations of ammonia and total NEFA were significantly higher than those found in sera ($P < 0.05$). No difference was observed in urea concentration between sera and follicular fluid.

DISCUSSION

Low reproductive efficiency is the most critical problem faced by the livestock industry despite significant gains in genetic selection for increased production output. This decline may be due to a change in the nutritional intake to meet the increased energy and protein demands for production. The pathogenesis of sub-fertility is a complex system involving many interactions between nutritional components and physiological signals. Reduced ovarian functions are responsible for low conception rates and early embryonic mortality. One of the main reasons of reduced ovarian functions are imbalance feeding (more protein diet, less energy diet), negative energy balance (NEB) and the associated endocrine and metabolic signaling pathways. This may be reflected in the microenvironment of the growing and maturing ovum, and likely result in the ovulation of a developmentally incompetent oocyte. Protein metabolite (ammonia) and metabolic parameters of NEB may be harmful to the follicle and oocyte developmental competence, but this has never been substantiated. Elevated metabolic stressors during oocyte maturation, may compromise fertility through a reduction in follicle and oocyte developmental competence and the viability of the subsequent embryo. These metabolites can adversely affect uterine function and indirectly cause early embryonic death. Early embryonic death can also result from a sub-optimal combination of genes arisen during fertilization. Ammonia and NEFA has recently received attention as metabolic stressors that may adversely affect oocyte and or embryo development. Elevation of ammonia and NEFA concentrations in the follicular fluid results in gamete or embryo toxicity and decreased reproductive efficiency. Our recent meta-analysis data showed that a complex multi-step ammonia metabolism

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and a negligible urea metabolism exist in ovary[6].

Our results are in agreement to those observed in cattle[3], goat[5,7], buffalo[8], sheep[9], pigs[10] and camel[11] and to those presented in a review[12]. However our observation of values of cholesterol and total NEFA were lowered than those observed in sheep by earlier[13]; however they collected follicular fluid from sheep fed with fatty acid diet. We found that glucose concentrations in follicular fluid were lower than those measured in serum. This means that glucose metabolism is less intensive in ovarian follicles. A relatively stable concentration of triglycerides is maintained in the bovine ovarian follicle, regardless of increases in serum due to physiological status or diet[14]. Triglycerides probably do not pass through the follicular membrane since they are transported primarily by the very low-density lipoprotein fraction (VLDL), which is too large to pass through this barrier[15]. Cholesterol is considered the precursor of all steroid hormones, including estrogen and progesterone. The low level of cholesterol in the ovarian follicle compared to serum may indicate the biotransformation of cholesterol to sex steroids. NEFA are transported in the blood by means of albumin, and this complex can easily penetrate the follicular wall. NEFA concentrations did not differ between the different follicle classes and tended to be higher in serum[3]. Our values for NEFA composition was in the same trends as those observed earlier in cattle[3,16]

Elevated ammonia concentrations in reproductive fluids may be a factor affecting embryo development and resulting in decreased reproductive efficiency in early lactation dairy cows[17]. An excess of protein and a deficit of energy in the feed ration increases the production of ammonia that, when converted into urea in the liver, causes embryo mortality through an exacerbation of negative energy balance and reduced plasma prtogesterone levels, an alteration of uterine pH and increased secretion of PGF-2α[18]. Although follicular fluid ammonia concentrations appear to be related to protein intake and blood urea nitrogen level, the exact mechanisms responsible for elevated concentrations of ammonia in follicular are unknown.

Follicular fluid, easily available material in IVF cycles, would be an optimal source on non-invasive predictors of oocyte quality[19]. Most studies aiming to find a good molecular predictor of oocyte quality in FF are mainly correlative and not performed on large-scale, prospective and well controlled basis. The metabolomic approach is a powerful tool to study such marker(s) in follicular fluid, but its application is still at the infancy stage; this technique is facing the problems arising from analysing a complex biological fluid such as follicular fluid[19]. Metabolomics of the follicular fluid is the dynamic quantitative assessment of all low molecular weight substances that are present in FF at a given time[20]. Being the end products of cell's metabolism, low-molecular weight metabolites can reveal the response of the follicle to all influences affecting its development. Metabolites are potentially more informative than the direct study of gene expression (genomics), mRNAs (transcriptomes) or proteins (proteomes)[19]. The metabolic profiling of follicular fluid collected from large antral follicles is more homogeneous that the one obtained with fluids collected from small follicles, reflecting differences in the biochemical profile linked to oocyte maturational stage[21].

In conclusions, the serum concentrations for glucose, cholesterol, triglycerides and non-esterified fatty acids were significantly higher compared to follicular fluid. The ammonia and beta-hydroxyburyric acids concentrations were significantly higher in follicular fluid.

TABLE 1 : Metabolic stressors in sera and follicular fluid of sheep and goat

Metabolic Stressors	Sheep		Goat	
	Sera	FF	Sera	FF
Glucose (mM)	1.89 ± 0.25^{a}	1.44 ± 0.05^{b}	1.76± 0.29^{a}	1.49 ± 0.07^{b}
Triglycerides (mM)	0.24 ± 0.04^{a}	0.18 ± 0.04^{b}	0.27 ± 0.02^{a}	0.24 ± 0.03^{b}
Cholesterol (mM)	3.62 ± 0.31^{a}	2.22 ± 0.29^{b}	3.98 ± 0.17^{a}	2.33 ± 0.14^{b}
Total NEFA (μM)	80.7± 4.26^{a}	70.4± 4.21^{b}	87.2± 3.21^{a}	67.5± 3.27^{b}
Stearic acid (μM)	12.4± 1.31^{a}	10.3± 1.27^{b}	13.6± 2.32^{a}	8.6± 1.21^{b}
Palmitic Acid (μM)	20.1± 1.26^{a}	17.6± 2.26^{b}	22.4± 1.27^{a}	16.0± 1.46^{b}
Oleic acid (μM)	32.2± 3.20^{a}	27.6± 4.21^{b}	34.7± 2.27^{a}	29.1± 2.27^{b}
β-hydroxybutyric acid (mM)	0.34 ± 0.04^{a}	0.48 ± 0.05^{b}	0.37 ± 0.05^{a}	0.44 ± 0.02^{b}
Ammonia (μM)	100.1± 16.13^{a}	130.2± 14.23^{b}	107.4± 14.20^{a}	142.7± 17.25^{b}
Urea (mM)	4.11± 0.17	4.16± 0.19	4.01 ± 0.11	4.14 ± 0.14

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REFERENCES

[1] G.H.Hardarson; Acta Veterinaria Scandinavica Supplement, **95**, 63-67 **(2001)**.

[2] B.L.Nielsen, A.B.Lawrence; Proceedings to the XIX World Buiatrics Congress, **1**, 79-82 **(1996)**.

[3] J.L.M.R.Leroy, T.Vanholder, B.Mateusen, A.Christophe, G.Opsomer, A.de Kruif, G.Genicot, A.Van Soom; Reproduction, **130**, 485-495 **(2005)**.

[4] N.Gerard, S.Loiseau, G.Duchamp, F.Seguin; Reproduction, **124**, 241-248 **(2002)**.

[5] O.P.Mishra, J.N.Pandey, P.G.Gawande; Asian Australasian Journal of Animal Sciences, **16**, 1711-1715 **(2003)**.

[6] S.Nandi, K.P.Suresh, S.Mondal; Indian Journal of Animal Sciences, **83(5)**, 557-559 **(2013)**.

[7] S.B.Deshpande, M.M.Pathak; Veterinary World, **3**, 221-223 **(2010)**.

[8] S.Nandi, V.Girish Kumar, B.M.Manjunatha; Biology of Reproduction, **77**, 155 **(2007a)**.

[9] S.Nandi, V.Girish Kumar, B.M.Manjunatha, P.S.P.Gupta; Development Growth and Differentiation, **49**, 61-66 **(2007b)**.

[10] W.T.Huang, S.G.Lu, P.C.Tang, S.C.Wu, S.P.Cheng, J.C.Ju; Asian-Australian Journal of Animal Science, **15**, 1403-1411 **(2002)**.

[11] K.H.El Shahat, A.M.A.El Maaty, A.R.Moawad; Animal Reproduction, **10**, 16-23 **(2013)**.

[12] N.M.K.Kor, K.Moradi; Annual Review & Research in Biology, **3(3)**, 246-255 **(2013)**.

[13] K.E.Wonnacott, W.Y.Kwong, J.Hughes, A.M.Salter, R.G.Lea, P.C.Garnsworthy, K.D.Sinclair; Reproduction, **139**, 57-69 **(2010)**.

[14] M.E.Wehrman, T.H.Welsh, G.L.Williams; Biology of Reproduction, **45**, 514-522 **(1991)**.

[15] R.R.Grummer, D.J.Carroll; Journal of Animal Science, **66**, 3160-3173 **(1988)**.

[16] B.Renaville, N.Bacciu, A.Comin, M.Motta, I.Poli, G.Vanini, A.Prandi; Reproduction in Domestic Animal, **45**, 118-121 **(2010)**.

[17] S.C.Hammon, G.R.Holyoak, T.R.Dhiman; Animal Reproduction Science, **86**, 195-204 **(2005)**.

[18] W.R.Butler; Journal Dairy Science, **81**, 2533-39 **(1998)**.

[19] A.Revelli, L.D.Piane, S.Casano, E.Molinari, M.Massobrio, P.Rinaudo; Reproductive Biology and Endocrinology, **40**, 7827-7840 **(2009)**.

[20] R.Goodacre, S.Vaidyanathan, W.B.Dunn, G.G.Harrigan, D.B.Kell; Trends in Biotechnology, **22**, 245-252 **(2004)**.

[21] N.Thomas, R.Goodacre, E.M.Timmins, M.Gaudoin, R.Fleming; Human Reproduction, **15**, 1667-1671 **(2000)**.