ISSN 1819-1878

Asian Journal of Animal Sciences

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Asian Journal of Animal Sciences 9 (6): 466-470, 2015
ISSN 1819-1878 / DOI: 10.3923/ajas.2015.466.470

Follicular Fluid Concentrations of Metabolic Stressors in Normal, Obese, Metabolic Stressed and Emaciated Ewes

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ABSTRACT

Somatic cell quality and oocyte quality may be influence by follicular fluid composition. The changes in metabolic composition in follicular fluid reflect the changes in biochemical composition of follicular fluid. The aim of this study was to examine the metabolic stressors composition of follicular fluid of sheep (obese, normal, metabolic stressed and emaciated). Follicular fluid samples were assayed for cholesterol, triglycerides, urea, ammonia, non-esterified fatty acids and β-hydroxybutryic acids. Metabolic stressors like total NEFA, urea and beta-hydroxy butyrate concentrations were significantly higher and cholesterol and triglycerides concentrations were significantly lowered in follicular fluid of metabolic stressed and emaciated ewes.

Key words: Follicular fluid, metabolic stressor, body condition, sheep

INTRODUCTION

During high production, alteration in the biochemical composition of follicular fluid might negatively impact on the reproductive performance of animals. During the postpartum period low reproductive efficiency might be caused by many factors, like including milk production, nutrition requirement and energy balance (Grummer, 2007). There is mobilization of store body reserves like triglycerides in adipose tissue at the time of onset of production. This will cause to a stressful situation and reduced welfare (Hardarson, 2002). At a certain level of stress i.e. metabolic stress animal is not challenged. However, when duration of stress is for very less time period and vice versa the animal remains largely unchallenged. When the stress i.e., metabolic load reaches a certain level, the animal will attempt to overcome by behavioral and physiological response. When, there is further increase in metabolic load leave, the animal unable to overcome and lead to pathological response (Hardarson, 2002). Follicular fluid was in part exudates of follicular fluid and was in addition partially composed of locally produced substances, which are related to the metabolic activity of the follicular cells. Most of the biochemical substances present in the Follicular Fluid (FF) diffuse freely into and out of follicle. Follicular fluid composition was under intensive investigation to know the follicular development, oocyte maturation and follicular atresia (Mishra *et al*., 2003; Nandi *et al*., 2007a, b; Leroy *et al*., 2004; Kor and Moradi, 2013). The present study was undertaken to investigate the levels of metabolic stressors (NEFA, ammonia, urea, cholesterol, triglycerides and beta- hydroxy butyric acids) in ovarian follicles in sheep (obese, normal, metabolic stressed and emaciated) models.

MATERIALS AND METHODS

Collection and processing of FF: Two hundred ewes (*Ovis aries*) of 2.5-3 years old (Bellary breed; average body weight 26.4 kg) from Sira village, Tumkur, Karnataka were screened for these study. Forty mature, non-pregnant, cycling, parous ewes were chosen and categorized them into (a) Normal (n = 10, Average body weight: 32.3 kg), (b) Obese but not metabolic stressed (n = 10, Average body weight: 44.6 kg), (c) Emaciated but not metabolic stressed (n = 10, Average body weight:15.4 kg) and (d) Metabolic stressed (n = 20, Average body weight: 30.2 kg) based on body condition scoring (Thompson and Meyer, 2002) and blood sampling. The metabolic stressed ewes were identified/selected by estimating serum BUN and total NEFA (Nandi *et al*., 2013a). Further, the metabolic stressed group was divided into (i) Post parturient metabolic stress and (ii) Metabolic stress due to high protein diet (imbalance feeding, CP more than 20%). Follicular fluid and ovaries from all the animals included in the study were collected at the time of slaughter. Processing of follicular fluid was per described earlier (Nandi *et al*., 2007a). Ovaries with a mature functional CL and at least one large follicle were selected and transported to the laboratory within 1 h after slaughter in 0.9% saline, supplemented with gentamicin (50 mg mL^{-1}) and chilled to 4°C (to minimize metabolic changes). In the laboratory, ovaries were washed twice in chilled normal saline. The follicular fluid was collected by aspiration technique. For aspiration of FF, the follicles were held with forceps and a 22-gauge needle attached to a 5 mL plastic syringe was used. An antiproteolytic agent (20 mg mL^{-1} phenyl methyl sulfonyl fluoride, Himedia Lab. Pvt. Ltd., Mumbai, India) and an anticlotting factor (25 IU mL^{-1} heparin, Himedia Lab. Pvt. Ltd., Mumbai, India) were added to the FF. The FF was centrifuged at 1000×g for 5 min (4°C) and filtered through a 0.2 mm filter (Whatman, Mumbai, India). Aliquots of FF were put into 5 mL tubes (Tarsons, Kolkata, India) and stored (-80°C) pending biochemical analysis (done within 1 day after ovary collection). The ammonia estimation was done immediately after FF collection.

Estimation of metabolic stressors: The follicular fluids were subjected to biochemical analysis (cholesterol, triglycerides, urea, ammonia, total non-esterified fatty acids and β-a hydroxybutyrate). Metabolites were analyzed as per the Association of Official Analytical Chemists (1990) guidelines and also by using commercial kits. Reagent kits used for estimation of cholesterol, triglycerides and urea were from Span Diagnostics (Bangalore, India). Ammonia, Non-Esterified Fatty Acids (NEFA) and β-hydroxybutyric acid (β-hydroxybutyrate) kits were from Randox laboratories, 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 5%.

Statistical analysis: Four samples (replicates) from separate groups of ewes for each parameters 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 Mean±SEM. The data was analyzed by ANOVA followed by Tukey's multiple comparison tests. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The concentration of metabolic stressors in follicular fluid of obese, normal, metabolic stressed and emaciated sheep is presented in Table 1. The follicular fluid concentrations of total cholesterol were significantly lower in metabolic stressed (post parturient and Imbalanced feeding-HP diet) and emaciated ewes compared to obese and normal ewes. β-hydroxybutyrate (β-OHB) was

Table 1: Concentrations of metabolic stressors in ovarian follicular fluid of obese, normal, metabolic stressed and emaciated ewes

			Metabolic stressed		
Metabolic stressors	Normal	Obese	Post parturient	Imbalanced feeding-HP diet	Emaciated
Cholesterol (mM)	2.24±0.14[a]	2.68±0.16[a]	1.91±0.13[b]	1.97±0.16[b]	1.82±0.23[b]
Triglycerides (mM)	0.18±0.02[b]	0.32±0.14[a]	0.17±0.03[b]	0.19±0.02[b]	0.15±0.01[b]
Total NEFA (μM)	72.40±3.37[a]	73.10±3.11[a]	99.20±1.27[b]	78.40±1.31[a]	100.30±2.37[b]
Ammonia (μM)	129.10±11.21[b]	149.20±6.41[a]	132.30±10.24[b]	157.30±3.19[c]	135.60±3.39[b]
Urea (mM)	4.08±0.14[a]	4.11±0.11[a]	4.06±0.21[a]	6.06±0.21[b]	4.07±0.14[a]
β-OHB (mM)	0.48±0.05[a]	0.47±0.02[a]	0.72±0.14[b]	0.51±0.04[a]	0.69±0.21[b]

HP: High protein, Superscripts bearing different letters in the same row differ significantly ($p<0.05$)

significantly in higher concentration in follicular fluid of metabolic stressed (post parturient) and emaciated ewes compared to follicular fluid of obese and normal ewes. β-OHB level was also significantly higher in post parturient ewes compared to imbalanced (high protein diet) fed ewes ($p<0.05$). No significant change was obtained in follicular fluid triglyceride level in normal, metabolic stressed and emaciated ewes, while in follicular fluid of obese ewes triglycerides concentration were significantly higher compared to other groups. The follicular fluid concentration of total NEFA were significantly higher in metabolic stressed (post parturient) and emaciated ewes compared to obese and normal and metabolic stressed (imbalanced fed) group. No significant change was observed in total NEFA concentrations in obese and normal and imbalanced fed ewes. No difference was observed in urea concentration in follicular fluid of normal, obese, metabolic stressed (post parturient) and emaciated ewes. Significant increase in urea concentration in follicular fluid was observed in imbalanced fed ewes compared to other groups. Ammonia concentration was significantly higher in follicular fluid of metabolic stressed (imbalanced fed ewes) compared to normal, metabolic stressed (post parturient) obese and emaciated ewes. Similarly, ammonia concentration was significantly higher in obese group compared to normal, metabolic stressed (post parturient) and emaciated ewes. However, no significant difference in ammonia concentration was observed in follicular fluid of normal, metabolic stressed (post parturient) and emaciated group.

Decline in fertility is one of the most critical problems faced by the livestock industry. The possible reasons behind this decline fertility may be because of change in the nutritional intake to meet the increased energy and protein demands for production. Reduced ovarian functions are responsible for low conception rates and early embryonic mortality. One of the main reasons of reduced ovarian functions is imbalance feeding (more protein diet, less energy diet), Negative Energy Balance (NEB) and the associated endocrine and metabolic signaling pathways. 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.

The metabolomic approach has been 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 arise from analysing a complex biological fluid such as follicular fluid (Revelli *et al*., 2009). Metabolomics of the follicular fluid was the dynamic quantitative assessment of all low molecular weight substances that were present in FF at a given time (Goodacre *et al*., 2004).

Our results were in agreement to those observed in cattle (Leroy *et al*., 2004, 2005), goat (Mishra *et al*., 2003; Deshpande and Pathak, 2010), buffalo (Nandi *et al*., 2007a, b), sheep (Nandi *et al*., 2007a, 2013a), pigs (Huang *et al*., 2002) and camel (El-Shahat *et al*., 2013). Biochemical metabolites concentration in the follicular fluid fluctuates considerably with the stage

of cycle, follicle size and follicle status and presence of large follicles (Kor and Moradi, 2013). Cholesterol is known as precursor for steroid synthesis and in the follicular fluid only High-Density Lipoprotein (HDL) present resulting granulosa cells (avascular) of the follicles exclusively depended on the HDL (Mishra *et al*., 2003). However, our results for cholesterol and total NEFA were lowered than those observed in sheep in an earlier study (Wonnacott *et al*., 2010) though they collected follicular fluid from sheep fed with fatty acid diet. Stable concentration of triglycerides is maintained in the bovine ovarian follicle, regardless of increases in follicular fluid due to physiological status or diet (Wehrman *et al*., 1991). Our values for NEFA composition was in the same trends as those observed earlier in cattle (Leroy *et al*., 2005; Renaville *et al*., 2010). An excess of protein and a deficit of energy in the feed ration increased the production of ammonia, that when converted into urea in the liver, caused embryo mortality through an exacerbation of NEB and reduced plasma progesterone levels, an alteration of uterine pH and increased secretion of PGF-2α (Butler, 1998). Although follicular fluid ammonia concentrations appear to be related to protein intake and blood urea nitrogen level (Nandi *et al*., 2013b), the exact mechanisms responsible for elevated concentrations of ammonia in follicular were unknown. Our results are in agreement with an earlier report wherein increase in the concentration of ammonia and urea in ovarian follicular fluid was observed in cattle fed with rumen degradable protein or in an animal with negative energy balance (Armstrong *et al*., 2001; Sinclair *et al*., 2000). Our result also suggested an existence of complex multi-step ammonia and a negligible urea metabolism in an ovarian follicle (Jozwik *et al*., 2006; Nandi *et al*., 2013b). Our present study may confirm the hypothesis that the FF composition varies throughout the reproductive and productive status of animals (Orsi *et al*., 2005).

CONCLUSION

In conclusion metabolic stressors like total NEFA, urea and beta-hydroxybutyrate concentrations were significantly higher in follicular fluid of metabolic stressed and emaciated ewes. The work may encourage research on elucidation of the basic mechanisms involved in fertility in relation with nutrition and metabolic stress.

ACKNOWLEDGMENT

We are grateful to the Director, NAINP, Bangalore for providing necessary facility to carry out the research work. We also like to thank to Mr Gyan Prakash for his technical assistance.

REFERENCES

Armstrong, D.G., T.G. McEvoy, G. Baxter, J.J. Robinson and C.O. Hogg *et al*., 2001. Effect of dietary energy and protein on bovine follicular dynamics and embryo production *in vitro*: Associations with the ovarian insulin-like growth factor system. Biolo. Reprod., 64: 1624-1632.

Butler, W.R., 1998. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J. Dairy Sci., 81: 2533-2539.

Deshpande, S. B., and M.M. Pathak, 2010. Hormonal and biochemical profiles in follicular fluid of unovulated follicles in superovulated goats ovaries. Vet. World., 3: 221-223.

El-Shahat, K.H., A.M. Abo-El Maaty and A.R. Moawad, 2013. Follicular fluid composition in relation to follicular size in pregnant and non-pregnant dromedary camels (*Camelus dromedaries*). Anim. Reprod., 10: 16-23.

Goodacre, R., S. Vaidyanathan, W.B. Dunn, G.G. Harrigan and D.B. Kell, 2004. Metabolomics by numbers: Acquiring and understanding global metabolite data. Trends Biotechnol., 22: 245-252.

Grummer, R.R., 2007. Strategies to improve fertility of high yielding dairy farms: Management of the dry period. Theriogenology, 68: S281-S288.

Hardarson, G.H., 2002. Is the modern high potential dairy cow suitable for organic farming conditions? ACTA Veterinaria Scandinavica, 95: 63-67.

Huang, W.T., S.G. Lu, P.C. Tang, S.C. Wu, S.P. Cheng and J.C. Ju, 2002. Biochemical compositions of follicular fluid and the effects of culture conditions on the in vitro development of pig oocytes. Asian. Aust. J. Anim. Sci., 15: 1403-1411.

Jozwik, M., M. Jozwik, C. Teng and F.C. Battaglia, 2006. Amino acid, ammonia and urea concentrations in human pre-ovulatory ovarian follicular fluid. Hum. Reprod., 21: 2776-2782.

Kor, N.M. and K. Moradi, 2013. A review of biochemical metabolites concentration and hormonal composition of ovarian follicular fluid in domestic animals. Ann. Rev. Res. Biol., 3: 246-255.

Leroy, J.L.M.R., T. Vanholder, J.R. Delange, G. Opsomer and A. Van Soom *et al*., 2004. Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post partum. Theriogenolgy, 62: 1131-1143.

Leroy, J.L.M.R., T. Vanholder, B. Mateusen, A. Christophe and G. Opsomer *et al*., 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes *in vitro*. Reproduction, 130: 485-495.

Mishra, O.P., J.N. Pandey and P.G. Gawande, 2003. Study on biochemical constituents of caprine ovarian follicular fluid after superovulation. Asian-Aust. J. Anim. Sci., 16: 1711-1715.

Nandi, S., V.G. Kumar and B.M. Manjunatha, 2007a. Metabolite, electrolyte and enzymatic composition of bubaline follicular fluid. Biol. Reprod., Vol. 77.

Nandi, S., V.G. Kumar, B.M. Manjunatha and P.S.P. Gupta, 2007b. Biochemical composition of ovine follicular fluid in relation to follicle size. Dev. Growth Differ., 49: 61-66.

Nandi, S., K.P. Suresh and S. Mondal, 2013a. Ammonia and urea levels in blood and ovarian follicular fluid in cattle fed with normal and protein rich or imbalanced diet: A meta-analysis. Indian J. Anim. Sci., 83: 557-559.

Nandi, S., U.S.P. Shree and G.V. Kumar, 2013b. Metabolic stressors in ovine and caprine sera and ovarian follicular fluid. Applied Cell Biol., 2: 110-113.

Orsi, N.M., N. Gopichandran, H.J. Leese, H.M. Picton and S.E. Harris, 2005. Fluctuations in bovine ovarian follicular fluid composition throughout the oestrous cycle. Reproduction, 129: 219-228.

Renaville, B., N. Bacciu, A. Comin, M. Motta, I. Poli, G. Vanini and A. Prandi, 2010. Plasma and follicular fluid fatty acid profiles in dairy cows. Reprod. Domestic Anim., 45: 118-121.

Revelli, A., L.D. Piane, S. Casano, E. Molinari, M. Massobrio and P. Rinaudo, 2009. Follicular fluid content and oocyte quality: From single biochemical markers to metabolomics. Reprod. Biol. Endocrinol., Vol. 40. 10.1186/1477-7827-7-40

Sinclair, K.D., M. Kuran, F.E. Gebbie, R. Webband and T.G. McEvoy, 2000. Nitrogen metabolism and fertility in cattle: II. Development of oocytes recovered from heifers offered diets differing in their rate of nitrogen release in the rumen. J. Anim. Sci., 78: 2670-2680.

Thompson, J.M. and H. Meyer, 2002. Body condition scoring of sheep. Oregon State University, USA.

Wehrman, M.E., T.H. Welsh, Jr. and G.L. Williams, 1991. Diet-induced hyperlipidemia in cattle modifies the intrafollicular cholesterol environment, modulates ovarian follicular dynamics and hastens the onset of postpartum luteal activity. Biol. Reprod., 45: 514-522.

Wonnacott, K.E., W.Y. Kwong, J. Hughes, A.M. Salter, R.G. Lea, P.C. Garnsworthy and K.D. Sinclair, 2010. Dietary omega-3 and -6 polyunsaturated fatty acids affect the composition and development of sheep granulosa cells, oocytes and embryos. Reproduction, 139: 57-69.