Annual Review & Research in Biology
3(4): 397-404, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Follicular Fluid Concentrations of Biochemical Metabolites and Trace Minerals in Relation to Ovarian Follicle Size in Dairy Cows

Nasroallah Moradi Kor[1*], Kaveh Mohammadi Khanghah[2] and Ali Veisi[2]

[1]Young Researchers and Elites Club, Kerman Branch, Islamic Azad University, Kerman, Iran.
[2]Department of Animal Science, Razi University of Agricultural Sciences and Natural Resources, Kermanshah, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

Received 27th February 2013
Accepted 17th May 2013
Published 11th June 2013

ABSTRACT

In the present study, ovarian follicular fluid concentrations of trace elements and biochemical metabolites in relation to follicular size were investigated in dairy cows. Ovaries were recovered from 40 female adult Holstein Friesian cows 5–7 years of age with clinically normal reproductive tracts after slaughtering. The stage of the cycle in the cows slaughtered was diestrus determined post mortem. Visible follicles on the surface of the ovaries were classified, based on their diameter, into (i) small (3-5 mm), (ii) medium (6-9 mm) and (iii) large (10-20 mm) categories. Follicular fluid samples were analyzed for elements (iron, iodine, copper, manganese, zinc, cobalt, molybdenum and selenium) and biochemical metabolites (glucose, cholesterol, triglyceride, total protein, albumin, globulin, urea and creatinine). Results showed that concentrations of trace elements were different between follicles sized categories. Differences in follicular fluid concentrations of iodine and manganese between follicles sized categories were significant ($p \leq 0.05$). In addition, differences in follicular fluid concentration of glucose and cholesterol between follicles sized categories (Small, Medium and large follicles) were significant ($p \leq 0.05$). These results of the present study suggest that the levels of trace elements and biochemical metabolites composition in the follicular fluid were related to follicular size in dairy cows.

**Corresponding author: Email: Moradikor@iauk.ac.ir;*

Keywords: Dairy cow; follicular fluid; metabolites; trace elements.

1. INTRODUCTION

Follicular fluid (FF) is an avascular compartment within the mammalian ovary, separated from the perifollicular stroma by the follicular wall that constitutes a 'blood-follicle barrier [1,2]. Minerals are essential for growth and reproduction and are involved in a large number of digestive, physiological and biosynthetic processes within the body [3]. The most obvious function is as components of body organs and tissues and to provide structural support. In addition, they act as electrolytes, as constituents of body fluids and as catalysts in both enzyme and hormone systems. They therefore fulfil several important functions for the maintenance of animal growth and reproduction as well as health status [4]. Proper herd management should be designed to optimize the production of the highest quality product, while minimizing any adverse effects on the health and welfare of the animals [5]. In dairy cattle two key goals are adequate nutrition and adequate mammary health so as to produce wholesome milk. Recent data indicate that micronutrient management will enhance the production of good quality milk. The potential for minerals to play a significant role in herd fertility is indisputable [6]. The mineral elements that are of particular importance are categorised into major (calcium, phosphorous, potassium, sodium, chlorine, sulphur and magnesium) and trace elements (iron, iodine, copper, manganese, zinc, cobalt, molybdenum and selenium). The minerals that affect reproduction in cattle are generally found within the trace element group, although deficiencies of calcium and phosphorus can also affect fertility [7]. Organic minerals have a beneficial role to play in resumption of follicular growth and fertility in dairy cows. Reproductive problems are frequently reported in association with trace mineral deficiencies, particularly copper, selenium and manganese [8]. The key to the effectiveness of a mineral supplement is not necessarily its biological availability, but its biological activity [9]. Organic minerals have been shown to have several beneficial effects in ruminant and monogastric animals. There are still discernible differences among chelated mineral, mineral proteins and other organic minerals complexes. Proteinated minerals can possibly improve female reproduction through increased fertilization, lower embryo mortality, improved uterine environment and/or increased intensity of estrous behavior [5].Within the ovarian follicle, the developing oocyte is surrounded by the follicular fluid, which is a serum transudate modified by follicular metabolic activities. Besides meeting nutritional requirements of the developing oocyte, follicular fluid also maintains a proper environment for the maturation of the oocyte. Besides a serum transudate, follicular fluid also contains locally produced substances that share the metabolic activity of follicular cells [10]. This metabolic activity, together with the barrier properties of the blood–follicle barrier, has been shown to change significantly during the growth phase of the follicle [2,11]. The ovarian FF provides suitable microenvironment for the development, growth and maturation of the oocyte and is vital for the maintenance of fertility in the female [1]. The knowledge of the biochemical composition of FF can also provide useful information about the requirements for cell and oocyte growth and maturation. Moreover, such information may be used as a provisional guide for formulating suitable culture media for in vitro cell culture and oocyte maturation in a particular species [10]. Changes in the biochemical composition of FF may influence steroidogenesis, oocyte maturation and quality, ovulation and transport of the oocyte to the oviduct, as well as the preparation of the follicle for subsequent corpus luteum formation and function [12]. Biochemical metabolites concentration in the FF of the bovine ovary fluctuate considerably with the stage of cycle, follicle size and follicle status and presence of large follicles [1,13,14]. Leroy et al. [15] demonstrated that the oocyte and the granulosa cells grow and mature in a changing biochemical environment from small to large follicles. In the previous studies FF concentration of biochemical metabolites such as

glucose, cholesterol, triglycerides, total proteins, albumin and globulin have been determined in camel, cattle [16,17] and sheep [14]. These researchers demonstrated that the FF concentration of biochemical metabolites changes from small to large follicles. Kalmath and Ravindra [18] reported that the concentration of different mineral constituents significantly differed with size of the follicle and also with respect to the different months of the year. Before focusing on possible effects of mineral and metabolic changes within the follicle on oocyte quality, it seems necessary to investigate physiological concentrations of some minerals and metabolites in their fluid from small, medium and large follicles. Therefore, the aim of this study was to investigate the concentrations of Trace elements and biochemical metabolites composition of ovarian follicular fluid in relation to follicle size in dairy cows.

2. MATERIALS AND METHODS

This study was performed at the Razi University animal reproduction laboratory, located in the Kermanshah province; Iran (34º 18´ N and 47º 3´E) from December 2011 to February 2012 (winter). Ovaries were recovered from 40 female adult cows (Holstein Friesian) 5–7 years of age with clinically normal reproductive tracts after slaughtering. The ambient temperature during experiment ranged from 5–10ºC, with the relative humidity being 40–68% in winter season. The stage of the cycle in the cows slaughtered was diestrus determined post mortem.The presence of Corpus Luteum (CL) in ovaries determined that the cows are in diestrus cycle. Before slaughter, the age of each cow was determined by observing the conformation of teeth. The ovaries of the cows were excised immediately after slaughter and transported to the laboratory in 0.9% normal saline, supplemented with 1000 IU/mL penicillin G and 1000 mg/mL streptomycin sulfate, within 1 h after slaughter. Ovaries were identified by using the eartag number of the cow and transported on ice (4ºC) to the laboratory.

2.1 Collection of Follicular Fluid

At the laboratory, the ovaries were again washed in saline (0.9% NaCl, 4 °C) and each ovary was cleaned of the extraneous tissue. The diameter of various follicles present in each ovary was measured with the help of vernier caliper device. Inactive ovaries and associated with pregnant cattle and those that have any pathological lesions such as cystic follicles (>20 mm in diameter) were not included in the study. These follicles were placed in three groups according to their diameter, i.e. small (3–5 mm), medium (6–9 mm) and large (10–20 mm). Follicular fluid was aspirated from small, medium and large follicles using a sterile syringe and 22 G needle. In such cases, fluid collected from follicles of the same category from the same ovary of the same animal was pooled [10,15]. Follicles >20 mm in diameter with thick wall and containing red-coloured fluid were assumed to be cystic and were not included in the study. Follicular fluid samples were centrifuged (3000× g, 7 minutes) and the supernatant was collected. Sample preparation was completed within 3 h after slaughter and were stored at −20ºC for further analysis.

2.2 Biochemical Analysis of the Follicular Fluid

Follicular fluid samples were analysed for various trace elements and biochemical metabolites. The concentrations of trace elements were determined by ICP Chemical analyzer (Rigaku Miniflex 600 XRD, USA, Cat. # 3625-310A). The concentrations of glucose, cholesterol, triglycerides, total proteins, albumin, globulin, urea and creatinine were measured using commercially available kits. The determination of metabolites levels in FF

was estimated by a commercial clinical photometric analyzer (Model BT-3000, made in USA, Biotecnica-#738755203). Standard commercial kits were used for analysis and procedures were adopted as recommended by the manufacturer of kits. After processing samples and standards provided with the kits, absorbance of the standard and the samples was determined and the concentrations of respective metabolites in samples were computed, using the formula: concentration of a metabolite = absorbance of sample divided by absorbance of standard and multiplied by standard concentration.

2.3 Statistical Analysis

Mean values (±SD) for the concentrations of various trace elements and biochemical metabolites was calculated for follicular fluid in all cows. The concentration of each factor in the follicular fluid were compared between different sized follicles categories by ANOVA using general linear model procedure of SAS software [19]. Significance between means was tested using Duncan Multiple Range Test. A probability value of $P \leq 0.05$ indicated that the difference was statistically significant.

3. RESULTS AND DISCUSSION

The comparison between concentrations of various trace elements in follicular fluid from different sized follicles are presented in Table 1. The follicular fluid concentration of copper, zinc, cobalt, molybdenum and selenium in large follicles were higher when compared with the fluid from small follicles, Whereas the concentration of iodine and manganese in large follicles was higher than in medium and small follicles. The concentrations of iron did not differ between small, medium and large follicles ($p \geq 0.05$). Mean (± SD) concentrations of various biochemical metabolites in follicular fluid from different sized follicles are presented in Table 2. Differences in follicular fluid concentrations of glucose and cholesterol between different sized follicles categories were statisticaly significant ($p \leq 0.05$). The differences between follicle size categories for total proteins, albumin, globulin, urea and creatinine were not significant ($p \geq 0.05$).

In the present study, follicular fluid concentrations of trace element in large follicles were higher when compared with the fluid from small follicles. This implies that the principal source of trace element in follicular fluid is blood. A further reason for this observation could be the increased permeability of the blood–follicle barrier during follicular growth. Follicular fluid concentration of glucose in large follicles was significantly higher ($p \leq 0.05$) when compared with the fluid from small and medium follicles. These results were in agreement with previous reports in dairy cattle [20,15], goats [21], and ewes [14]. However, our results are in contrast with the finding of Rahman et al. [16] who demonstrated that the glucose concentration was significantly lower ($p \leq 0.05$) in fluid from large when compared with the small follicles in camels.

Table 1. Mean (± SD) concentrations of various trace elements in follicular fluid from different sized follicles

Trace elements	Three follicle classes		
	Small follicles (3-5mm)	Medium follicles (6-9mm)	Large follicles (10-20mm)
Iron (mg/dl)	2.16 ± 1.72	2.34 ± 1.34	2.94 ± 1.22
Iodine (mg/dl)	7.41 ± 1.03^{c}	10.63 ± 1.41^{b}	12.82 ± 1.27^{a}
Copper (mg/dl)	5.28 ± 0.49^{b}	5.32 ± 0.61^{b}	7.81 ± 1.02^{a}
Manganese (mg/dl)	6.35 ± 0.74^{c}	9.71 ± 0.95^{b}	11.91 ± 0.89^{a}
Zinc (mg/dl)	1.64 ± 0.58^{b}	2.13 ± 0.47^{ab}	3.08 ± 0.72^{a}
Cobalt (mg/dl)	5.84 ± 1.07^{b}	9.04 ± 1.12^{a}	9.23 ± 1.67^{a}
Molybdenum (mg/dl)	3.21 ± 0.38^{b}	3.78 ± 0.52^{b}	5.23 ± 0.37^{a}
Selenium (mg/dl)	2.64 ± 1.34^{b}	3.31 ± 1.02^{b}	5.27 ± 1.57^{a}

Values with different superscripts (a>b>c) in the same row differ significantly (P<0.05).

Table 2. Mean (± SD) concentrations of various metabolites in follicular fluid from different sized follicles

Metabolites	Three follicle classes		
	Small follicles (3-5mm)	Medium follicles (6-9mm)	Large follicles (10-20mm)
Glucose (mg/dl)	40.99 ± 1.02^{c}	47.81 ± 2.14^{b}	54.44 ± 2.32^{a}
Cholesterol (mg/dl)	29.81 ± 2.51^{a}	25.65 ± 2.07^{b}	22.48 ± 2.23^{c}
Triglyceride (mg/dl)	33.33 ± 2.53^{a}	30.69 ± 2.23^{b}	30.24 ± 1.41^{b}
Total proteins (g/dl)	6.49 ± 1.34^{b}	6.78 ± 1.45^{b}	7.01 ± 1.39^{b}
Albumin (g/dl)	4.20 ± 0.93^{b}	4.57 ± 1.27^{b}	4.79 ± 0.85^{b}
Globulin (g/dl)	2.73 ± 0.18^{b}	2.96 ± 0.12^{b}	3.17 ± 0.07^{b}
Urea (mg/dl)	15.62 ± 1.08^{b}	16.81 ± 0.82^{b}	16.93 ± 1.25^{b}
Creatinine (mg/dl)	2.83 ± 0.78^{b}	1.87 ± 0.62^{b}	1.46 ± 0.55^{b}

Values with different superscripts (a>b>c) in the same row differ significantly (P<0.05).

The metabolism of glucose is less intensive in large follicles compared with small ones, resulting in lower consumption of glucose from fluid of large follicles. Perhaps this is because the large follicles have the ability to filter and reserve the high concentrations of glucose from blood for utilization in their development to the mature Graafian follicle [1]. A further reason for this observation could be the increased permeability of the blood–follicle barrier during follicular growth [2,22]. In the present study follicular fluid concentration of cholesterol decreased with the increase in follicular size, which was in agreement with the findings of Thangavel and Nayeem [23] in buffalo and Huang et al. [24] in pigs and in contrary to the study of Leroy et al. [15] and Brantmeier et al. [25] in cattle, Nandi et al. [14] in sheep, and Thakur et al. [21], Bordoloi et al. [26], and Mishra et al. [27] in goat. The result of the present study confirms the results of Rahman et al. [16] who demonstrated that the low cholesterol concentration in the FF indicated biotransformation of cholesterol to steroid hormones. Cholesterol is known to be a precursor of all steroid hormones, including oestrogen and progesterone in females [16]. Cholesterol in the FF was in the form of a constituent of high-density lipoprotein [25]. Therefore, the avascular granulosa cells of the follicles totally depended on the cholesterol from high-density lipoprotein, which was derived from the blood plasma by crossing the basement membrane of granulosa cells [27]. Endresen et al. [28] reported that granulosa cells have a large store of cholesteryl esters that may provide free

cholesterol for the preovulatory progesterone or steroidogenesis. Follicular fluid concentration of triglyceride in fluid from small follicles was significantly higher ($p \leq 0.05$) when compared with the fluid from medium and large follicles. The triglycerides concentration were higher in small follicles because they might be the alternate sources of energy for the cells in follicles [17,15]. A similar trend was found in the triglycerides concentration, with a higher triglycerides concentration in the fluid from smaller than the larger follicles in porcine and bovine ovaries, as reported by Chang et al. [29] and Leroy et al. [15] respectively. Triglycerides are the storage form of lipids, and their hydrolysis yields one molecule of glycerol and three molecules of fatty acids, and the energy needed for the growing follicle. Thus, continuous and rapid utilization of triglycerides might have resulted in their low concentrations in large compared with small follicles [16]. Another reason for the high concentrations of triglycerides in small follicles was that triglycerides did not pass through the follicular membrane [30] and follicular triglyceride levels are mainly a result of local metabolic processes [15]. Follicular fluid concentration of total proteins, albumin, globulin, urea and creatinine did not differ between follicle classes ($p \geq 0.05$). In the mammalian ovary, the FF contains proteins and peptides that play an important role in the growth, development, and maturation of oocytes [31]. Similar to our result, Leroy et al. [15] in dairy cows and Arshad et al. [32] in buffalos reported that protein concentration was relatively uniform throughout the follicular development. However, our results differed from those of Brantmeier et al. [25] and Wise [33] in cattles and Thangavel and Nayeem [23] in buffalos, who reported a decrease in the total protein concentration as the follicle size increased. Albumin plays a vital role in the development of a colloidal pressure which might contribute to the high viscosity of the follicular fluid. It can be speculated that albumin may increase osmotic pressure and the movement of solvent, i.e. water, and contribute towards the low concentration of albumin in the fluid from large follicles [16]. Our data was in agreement with the results reported for FF from pigs [34] and in contrary to the study of Rahman et al. [16] in camel. The globulin present in FF, though in small quantity, might be necessary for protecting the follicle from external environments [32].

4. CONCLUSION

From the present study it was concluded that the concentrations of trace elements and biochemical metabolites composition in the follicular fluid of the bovine ovary fluctuate considerably with the follicle size.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Mohsen Akbari for his useful and kindly help. The authors would also like to specially thank the director of the Bistun abattoir for providing the research facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abd Ellah MR, Hussein HA, Derar DR. Ovarian follicular fluid constituents in relation to stage of estrus cycle and size of the follicle in buffalo. Vet World. 2010;3(6):263-267.

2. Albomohsen H, Mamouei M, Tabatabaei S, Fayazi J. Metabolite Composition Variations of Follicular Fluid and Blood Serum in Iranian Dromedary Camels During the Peak Breeding Season. J Anim Vet Adv. 2011;10(3):327-331.
3. Arshad HM, Ahmad N, Rahman ZU, Samad HA, Akhtar N, Ali S. Studies on some biochemical constituents of ovarian follicular fluid and peripheral blood in buffaloes. Pak Vet J. 2005:25(4):189-193.
4. Bagavandoss P, Midgley AR, Wicha M. Developmental changes in the ovarian follicular basal lamina detected by immunofluorescence and electron microscopy. J Histochem Cytochem. 1983:31:633–640.
5. Bordoloi PK, Sarmah BC, Dutta DJ, Deka BC. Follicular fluid cholesterol in goat ovary. Indian Vet J. 2000;77:638–639.
6. Brantmeier SA, Grummer RR, Ax RL. Concentrations of high density lipoproteins vary among follicular sizes in the bovine. J Dairy Sci. 1987;70:2145–2149.
7. Chang SCS, Jones JD, Ellefson RD. The porcine ovarian follicle: I. Selected chemical analysis of follicular fluid at different developmental stages. Biol Reprod. 1976;15:321–328.
8. Close WH. The role of organic trace mineral proteinates in pig nutrition. In: Biotechnology in the Feed Industry. 1998;Proc Alltech's 14th Annual Symp:469-483.
9. Endresen MJ, Haug E, Abyholm T, Henriksen T. The source of cholesterol for progesterone synthesis in cultured preovulatory human granulosa cells. Acta Endocrinol. (Copenh). 1990;123:359–364.
10. Fortune JE, Hansel W. Concentrations of steroids and gonadotropins in follicular fluid from normal heifers and heifers primed for superovulation. Biol Reprod. 1985;32:1069-1079.
11. Gerard N, Loiseau S, Duchamp G, Seguin F. Analysis of the variations of follicular fluid composition during follicular growth and maturation in the mare using proton nuclear magnetic resonance. Reproduction. 2002;124:241–248.
12. Gosden RG, Hunter RHF, Telfer E, Torrance C, Brown N. Physiological factors underlying the formation of ovarian follicular fluid. J Reprod Fertil. 1988;82:813–825.
13. Grummer RR, Carroll DJ. A review of lipoprotein cholesterol metabolism: importance to ovarian function. J Anim Sci. 1988;66:3160–3173.
14. Harmon RJ. Trace minerals and dairy cattle: importance for udder health. In: Biotechnology in the Feed Industry. 1988;Proc Alltech's 14 th Annual Symp:485-495.
15. Hidiroglou, M. Manganese in ruminant nutrition. Can J Anim Sci. 1979;59:217-236.
16. Huang WT, Lu SG, Tang PC, Wu SC, Cheng SP, Ju, JC. Biochemical compositions of follicular fluid and the effects of culture conditions on the in vitro development of pig oocytes. Asian Aust J Anim Sci. 2002;15:1403–1411.
17. Kalmath GP, Ravindra JP. Mineral profiles of ovarian antral follicular fluid in buffaloes during follicular development. Indian. J Anim Res. 2007;41(2):87–93.
18. Landau S, Braw-Tal R, Kaim M, Bor A. Preovulatory follicular status and diet affect the insulin and glucose contents of follicles in high yielding dairy cows. Anim Reprod Sci. 2000;64:181–197.
19. Leroy JLMR, Vanholder T, Delanghane JR, Opsomer G, Van-Soom A, Bols PEJ, de Kruif A. Metabolite and ionic composition of follicular fluid from different-sized follicles and their relationship to serum concentrations in dairy cows. Anim Reprod Sci. 2004;80:201–211.
20. Lyons TP. Bioscience Centres: Forging links between industry and academia. In Biotechnology in the Feed Industry", Proc. 1993; Alltech's 9th Sym:1-25.
21. Maas J. Relationship between nutrition and reproduction in beef cattle. Veterinary Clinics of North America: Food Animal Practice. 1987;3:633-646.

22. McDowell LR. Minerals in Animal and Human Nutrition. Academic Press Inc. Harcourt Brace Jovanovich Publishers, San Diego, CA; 1992.
23. McGaughey RW. A comparison of the fluids from small and large ovarian follicles of the pig. Biol Reprod. 1975;13:147–153.
24. Mishra OP, Pandey JN, Gawande PG. Study on biochemical constituents of caprine ovarian follicular fluid after superovulation. Asian Aust Focus. 2003;16:1711–1715.
25. Mukesh V, Sharma MK., Singh D. Effect of follicular fluid proteins and gonadotropins on progesterone secretion by buffalo granulosa cells in vitro. Asian-Aust J Anim Sci. 2004;17(11):1496-1500.
26. Nandi S, Girish Kumar V, Manjunatha BM, Gupta PSP. Biochemical composition of ovine follicular fluid in relation to follicle size. Develop Growth Differ. 2007;49:61–66.
27. Rahman Zia-Ur, Bukhari SA, Ahmad N, Akhtar N, Ijaz A, Yousaf MS, Haq IU. Dynamics of Follicular Fluid in One-humped Camel (Camelus dromedarius). Reprod Dom Anim. 2008;43:664–671.
28. Statistical Analysis Systems Institute 1998 SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC.
29. Shujait A, Ahmad N, Akhtar N, Rahman Zia-ur, David EN. Metabolite contents of blood serum and fluid from small and large sized follicles in dromedary camels during the peak and the low breeding seasons. Anim Reprod Sci. 2008;108:446–456.
30. Tabatabaei S, Mamoei M, Aghaei A. Dynamics of ovarian follicular fluid in cattle. Comp Clin Pathol. 2011;20:591–595.
31. Thakur RS, Chauhan RAS, Singh BK. Studies on biochemical constituents of caprine follicular fluid. Indian Vet J. 2003;80:160–162.
32. Thangavel A, Nayeem M. Studies on certain biochemical profile of the buffalo follicular fluid. Indian Vet J. 2004;81:25–27.
33. Underwood EJ. The mineral nutrition of livestock. CAB London England; 1981.
34. Wise T. Biochemical analysis of bovine follicular fluid: albumin, total protein, lysosomal enzymes, ions, steroids and ascorbic acid content in relation to follicular size, rank, atresia classification and day of estrous cycle. J Anim Sci. 1987;64:1153–1169.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=239&id=9&aid=1462