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Impact of acute metabolic acidosis on the acid-base balance in follicular fluid and blood in dairy cattle

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ABSTRACT

Acid-base balance is one of the most vigorously regulated variables of the body, including genital organs. Subacute ruminal acidosis is a common disturbance in dairy cows that disturbs several biochemical indices in the blood, cerebrospinal fluid, and urine. The possible negative effects of metabolic acidosis on the follicular fluid (FF) composition and, subsequently, on oocyte quality, are not fully elucidated. This study aimed to evaluate the changes in acid-base balance (ABB) in FF and blood during acute metabolic acidosis in dairy heifers. Ten Holstein heifers were stimulated with FSH in eight decreasing doses at 12-hour intervals (D0–D3). Acidosis was induced by oral administration of sucrose at 9 g/kg of body weight, dissolved in 10 L of warm tap water, at D3. Samples were collected from each cow at 0, 8, 12, 16, 24, 32, 40, and 48 hours after treatment. Samples of FF, obtained by transvaginal follicular aspiration, and peripheral blood were examined for ABB parameters: pH, pCO_2, pO_2, HCO_3^-, and base excess (BE). A significant decrease in pH, HCO_3^-, and BE values in the blood, as well as FF, occurred after sucrose treatment. The lowest pH values occurred in blood at 16 hours, and in FF at 24 hours, after treatment (7.30 ± 0.05 and 7.33 ± 0.05, respectively). The lowest HCO_3^- values in blood (18.75 ± 3.2 mmol/L) and FF (18.07 ± 2.84 mmol/L) occurred 24 hours after treatment, as did the lowest BE values (−6.61 ± 3.7 mmol/L and −7.53 ± 3.89 mmol/L, in blood and FF, respectively). Significant correlations for HCO_3^- (r = 0.928), BE (r = 0.946), pH (r = 0.889), and pCO_2 (r = 0.522) existed between blood and FF samples. The results demonstrated that metabolic acute acidosis substantially influences the characteristics of both serum and FF.

1. Introduction

The acid-base balance (ABB) of animals depends on the intricate association between anions and cations in the blood. Extracellular H^+ is one of the most vigorously regulated variables of the body. The vital limits of pH variation for mammals are between pH 7.36 and 7.44 [1]. Under normal conditions, acids and bases are added continuously to the body fluids as a result of either ingestion or production during cellular metabolism. The body combats any changes in the normal ABB, by using three fundamental mechanisms: chemical buffering, respiratory adjustment of blood carbonic acid, and excretion of H^+ or $HCO3^-$ by the kidneys [1]. Physiological parameters for pH, partial pressure of carbon dioxide (pCO_2), and concentrations of base excess ([BE]), and standard bicarbonate ([HCO_3^-]) are 7.38–7.43; 5.2–6.4 kPa; −0.5 to 4.5 and 23.5–27 mmol/L, respectively, in the blood of dairy cattle [2].

Ruminal acidosis in dairy cattle is caused by feeding highly concentrated rations with an inadequate intake of fiber. It can lead to subacute ruminal acidosis (SARA) which occurs in 11% to 26% of cows [3,4], and even over 40% in some herds [5], during intensive dairy production. Systemic effects of ruminal acidosis, include disturbances in several biochemical indices in the blood [6], cerebrospinal fluid, and urine [7]. Blood gas analysis has been reported as a valuable tool to diagnose acidosis in dairy cows because it

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provides a good assessment of acidosis and is also less invasive than rumen pH analysis [8].

Mammalian oocytes grow within ovarian follicles in which the oocyte is coupled to surrounding somatic granulosa cells by gap junctions. Oocyte growth is absolutely dependent on its association with granulosa cells [9]. The intrafollicular microenvironment is crucial for optimal follicle and oocyte development. Coordinated somatic cell-oocyte interactions attempt to balance cellular metabolism with energy requirements during folliculogenesis and meiotic resumption. Perturbation of these cellular mechanisms by metabolic disease can harm the oocyte [10]. The effect of acidosis on oocytes and embryos at the cellular level has been investigated and the mechanism by which the intracellular pH is regulated in bovine oocytes and embryos has been reported [11,12]. Most cellular processes are acutely pH-sensitive and impairment of pH regulation can critically compromise cell function and viability. The mammalian cell has, therefore, been equipped to maintain a normal pH in response to changing environments and metabolic acid generation [13]. To correct acidosis, mammalian cells possess Na^+/H^+ exchangers (NHEs) of the NHE gene family, which export H^+ in exchange for Na^+, thereby raising pH [14]. However, changes in ABB in follicular fluid (FF) during acidosis in dairy have not yet been reported.

Ultrasound-guided transvaginal aspiration is commonly used to collect FF when various biochemical or endocrine examinations of FF are required [14–20]. Blood gases and ABB are other possible parameters that can be investigated. Several articles in human medicine have discussed these factors [21–23]. These articles specifically focused on CO_2 concentrations and pH in FF. However, the sampling of FF for ABB and gas analysis from live animals is rarely described [19,24–26], perhaps due to the technical difficulties involved in FF sampling [27]. To the best of our knowledge, no studies have been published regarding acidosis and its impact on FF quality. This study aimed to evaluate ABB changes in FF and blood during induced metabolic acidosis in dairy heifers.

2. Materials and methods

2.1. Animals and treatments

Ten Holstein heifers (aged 15 months, average body weight of 430 ± 15 kg) were housed at the Ruminant and Swine Clinic at the University of Veterinary and Pharmaceutical Sciences Brno. The cows were synchronized by cloprostenol (500 μg IM per cow, Oestrophan, Bioveta, Ivanovice na Hane, Czech Republic). After 7 days, dominant follicles were ablated to start the new follicular wave. After 2 days (Day 0, D0), stimulation using FSH was initiated. A total dose of 345 μg FSH (Stimufol, ULg, FMV, Liège, Belgium) was administered IM in eight doses at 12-hour intervals (D0–D3) to induce FF production during the whole experimental period (Table 1). A metabolic acidosis was induced by oral administration of sucrose, at 9 g/kg of body weight, dissolved in 10 L of warm tap water, given as a ruminal drench on D3. A similar method to induce acidosis using saccharosis in sheep was described previously [28]. After, the heifers were not fed until the last sample was collected on D5.

Table 1
Treatment schedule.

Day	Hours	Treatment	Dose
D−9		Cloprostenol	500 μg
D−2		TVFA	
D0	0	FSH	55 μg
	12	FSH	55 μg
D1	24	FSH	50 μg
	36	FSH	45 μg
D2	48	FSH	35 μg
	60	FSH	35 μg
D3	72	FSH	35 μg + sucrose
	84	FSH	35 μg

TVFA = aspiration of all follicles exceeding 5 mm; FSH = superstimulation using FSH; sucrose = treatment of sucrose 9 g/kg of bodyweight.

2.2. Sampling schedule

Venous blood and FF samples collected at 06:00 hours, were used as control samples. Immediately after, heifers received the sucrose treatment (ST) (0 hours). Next, samples were collected at 8, 12, 16, 24, 32, 40, and 48 hours after ST. Thus, eight samples from each heifer were collected.

2.3. Follicular fluid sampling and analysis

The FF was collected using a new device to aspirate FF for ABB analysis (Figs. 1 and 2) [29]. The transvaginal aspiration was performed after epidural anesthesia (4 mL, 2% lidocaine, Fatro, Ozzano Emilia, Italy), rectum evacuation, and disinfection of the vulva and perineum. A real-time B-mode ultrasound machine (Aloka SSD-500, Tokyo, Japan), equipped with a convex ultrasound transducer (7.5 MHz, Aloka UST 9125, Tokyo, Japan) placed in a plastic holder, was used to control the follicular aspiration. The probe holder was inserted as deep as possible into the *fornix vaginae*. The technician performing the procedure manipulated the ovary through the rectal wall and located the target follicle, for aspiration, on the scanner screen. The syringe holder, with attached aspiration syringe and needle, was inserted into a guide tube and the needle was then inserted into the center of the target follicle by manipulation of the end of the syringe holder. Then, a guide ring with a connecting rod and attached syringe piston were slowly pulled back, and the fluid was aspirated into the syringe. All follicles exceeding 5 mm were aspirated.

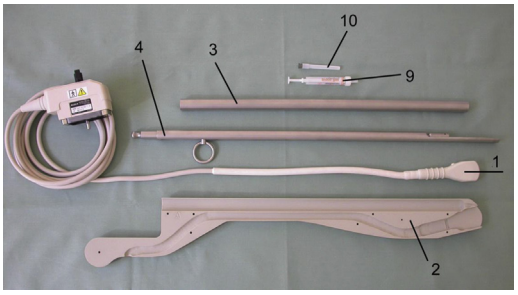

Fig. 1. The device used to aspirate follicular fluid for acid-base balance analysis. 1 = ultrasound transducer, 2 = probe holder, 3 = metal guide tube, 4 = syringe holder, 9 = aspiration syringe, 10 = needle.