

## A new device for the aspiration of follicular fluid for acid-base balance analysis in cattle

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**ABSTRACT:** The aim of this study was to evaluate a new device for the ultrasound-guided transvaginal aspiration of follicular fluid for acid-base balance analysis (ABB set) in comparison with the original modified commercial OPU set. In the ABB set, an aspiration syringe was placed in the front part of the new tool's handle, next to the transducer, so as to enable direct collection of the sample into the syringe. To obtain a sufficient amount of testable fluid, reservoirs of urine (rubber balloons) were used for later aspiration under laboratory conditions in Experiment 1. Fifteen triads of samples (each triad with two punctures) were collected. While the first sample of each triad was taken using the ABB set (ABB sample), two samples were taken by one puncture using the original modified commercial OPU set: aerobic phase of sampling (AE sample) with air present in the tubing at the start of sampling and the subsequent anaerobic phase of sampling (AN sample). Values determined in the second sample from the triad (AE) varied from the values in both ABB and AN samples (pH 7.685 vs. 7.704 vs. 7.692, pCO<sub>2</sub> 11.13 vs. 10.3 vs. 10.85, pO<sub>2</sub> 6.87 vs. 8.67 vs. 7.02). In Experiment 2, ultrasound-guided transvaginal aspirations were carried out in 13 cows bearing ovarian cysts with diameters of at least 3 cm, using plastic aspiration syringes (Experiment 2P) and in 12 cows using glass aspiration syringes (Experiment 2G). The sequence of samples was the same as in Experiment 1. We found a significantly higher pH in AE in comparison to AN (7.357 vs. 7.348), lower pCO<sub>2</sub> (6.85) and higher O<sub>2</sub> (14.12) in samples of AE in comparison to samples of ABB and AN (pCO<sub>2</sub> 7.36, 7.30; O<sub>2</sub> 9.95, 10.63 respectively) in cystic fluid in Experiment 2P. We found a significantly higher pH (7.4), lower pCO<sub>2</sub> (5.98) and a higher pO<sub>2</sub> (12.35) in AE samples in comparison to ABB and AN samples of cystic fluid (pH 7.386, 7.385; pCO<sub>2</sub> 6.39, 6.35 and O<sub>2</sub> 10.56, 10.65, respectively) from Experiment 2G. We conclude that the acid-base balance assay was affected by air, present in the tubing during aerobic sampling in comparison to anaerobic and ABB set sampling. These pre-analytical changes can be prevented by the use of the ABB set because the results obtained with the ABB set were not different from that of the AN samples. We also confirmed pre-analytical changes in acid-base balance parameters in the cystic fluid after it had been stored in plastic aspiration syringes. Our new ABB set equipped with a glass aspiration syringe is suitable for sampling follicular fluid for both acid-base balance and gas analysis.

**Keywords:** cow; acid-base parameters; ovarian cysts; cystic fluid collection; ovum pick-up

### List of abbreviations

**ABB** = acid base balance, **AE** = aerobic, **AN** = anaerobic, **OPU** = ovum pick-up, **TVFA** = transvaginal follicular aspiration

Follicular fluid is derived from blood flowing through the thecal capillaries as a result of the activity of granulosa cells, generating an osmotic gra-

dient. The movements of granulosa cells are relative to each other and the remodelling of cell-to-cell junctions allows the fluid to accumulate (Rodgers

Supported by the Internal Grant Agency of University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic (Grant No. 70/2012/FVL) and Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM 6215712403).

and Irving-Rodgers 2010). Follicular fluid sampling has been performed for many years in order to evaluate the oocyte microenvironment in ovaries obtained from slaughtered animals (Edwards 1974; Wise 1987; Leroy et al. 2004a; Orsi et al. 2005) or from live animals (Ginther et al. 1997; Kendrick et al. 1999; Gerard et al. 2002; Jorritsma et al. 2003; Leroy et al. 2004b). The most frequently applied method of *in vivo* aspiration is ultrasound-guided transvaginal follicular aspiration (TVFA), first described by Pieterse et al. (1988) and known as the ovum pick-up method (OPU). Since then, this method has been used for oocyte collection in live cattle and horses (Pieterse et al. 1988; Bruck et al. 1992) and later for follicular fluid collection as well, when various biochemical or endocrinological examinations of follicular fluid collected by TVFA are carried out (Vos et al. 1994; Kohram et al. 1998; Moallem et al. 1999; Landau et al. 2000; Walters et al. 2002; de Castro e Paula et al. 2008; Shehab-El-Deen et al. 2010). Acid-base balance and blood gases are other possible parameters that can be investigated. Several articles in human medicine have addressed these factors (Shalgi et al. 1972; Daya 1988; Imoedemhe et al. 1993) and have focused especially on concentrations of carbon dioxide and pH in follicular fluid. However, the recovery of follicular fluid for acid-base balance and gas analysis from live animals is rarely described (Berg et al. 2003, 2005; Cech et al. 2007; de Castro e Paula et al. 2008), perhaps due to the technical difficulties involved in follicular fluid sampling. Peripheral blood collection for acid-base balance analysis is performed routinely. This procedure is much easier than the recovery of follicular fluid. Nevertheless, for this analysis, there are many potential pre-analytical errors in blood sampling-e.g., caused by the underfilling of the syringe (Bandi 1981; James et al. 1997), the syringe material itself or conditions of sample storage (Szenci and Besser 1990; Wu et al. 1997; Beaulieu et al. 1999; Knowles et al. 2006; Cingi et al. 2009). Because similar errors should be expected during and after the collection of follicular fluid (Redding et al. 2006), requirements for anaerobic follicular fluid collection have been reported (Berg et al. 2005; Redding et al. 2006). There is evidence that changes in the quality of human follicular fluid can occur even before aspiration, due to increasing exposure to intraperitoneal CO<sub>2</sub> during artificial pneumoperitoneum with laparoscopic procedures for follicular aspiration. This has been associated with a decrease in the pH

of follicular fluid (Daya 1988; Imoedemhe et al. 1993). In our previous study we also observed pre-analytical changes in O<sub>2</sub>, CO<sub>2</sub> and pH values when the samples of fluid from ovarian cysts were not taken under strictly anaerobic conditions (Cech et al. 2011), and therefore we have formulated requirements necessary for the development of a new set for follicular fluid collection. The purpose of our study was to evaluate a new set for the aspiration of follicular fluid to be used in determining acid-base balance (ABB set).

## MATERIAL AND METHODS

### Animals

**Experiment 1: Evaluation of the ABB set under laboratory conditions.** Three non-lactating dairy cows housed at the Ruminant Clinic of the University of Veterinary and Pharmaceutical Sciences, Brno were used. Urine from the cows was collected by catheterisation into rubber balls to create sufficient amounts of homogenous fluid for ABB analyses.

**Experiment 2: Evaluation of the ABB set under *in vivo* conditions.** Thirty-three Holstein dairy cows bearing follicular cysts were used for the collection of cystic fluid by means of ultrasound-guided transvaginal aspiration. The animals were housed at a commercial dairy farm, and cysts larger than 30 mm were diagnosed by means of ultrasonographic examinations during regular visits.

The experiments were approved by the institutional care committee.

### Aspiration equipment and sampling protocol

**ABB set.** A convex ultrasound transducer (7.5 MHz Aloka UST 9125, Japan) (1) is placed in a holder (2) at an angle allowing for good visibility of the aspiration needle on the monitor of a real-time B-mode ultrasound scanner (SSD-500, Aloka, Japan). A probe holder of the usual shape and appropriate size is made from two plastic parts connected by means of screws. A metal guide tube (3) is placed in the upper part of the ultrasound probe holder. This is used for insertion and free movement of the syringe holder. The syringe holder (4) makes up the main inner part of the device and is manipu-

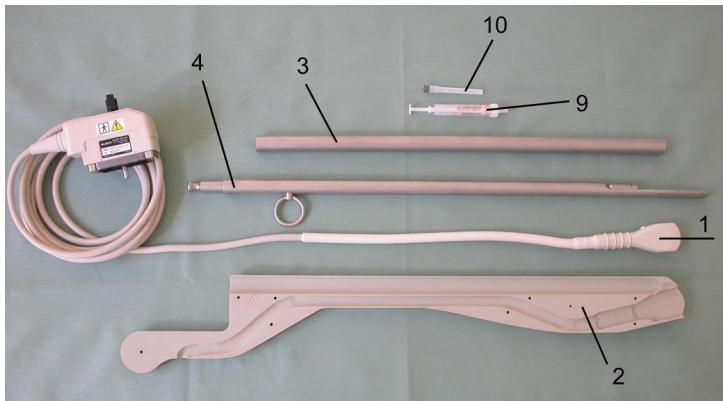


Figure 1. The ABB device. 1 = ultrasound transducer, 2 = probe holder, 3 = metal guide tube, 4 = syringe holder, 9 = aspiration syringe, 10 = needle

lated by the examiner together with an attached syringe (9) and needle (10). The aspiration syringe (5) is placed in the front part of the syringe holder. A connecting rod (6) controlling a syringe piston goes through the holder's corpus to the rear end of the device. The examiner moves the connecting rod through a guide ring (7) that enables the management of the aspiration from outside. The end of the syringe holder (8) is formed so as to allow for easy manipulation. A plastic syringe designed for blood acid-base analysis (Monovette<sup>®</sup> 2 ml, Sarstedt, Germany) or a specially modified 2-ml glass syringe (Chirana, Stara Tura, Slovak Republic) is used for aspiration. Standard disposable Luer-type needles are used for aspiration (mainly 0.7 × 40 mm, 22 G) (Figure 1 to 3). All parts of the ABB set were originally made by Medin, a.s., Nove Mesto, Czech Republic.

**Modified ovum pick-up equipment.** A convex ultrasound transducer (5 MHz Aloka UST 974-5, Japan) mounted on a plastic handle (Eickemeyer 303922, Germany) with a stainless-steel needle guide was used to control aspiration. A 17-gauge, 60 cm long aspiration needle (V-OPAA-1760, Cook, Australia) with a shortened Cook aspiration line (with a length of 20 cm), and a 18G – needle (Terumo, Japan) was inserted into the end of the tubing. The total volume of the aspiration needle and aspiration line was 0.9 ml. Aspirations were performed manually into 2 ml plastic syringes

(Monovette<sup>®</sup>, Sarstedt, Germany) or specially modified 2 ml glass syringes (Chirana, Stara Tura, Slovak Republic) connected to an 18G needle at a speed of approximately 0.2 ml/s (negative pressure 50 mm Hg) (Figure 4).

### Sample collection and analysis

**Experiment 1: Urine sample collection.** Urine was obtained from dairy cows by means of bladder catheterisation into five rubber balloons that were tied up to create reservoirs of homogenous biological fluid. Three groups of samples were aspirated from each reservoir, with the puncture point in each case being closed by haemostatic forceps after aspiration. The first sample from the group was collected by means of the aspiration part (syringe holder, syringe, needle) of the ABB set (ABB sample) with a commercial 22 gauge, 4 cm-long needle. The subsequent two samples were collected in sequence by means of the aspiration part (17 gauge, 60 cm-long aspiration needle and syringe) of the modified ovum pick-up equipment (OPUe): aerobic collection (AE sample) with air present in the aspiration tubing and anaerobic collection (AN sample) immediately after AE, when air had already been flushed from the tubing. Plastic syringes designed for blood acid-base analysis (Monovette<sup>®</sup> 2 ml, Sarstedt, Germany) were used for urine aspiration.



Figure 2. Detailed depiction of attachment of the aspiration syringe. 5 = site of attachment of aspiration syringe, 6 = connecting rod



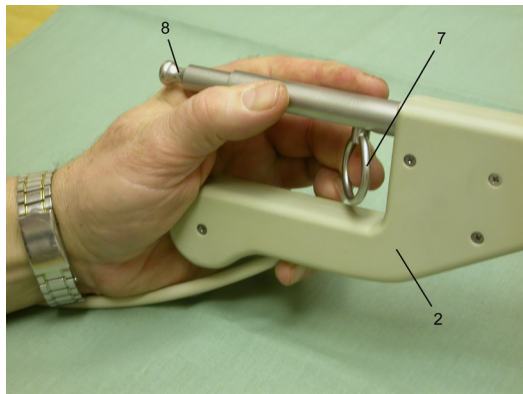


Figure 3. Detailed depiction of manipulation with the guide ring. 2 = probe holder, 7 = guide ring, 8 = end of the syringe holder

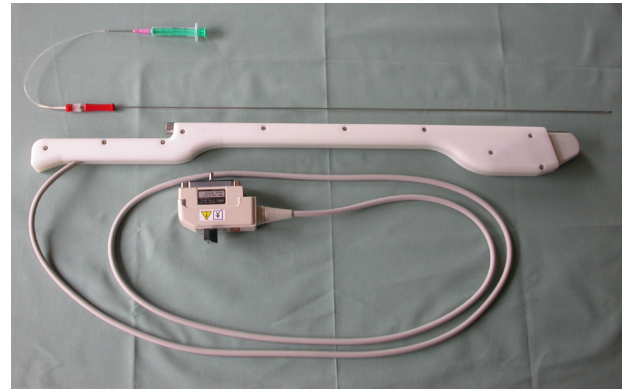


Figure 4. The modified ovum pick-up equipment

The volume of all samples was 1 ml. Air bubbles were expelled from the syringes immediately after collection and the samples were kept on ice until analysis. An acid-base balance analysis was performed on a Rapidlab<sup>®</sup> 855 analyser (Bayer, USA) within 30 min after sampling. The values of pH and the partial pressures of CO<sub>2</sub> (pCO<sub>2</sub>) and O<sub>2</sub> (pO<sub>2</sub>) were compared.

**Experiment 2: Cystic fluid aspiration.** Animals received an epidural anaesthesia (5 ml of 2% lidocaine, Lidocaine 2%, Fatro, Italy) to prevent straining during aspiration. After emptying the rectum, the vulva and perineal area were thoroughly cleaned and disinfected. The first sample of cystic fluid was aspirated using the ABB set. The probe holder was inserted deep into the fornix vaginae until the vaginal wall tightened. An examiner manipulated the ovary through the rectal wall and located the target cyst for aspiration on the scanner screen. The syringe holder, with the aspiration syringe and needle attached, was then inserted into a guide tube, and the examiner inserted the needle into the centre of the aspirated structure by manipulating the end of the syringe holder. Subsequently, the guide ring with its connecting rod and attached syringe piston were retracted slowly using the middle finger, and the fluid was aspirated into the syringe. The second and third samples were collected using the modified ovum pick-up equipment. After placing the OPUe holder with a transducer into the vagina, the aspiration needle connected to an aspiration line and syringe was inserted into the guide and advanced through the vaginal wall into the cyst lumen. Samples were aspirated in the same order as in Experiment 1: aerobic collection (AE) with air

present in the aspiration tubing and anaerobic collection (AN) immediately after AE. Plastic syringes designed for blood acid-base analysis (Monovette<sup>®</sup> 2 ml, Sarstedt, Germany) were used for cystic fluid aspiration in thirteen cows (Experiment 2P), while glass syringes (Chirana, Stara Tura, Slovak Republic) were used for cystic fluid aspiration in twelve cows (Experiment 2G). The air bubbles were expelled from the syringes immediately after collection and the samples were kept on ice until analysis. The volume of all samples was 1 ml. Cystic fluid samples with obvious blood contamination (pink or red colour,  $n = 8$ ) were omitted from further processing. An acid-base balance analysis was performed on the Rapidlab<sup>®</sup> 855 analyser (Bayer, USA) within 2–4 h after sampling. The values of pH and the partial pressures of CO<sub>2</sub> (pCO<sub>2</sub>) and O<sub>2</sub> (pO<sub>2</sub>) were compared.

### Statistical analysis

The results are collated as mean values with standard deviation. The evaluations of the statistical significance of the difference between samples were performed using Student's paired *t*-test. The analysis was carried out using Excel software.

## RESULTS

### Experiment 1

The values of the pH and gas analysis in the urine are listed in Table 1. The values of pH and pO<sub>2</sub>

Table 1. Values of pH, pCO<sub>2</sub> and pO<sub>2</sub> in urine after different sampling procedures

Urine (n = 15)	pH	pCO <sub>2</sub>	pO <sub>2</sub>
ABB	7.685 ± 0.305 <sup>a</sup>	11.13 ± 0.82 <sup>a</sup>	6.87 ± 2.59 <sup>a</sup>
AE	7.704 ± 0.305 <sup>b**</sup>	10.3 ± 0.61 <sup>b***</sup>	8.67 ± 1.47 <sup>b*</sup>
AN	7.692 ± 0.308 <sup>a</sup>	10.85 ± 0.62 <sup>a</sup>	7.02 ± 2.07 <sup>a</sup>

ABB = collection by the ABB device, AE = aerobic collection using modified ovum pick-up equipment, AN = anaerobic collection using modified ovum pick-up equipment  
 Values with different superscripts are different: \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001

were significantly higher and values of pCO<sub>2</sub> significantly lower in samples collected using OPUe under aerobic conditions. The values of estimated parameters in samples collected using the ABB set and the OPUe under anaerobic conditions were similar.

### Experiment 2

Ultrasound-guided transvaginal aspiration of cystic fluid using the ABB set was performed successfully in 33 cows (100%). Contamination of cystic fluid with blood occurred in eight cows (24.2%) in the second group of samples (modified OPU equipment). In those cows, sampling was suspended and the samples were not analysed. The pH values and gas analysis in the cystic fluid collected in plastic syringes are shown in Table 2. The values of pO<sub>2</sub> were significantly higher and values of pCO<sub>2</sub> significantly lower in samples collected using OPUe under aerobic conditions. The pH values in

samples collected using OPUe under aerobic conditions were higher than the pH values in samples collected using OPUe under anaerobic conditions. pH values, pO<sub>2</sub> and pCO<sub>2</sub> in samples collected using the ABB set and using OPUe under anaerobic conditions were similar.

The pH values and gas analysis of the cystic fluid collected using glass syringes are shown in Table 2. The pH values and pO<sub>2</sub> were significantly higher and values of pCO<sub>2</sub> significantly lower in samples collected using OPUe under aerobic conditions. The values of estimated parameters in samples collected using the ABB set and OPUe under anaerobic conditions were similar.

### DISCUSSION

In the present study we evaluated a new ABB set for follicular fluid collection that should prevent samples from coming into contact with air during aspiration. The construction of our device is based on the principle that the aspiration syringe should be placed in the front part of the probe holder. Therefore, after insertion of the holder into the vagina it is present immediately in front of the ovary. Aspirated fluid does not have to travel as long a distance, as is the case for the older OPUe device, and thus, is not contaminated by air present in the aspiration system, as we demonstrated in a previous study (Cech et al. 2011). We used our new ABB set for the aspiration of the first sample from each sequence, because we needed two repeated aspirations of each cyst; disposable 22G needles used in the ABB set cause lower mechanical trauma to the aspirated structure than the 17G needle used in the modified OPUe. Despite this, traumatization of the cysts as a result of the first puncture caused blood

Table 2. Values of pH, pCO<sub>2</sub> and pO<sub>2</sub> in cystic fluid after different sampling procedures, samples collected in plastic and glass syringes

	Cystic fluid (n = 13) plastic syringes			Cystic fluid (n = 12) glass syringes		
	pH	pCO <sub>2</sub>	pO <sub>2</sub>	pH	pCO <sub>2</sub>	pO <sub>2</sub>
ABB	7.332 ± 0.057 <sup>a</sup>	6.59 ± 0.86 <sup>a</sup>	10.17 ± 2.24 <sup>a</sup>	7.386 ± 0.055 <sup>a</sup>	6.39 ± 0.65 <sup>a</sup>	10.56 ± 2.28 <sup>a</sup>
AE	7.334 ± 0.057 <sup>a</sup>	6.22 ± 0.78 <sup>b***</sup>	12.68 ± 2.57 <sup>b*</sup>	7.400 ± 0.055 <sup>b***</sup>	5.98 ± 0.62 <sup>b***</sup>	12.35 ± 1.77 <sup>b**</sup>
AN	7.329 ± 0.054 <sup>b**</sup>	6.54 ± 0.87 <sup>a</sup>	9.99 ± 2.54 <sup>a</sup>	7.385 ± 0.054 <sup>a</sup>	6.35 ± 0.64 <sup>a</sup>	10.65 ± 2.27 <sup>a</sup>

ABB = collection by the ABB device, AE = aerobic collection using modified ovum pick-up equipment, AN = anaerobic collection using modified ovum pick-up equipment  
 Values with different superscripts are different: \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001

contamination during the second sampling in eight cows, and these samples had to be omitted from further investigation. We assume that, apart from accidental direct damage of blood vessels, the speed with which the ABB set and OPUE holders were changed after collecting the first sample is a critical factor for the prevention of blood contamination in the second sample. Nevertheless, we collected the first sample successfully in all thirty-three aspirated cows. Therefore, we conclude that the ABB set is suitable for the collection of fluid from ovarian structures. In Experiment 1, we compared the aspirating part of the ABB set to the aspirating part of the OPUE device under laboratory conditions within the urine reservoirs. In this experiment, we found significantly higher values of pH and  $pO_2$  and significantly lower values of  $pCO_2$  in urine samples collected using OPUE under aerobic conditions. The values of estimated parameters in samples collected using the ABB set and the OPUE under anaerobic conditions were similar. With these results we have confirmed our previous findings (Cech et al. 2011) that  $O_2$  from air present in the aspiration needle and line passes into the sample, and that  $CO_2$  escapes from the sample into the air present in the aspiration needle and line according to a concentration gradient. As a consequence of lower  $pCO_2$  we found higher pH values. The differences among estimated parameters were absolutely stable for all 15 triads of urine samples. This is in agreement with studies performed with animal blood in which it has been reported that  $CO_2$  can escape from the serum to the partial vacuum above by underfilling of the syringe or blood collection tubes (Bandi 1981; James et al. 1997; Constable 2008). Therefore, we conclude that the ABB set allows for anaerobic sampling of the fluid from a reservoir that is required for follicular fluid collection as well (Berg et al. 2005; Redding et al. 2006). This set allows for the study of changes in pH,  $pO_2$  and  $pCO_2$  during the final part of follicular development similarly as in human follicular fluid (Fischer et al. 1992), where a relationship between the developmental potential of the human oocyte and the dissolved oxygen content of follicular fluid has been proven (Van Blerkom et al. 1997). In Experiment 2P, we estimated the acid-base parameters of fluid obtained from ovarian cysts in the plastic syringes. The results were similar to those of Experiment 1, except that the pH values were similar to that of cystic fluid collected using the ABB set and the aerobic phase of the OPUE device. We assume

that the reason for these observations is the longer amount of time (2–4 h) necessary for transporting the samples from farms into the laboratory. During this time, small, insignificant changes in  $pO_2$  and  $pCO_2$  stored in plastic syringes occurred, probably causing changes in pH values in comparison to Experiment 1. In addition, there were differences in some of the groups of three samples of cystic fluid, and the values of estimated parameters were not as stable as in Experiment 1. In Experiment 2G, when samples of cystic fluid were collected in glass, we obtained the same results as in Experiment 1 with absolute stability in the differences for all 12 triads of samples. Our results are supported by evidence that syringe material and conditions and length of sample storage, significantly influence acid-base balance values (Szenci and Besser 1990; Wu et al. 1997; Beaulieu et al. 1999; Knowles et al. 2006; Cingi et al. 2009). Despite the fact that we have not performed a direct comparison of the samples from the same source stored in glass and plastic syringes, we agree with the assertion that if a delay in acid-base balance analysis is expected, the samples should be drawn and stored in glass (Knowles et al. 2006). We conclude that our new ABB set equipped with a glass aspiration syringe is useful for the anaerobic collection of fluid from ovarian follicular structures in cattle and for acid-base balance and gas analysis. In addition, we confirmed the effect on acid-base balance and gas analysis of the air present in the aspiration tubing, as well as the occurrence of pre-analytical changes in pH values in cystic fluid after lengthy storage in plastic aspiration syringes.

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Received: 2013–07–30

Accepted after corrections: 2013–10–31

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