Sel'skokhozyaistvennaya Biologiya [*Agricultural Biology*], 2013, № 4, p. 99-104

UDC 636.2-611.2.013:619:591.121

doi: 10.15389/agrobiology.2013.4.99rus
doi: 10.15389/agrobiology.2013.4.99eng

FUNCTIONAL FORMATION OF RESPIRATORY SYSTEM IN NEONATAL CALVES WITH DIFFERENT VIABILITY

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Received April 1, 2013

A b s t r a c t

Start of spontaneous breathing is one of the most important factors in adaptation of the newborn to environment. In contrast to the anatomical and physiological features, transient states of newborn appear at delivery or after the birth and stop when the adaptation period ends. However, under the influence of certain factors, such as the peculiarities of prenatal period and delivery, external conditions, feeding and maintenance of the newborn, they may become pathologic. At an industrial complex (Voronezh region), we studied the peculiarities of formation of the respiratory functions and respiratory water release in neonatal Red-and-White calves with different viability. Spirometric investigations were supplemented with measuring pH and concentration of electrolytes (sodium, potassium, calcium, magnesium) in the exhaled breath condensate with the ion-selective electrodes on the Olympus-400 analyzer («Beckman Coulter», USA), and atomic adsorption spectrophotometer («Perkin Elmer», USA). Acid-base status and venous blood gas composition also was tested with ABL-330 («Radiometer», Denmark). The role of physiological hyperventilation in the normalization of acid-base status and blood gases balance of the neonate, the dilatation of the bronchial tubes and lungs unfolding was shown. Breath water excretion in calves is closely connected with liquid secretion, produced in the alveoli and bronchi, the excess of which is absorbed by the epithelium of the upper airways. Hypoxia and acidosis dramatically increase the parasympathetic effects, causing bronchospasm and increased secretion in bronchial glands, which affects the intensity of the respiratory water generation and physico-chemical properties of the exhaled breath condensate.

Keywords: neonatal calves, respiratory system, viability, acid-base state, gas exchange, respiratory water release, exhaled breath condensate.

Adaptation to postnatal life is accompanied by the changes practically in all functional systems of an organism. In contrast to anatomico-physiological peculiarities borderline (transient) states of the newborn become evident during labor process or after birth and finish at the end of adaptation period [1, 2]. These states are physiological for healthy and viable animals but under certain conditions (depending on gestational age at birth, peculiarities of intrauterine period and labor course, feeding and keeping conditions for the newborn) they can become pathological [1]. So, it is necessary to study and control them.

The start of spontaneous breathing is one of the most important adaptation factors of the newborn to postnatal life.

Formation of spontaneous breathing function in children is rather completely described in a number of works [1-4]. Considering animals, this function is studied to a lesser degree [5-7], whereas metabolic and respiratory water release functions of the lungs are not studied yet.

The aim of this work was to study the peculiarities of respiratory and respiratory water release functions of respiratory organs in newborn calves with different viability.

Technique. The observations were held in 2011-2012 at and included newborn Red-Motley calves: group I (n = 10) – with normal viability, group II (n = 9) – with low viability. Body weight, temperature, heart rate, respiratory rate (RR), state of visible mucous membranes, number of incisors, muscle tone, the time of sucking reflex and confident standing pose start [8] were considered when evaluating the viability. Spirometric investigations were daily realized using spirometer SSP (Russia) and valve masks. RR, respiratory minute volume (RMV) and respiratory volume (RV) were determined. Exhaled breath condensate (EBC) in calves was sampled on the 1st, 3rd and 14th days of life during morning hours before feeding using the device, which we developed according to the method described earlier [9]. We considered EBC forming in calves during 1 minute and out of 100 L of exhaled breath. EBC samples were frozen and kept in liquid nitrogen at -196°C after receiving. Concentration of electrolytes (sodium, potassium, calcium, magnesium) in EBC and pH were measured immediately after unfreezing the samples. Sodium and potassium content in EBC was determined using ion-selective electrodes on the Olympus-400 analyzer ("Beckman Coulter", USA), calcium and magnesium - on atomic adsorption spectrophotometer model 703 ("Perkin Elmer", USA). Blood was sampled from calves' jugular veins during the same period as EBC. Acid-base balance (ABB) and gas composition of venous blood were tested on microanalyzer ABL-330 ("Radiometer", Denmark) for the following indices: pH, partial pressure of carbon dioxide (pCO_2) and oxygen (pO_2), concentration of carbonic acid (H_2CO_3), true bicarbonate (AB), sum (BB), excess or deficit (BE) of buffer bases (BE), hemoglobin saturation with oxygen (Sat. O_2).

Statistical data manipulation was realized using program Statistica v. 6.0. Significance of differences was evaluated by the method of pairwise comparisons using Student's t-criterion. The differences were considered reliable at significance level (error probability) of $p < 0.05$.

Results. Transient hyperventilation was typical of all newborn calves during the first days of life. It stopped in healthy viable calves during the first 48 h after birth. Heart rate and respiratory rate decreased by the 3rd day of life in comparison with the indices at the age of 1 day by 9.9 and 20.5% ($p < 0.05$), respectively, respiratory minute volume did not significantly change but respiratory volume increased by 44.2% ($p < 0.05$). Respiratory minute volume and respiratory volume significantly decreased on the 7th day comparing with the age of 1 day by 24.3 and 39.4% ($p < 0.05$), respectively. RMV and RV were the same as at the age of 3 days (table 1).

1. Age dynamics of indices of external respiration in Black-Motley calves in groups with different viability (*X*±*x*, working environment, Novousmanskiy Region, Voronezh Province)

Age, days	Index		
	RR, min.	RMV, L	RV, ml
1st	63.5±3.5	13.19±0.92	209.4±18.2
	51.3±4.9[b]	8.75±0.73[b]	172.5±9.4
3rd	50.5±3.5[a]	15.40±2.20	302.0±21.0[a]
	44.2±1.9[b]	10.08±0.73[b]	225.8±9.35[a, b]
7th	38.5±0.9[a]	11.60±0.84	301.0±9.8[a]
	36.3±2.3[a]	6.98±0.50[b]	195.6±11.7[b]
14th	30.7±1.8[a]	10.60±1.01	345.0±26.5[a]
	52.5±4.0[b]	11.34±2.13	215.3±25.8[b]

Note. RR, RMV and RV – respiratory rate, respiratory minute volume and respiratory volume, respectively. Above the line – indices in group I, under the line – indices in group II; [a] - the differences are significant relatively to the indices at the age of 1 day ($p < 0,05$), [b] - the differences are significant relatively to the indices in group I.

Calves with decreased viability demonstrated insignificantly decreased heart rate and respiratory rate on the 3rd day of life comparing with the age of 1 day by 7.0 and 13.8% ($p < 0.05$), respectively, respiratory volume in spite of increase by 29.2% ($p < 0.05$) remained significantly (by 25.2%, $p < 0.05$) lower than in healthy viable animals. RV increase on the 3rd day of their life was temporary and even on the 7th-14th day respiratory volume statistically did not significantly differ from the index at the age of 1 day.

Transient hyperventilation in calves is directed at elimination of postpartum metabolic acidosis. According to D.O. Melnichuk et al. [10] stabilization of metabolic parameters of ABB in calves occurs during the first hours after birth, whereas respiratory parameters come to normal in 24-36 h.

2. Age dynamics of indices of acid-base balance and gas composition blood indices in Red-Motley calves in groups with different viability (*X±x,* working environment, Novousmanskiy Region, Voronezh Province)

Index	Age, day		
	1st	3rd	7th
pH	7.33±0.01 7.27±0.01[b]	7.34±0.01 7.28±0.01[b]	7.34±0.01 7.29±0.01[b]
pCO_2, mm Hg	50.9±1.42 57.0±1.10[b]	51.8±2.01 54.6±2.38	52.5±2.40 55.1±1.63
AB, mmol/L	26.7±0.60 25.3±0.48[b]	26.5±0.51 24.5±1.71	26.8±0.86 25.7±0.79
BE, mmol/L	+0.24±0.17 –2.10±0.46[b]	+2.50±0.47[a] –2.93±1.48[b]	+2.64±0.76[a] –0.98±1.41[b]
H_2CO_3, mmol/L	1.53±0.03 1.72±0.03[b]	1.56±0.06 1.64±0.05	1.58±0.07 1.66±0.06
AB/H_2CO_3	17.5±0.24:1 14.7±0.36:1[b]	17.6±0.98:1 14.9±0.38:1[b]	17.2±0.81:1 15.5±0.63:1[b]
pO_2, mm Hg	24.9±1.,29 20.3±1.77[b]	29.1±0.28[a] 20.7±3.05[b]	38.8±1.17[a] 24.6±1.45[b]
Sat.O_2, %	31.7±3.04 22.8±5.00[b]	38.8±0.62[a] 23.9±7.56[b]	40.6±3.17[a] 30.4±2.96[b]
BB, µmol/L	41.7±0.24 39.9±0.50	44.5±0.34[a] 39.1±1.47[b]	44.6±0.42[a] 41.0±0.71[b]

Note. pCO_2 – partial pressure of carbon dioxide and oxygen, respectively, H_2CO_3 – concentration of carbonic acid, AB – concentration of true bicarbonates, BB - sum of buffer bases, BE – excess and deficit of buffer bases, Sat.O2 – hemoglobin saturation with oxygen. Above the line – in group I, under the line – in group II; [a] - the differences are significant relatively to the indices at the age of 1 day ($p < 0,05$), [b] - the differences are significant relatively to the indices in group I.

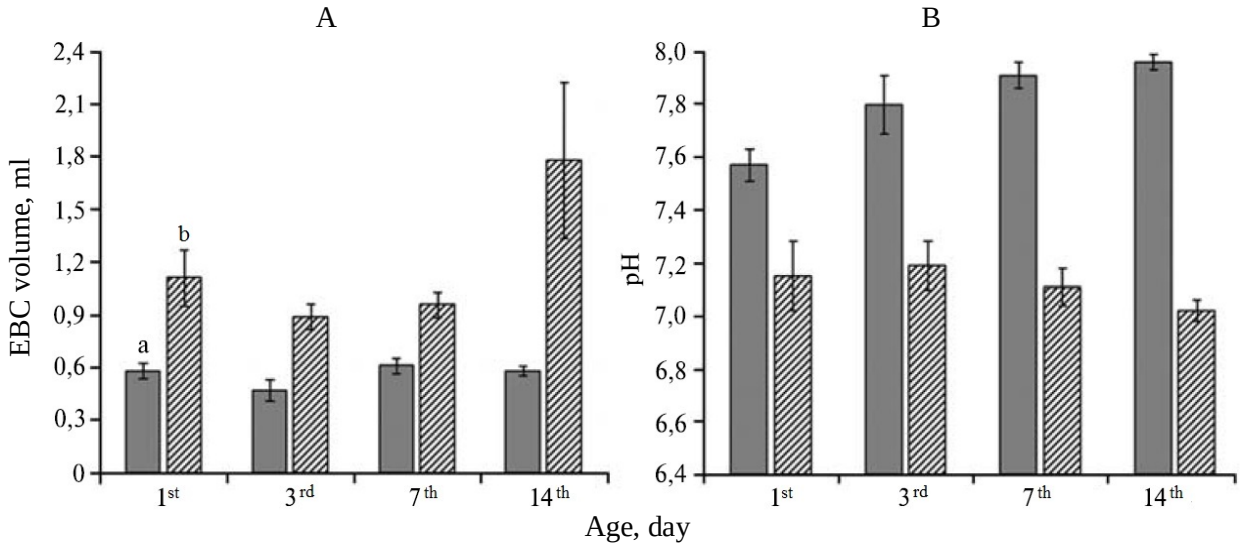
ABB in healthy viable calves in 24 h after birth was characterized as compensated respiratory acidosis (table 2). Normal blood pH indices, high partial pressure of carbon dioxide, excess of buffer bases and increased blood concentration of true bicarbonates proved it. It was possible to determine ABB in calves with decreased viability at the age of 1 day as a compensatory respiratory-metabolic acidosis that was proved by blood pH indices corresponding with the lowest norm threshold, increased partial pressure of carbon dioxide (respiratory component of ABB) and deficit of buffer bases (metabolic component of ABB) under normal blood content of true bicarbonates.

At the end of transient hyperventilation on the 3rd day of life in calves of group I an excess of blood buffer bases (alkali reserve) increased 10.4 times ($p < 0.05$) in comparison with the index at the age of 1 day, the sum of buffer bases – by 6.7% ($p < 0.05$) that proves complete metabolic compensation of postpartum acidosis. At the same time partial pressure of oxygen and hemoglobin saturation with oxygen increased by 16.9 and 22.4% ($p < 0.05$), respectively, the increase was even greater (by 55.8% and 28.1%, $p < 0.05$, respectively) by the 7th day. Other ABB indices (blood pH, partial pressure of carbon dioxide, bicarbonate ratio of AB/H_2CO_3) came to the norm during the first 24 hours after birth and did not significantly change after it.

Partial pressure of oxygen and hemoglobin saturation with oxygen in calves from group II since the 1st and 3rd days of life did not significantly change and only on the 7th day increased by 21.2 and 33.3% ($p < 0.05$), respectively. While healthy viable animals demonstrated stabilization of metabolic parameters of ABB during the 1st day after birth, animals with decreased viability demonstrated hypoxic state and respiratory-metabolic acidosis till the 7th day of life that is proved by low partial pressure of oxygen (24.60±1.45 mm Hg) and hemoglobin saturation with oxygen (30.40±2.96%), deficit of buffer bases (-0.98±1.41 mmol/L), low indices of sum of buffer bases (41.00±0.71 mmol/L) and blood pH (7.30±0.01) and physiological hyperventilation became pathological.

Emission of water vapors in calves when breathing is connected with production of liquid secretion in alveoli and bronchi. An excess of this secretion is absorbed by epithelium of upper air passages [9]. Respiratory water release is determined by filtration of water out of vessels of lesser circulation and upper air passages and regulated by blood supply. Water successively passes membranes and cytosol of capillaries of endothelium, basal membrane, alveolar

epithelium, highly selective layer of glycocalix and surfactant and also moves through intercellular slits and unions. At the same time endothelium of pulmonary capillaries actively participate in transport and control of number of hormones, enzymes and other biologically active substances [9, 11]. Hypoxia and acidosis sharply enhance parasympathetic effects causing bronchiospasm and increased secretion of bronchial glands [12] that naturally influences the intensity of respiratory water release.



Age dynamics of condensate volume forming out of 100 L of exhaled breath condensate (EBC), (A) **and pH of EBC** (B**) in Red-Motley calves with normal** (a, group I) **and decreased** (b, group II) **viability** (working environment, Novousmanskiy Region, Voronezh Province).

Respiratory water release volume in healthy viable calves since the 1st till the 14th day of life did not significantly change (fig.). At the end of transient hyperventilation under compensation of postpartum metabolic acidosis EBC pH in calves of group I increased by 3.0-5.2% ($p < 0.05$) in comparison with index at the age of 1 day (see fig.). Animals with decreased viability demonstrated no increase of EBC pH after some time and EBC volume forming from 100 L of expired air exceeded mean values of healthy viable animals by 57.3-91.4% ($p < 0.05$). Increased respiratory water release in calves of group II is probably connected not only with the intensification of bronchial gland secretion but also with the damage of cellular biomembranes of respiratory tract and disorders of processes of epithelial absorption and secretion of water and electrolytes.

3. Age dynamics of electrolyte concentration (mmol/L) in exhaled breath condensate in Red-Motley calves in groups with different viability (*X±x*, working environment, Novousmanskiy Region, Voronezh Province)

Age, day	Sodium	Potassium	Calcium	Magnesium
1st	3.32±0.16	0.32±0.03	0.16±0.02	0.10±0.01
	4.33±0.30[b]	0.42±0.04[b]	0.11±0.02[b]	0.07±0.01[b]
3rd	3.53±0.11	0.29±0.04	0.18±0.02	0.11±0.01
	4.37±0.46[b]	0.56±0.05[a, b]	0.10±0.02[b]	0.06±0.01[b]
7th	3.53±0.08	0.29±0.04	0.18±0.02	0.11±0.01
	4.44±0.27[b]	0.61±0.03[a, b]	0.11±0.02[b]	0.06±0.01[b]
14th	3.57±0.12	0.36±0.02	0.20±0.03	0.11±0.01
	4.25±0.10[b]	0.65±0.05[a, b]	0.10±0.01[b]	0.06±0.01[b]

Note. Above the line – indices in group I, under the line – indices in group II; [a] - the differences are significant relatively to the indices at the age of 1 day ($p < 0,05$), [b] - the differences are significant relatively to the indices in group I.

Ionic composition of EBC in some degree reflected these processes (tab. 3). Calves with decreased viability in 24 h after birth demonstrated higher sodium and potassium content in EBC than in animals of group I by 30.4 and 31.3% ($p < 0.05$), respectively, and calcium and magnesium – lower by 31.3 and 30.0% ($p < 0.05$), respectively. Potassium and EBC content increased in 72 hours after birth in comparison with the index at the age of 1 day by 33.3% ($p < 0.05$), by the 7th and 14th days of life – by 45.2 and 54.8% ($p < 0.05$), respectively. At the same

time concentration of other elements did not significantly change. Electrolytic EBC content in healthy viable calves did not significantly increase with growth. With the termination of cardiorespiratory adaptation by the 7^{th} day of life sodium content in EBC of calves of group II became higher than in animals of group I by 25,8 and 110,3% ($p < 0.05$), respectively, calcium and magnesium – lower by 38.9 and 45.5% ($p < 0.05$), respectively. An increase of respiratory water, sodium and potassium release and decreased calcium and magnesium release observed in calves of group I under breathing apparently reflect disorders of respiratory tract epithelium functions and surfactant system of lungs [13].

Thus, newborn calves with normal and decreased viability of formation of respiratory and water release functions of respiratory organs have significant differences during postnatal period. Transient hyperventilation terminates by the 3^{rd} day of life in healthy viable animals and leads to lung straighten that expresses through the increase of respiratory volume (respiration depth) under an invariable amount of respiratory minute volume. With the termination of physiological hyperventilation and elimination of postpartum metabolic acidosis pH of exhaled breath condensate increases in comparison with the index at the age of 1 day by 3.0-5.2% ($p < 0.05$) whereas the intensity of respiratory water release does not significantly change. Transient hyperventilation is less evident in calves with decreased viability and lasts till the 7^{th} day. Respiratory volume and pH of EBC in comparison with the indices at the age of 1 day do not significantly change and respiratory water volume released under breathing exceeds mean values in healthy viable calves 1.6-3.1 times ($p < 0.05$).

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