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*Short communication*

# The influence of selected techniques of bovine leukocyte isolation on their viability and metabolism

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## Abstract

The aim of the study was to assess the effect of selected isolation methods on the viability and metabolism of bovine leukocytes. The cells were isolated using a Ficoll 1077, Histopaque 1083 gradient and osmotic shock method, and Ficoll or Histopaque with osmotic shock. Evaluation were made of the total number of cells, viability after isolation and in 24h culture on RPMI 1640 medium and metabolism with NBT reduction assay. Microscopic and cytometric evaluation of the leukocytes revealed that the isolation methods applied had an influence on their number and viability. Based on the results it can be concluded that isolation methods of cells in a Histopaque or Ficoll yield highly pure cell fractions with high viability.

**Key words**: cell cultures, leukocytes, flow cytometry, microscope analysis

## Introduction

Blood is one of the main tissues used in laboratory tests for evaluating health, immune status and has an important role in scientific research. Isolation of leukocytes should make it possible to obtain a high number of cells, cell viability, membrane integrity, and stability of intracellular structures. Current cell isolation methods, such as Ficoll (van der Akker et al. 2008), Percoll or Histopaque (Halliday et al. 2005) are based on density gradient. Isolation is critical for cell metabolism; activity of enzymes, which leads to a reduction in generation of ROS, thus impairing the respiratory burst mechanism.

Taking into account the effect of different methods for isolating cells on their vital functions, and the widespread use of these cells in *in vitro*, the aim of the study was to evaluate the influence of isolation methods on viability and metabolism of bovine leukocytes.

## Materials and Methods

The blood was collected from healthy Holstein-Friesian cows from the external jugular vein into blood collection tubes (Greiner BioOne, Ge). Leukocytes isolated by osmotic shock were treated

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Table 1. Viability of leukocytes isolated with different technics after isolation and from 24h cultures in RPMI 1640 stained with trypan blue (TB) and propidium iodine (PI) (x ± SD)

| Viability (%) | Osmotic shock | | Ficoll | | Histopaque | | Ficoll ± o.s. | | Histopaque ± o.s. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TB | IP | TB | IP | TB | IP | TB | IP | TB | IP |
| After isolation | 95 ± 4 | 96 ± 3 | 99 ± 3 | 99 ± 2 | 98 ± 2 | 99 ± 3 | 93 ± 3 | 93 ± 4 | 95 ± 4 | 96 ± 3 |
| After 24h incubation | 85 ± 5 | 84 ± 8 | 91 ± 5 | 92 ± 4 | 91 ± 4 | 92 ± 4 | 83 ± 5 | 60 ± 4[a] | 87 ± 5 | 86 ± 4 |

[a] Significant differences in comparison to the leukocytes isolated by other technics ($p \leq 0.05$)

Table 2. The average (x ± SD) of viability of bovine leukocytes after isolation and from 24th cultures detected with cytometry

| Viability (%) | Osmotic shock | | Ficoll | | Histopaque | | Ficoll ± o.s. | | Histopaque ± o.s. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | L | N | L | N | L | N | L | N | L | N |
| After isolation | 97 ± 5[a] | 96 ± 6[a] | 99 ± 1 | 98 ± 4[a] | 99 ± 1 | 99 ± 1[a] | 94 ± 5[a] | 94 ± 6[a] | 96 ± 5 | 94 ± 2 |
| After 24h incubation | 89 ± 5 | 83 ± 5 | 96 ± 3 | 88 ± 5 | 96 ± 2 | 86 ± 8 | 61 ± 7* | 58 ± 8* | 90 ± 4 | 86 ± 6 |

* significant differences in comparison to leukocytes isolated by other technics ($p \leq 0.05$)
[a] significant differences in comparison to leukocytes from 24 th cell cultures ($p \leq 0.05$), Abbreviations: L-lymphocytes, N-neutrophils, o.s. – osmotic shock

according to Sláma et al. (2006). Isolation in Histopaque 1083 and Histopaque with osmotic shock was performed (Halliday et al. 2005). Isolation in Ficoll 1077 and Ficoll with osmotic shock was performed according van der Akker et al. (2008). The cells were suspended in RPMI 1640 medium with 10% FBS. The leukocyte culture was prepared (Urban-Chmiel et al. 2009). Cell viability was evaluated after isolation and after 24h incubation in RPMI 1640 staining with trypan blue, using a light microscope and propidium iodine staining, with cytometry (Slàma et al. 2006). The metabolism of the cells was determined with NBT colorimetric assay. The results were analysed with Statistica 9.0.

## Results and Discussion

Isolation methods used in this study have influence on viability, stability of intracellular structures and metabolism in leukocytes. Microscopic analysis of the cells showed that the isolation methods influence the number of cells obtained. The highest values 9.7 mln cells/ml were obtained by the osmotic shock, the lowest 6.6 and 7.6 mln cells/ml, respectively, were obtained in Ficoll or Histopaque with osmotic shock. The results indicate that the most useful method, with highest % of cells are Ficoll and Histopaque. The results are similar to those obtained by Michal et al. (1994). The experiments showed that the isolation methods had an influence on the viability of leukocytes. The highest % of live cells was obtained with the Ficoll and Histopaque. The least useful method for obtaining a pure leukocyte is the osmotic shock. These results are confirmed by results of Caswell et al. (1999). The lower percent of viability of the cells may have been due to the cells' high sensitivity to the isolation techniques applied. The viability of the leukocytes from the 24h culture was >90% in Ficoll and Histopaque. The values were higher ($p \leq 0.05$) than those found in cells isolated by osmotic shock and Ficoll with osmotic shock (Table 1). The lowest ($p \leq 0.05$) viability in the 24h culture was observed after isolation in Ficoll with osmotic shock (Table 2). The decrease ($p \leq 0.05$) revealed by cytometric analysis in the viability of cells from the 24h culture isolated in Ficoll with osmotic shock showed this method as most destructive. The increased mortality of the cells in the 24h culture may be the result of intracellular oxidation, which induce the release of proteolytic enzymes from intracellular granules, causing damage to the remaining cells in the culture. Analysis of the metabolism of leukocytes in NBT showed the highest absorbance (0.29±0.06) in case of the osmotic shock, Histopaque or Ficoll with osmotic shock. The lowest absorbance was observed in cells isolated in Histopaque (0.25±0.06) or Ficoll (0.24±0.02). The results were lower ($p \leq 0.05$) than those in the cells isolated by osmotic shock or Ficoll and Histopaque with osmotic shock. The findings obtained in NBT indirectly indicates the level of oxidative stress. The lowest absorbance ($p \leq 0.05$) in cells isolated in Histopaque and Ficoll showed that these methods had an insignificant effect on metabolism and oxidative stress. This is con-

firmed by the correlation between the viability and the rate of NBT. In cells isolated by osmotic shock, Histopaque or Ficoll with osmotic shock, despite the high viability of the cells, a negative effect was observed on metabolism resulting in an increase of absorbance in NBT. The correlation coefficients were over r=0.68 in cells isolated by osmotic shock and r=0.53 in Ficoll with osmotic shock. In Histopaque or Histopaque with osmotic shock the correlation was r=0.19 and r=0.22 respectively. This indicates a dependence between the isolation, the viability and metabolism of the cells. In conclusion, the results of the experiments indicate the isolation in Histopaque and Ficoll gradient, is most useful for obtaining of highly pure cells with high viability and stable intracellular structures.

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