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MILK ANALYSIS BY FLOW CYTOMETRY TO IDENTIFY SUBCLINICAL MASTITIS

Subclinical mastitis in cows is an expensive disease that is difficult to detect without special tests for diagnosis. The number of somatic cells (SCC) in milk is used as an important indicator of the udder health since SCC are parts of the innate immune system and are involved in protecting the mammary glands from infection.

The aim of our study was determined whether it is possible to identify subclinical mastitis in cattle at an early stage by a simple and fast flow cytometric method, and then presume the main cell populations in flow cytometric dot plots and, with these, to elaborate a method of mastitis prognostics. As a result of the microscopic method, three samples with contagious infection were identified. It was shown that milk from cows with mastitis contained populations of cells accompanying inflammation (suspected macrophages, granulocytes) that can be detected using frontal light scattering (FS) and right signals (SS).

Methods. Samples of milk were collected from 20 cows. Centrifugation was performed to isolate cells from milk, and fat was removed using filter

paper. Microscopy of cow milk cells was performed to exclude debris in the samples. After preparation, the milk cells were incubated with propidium iodide (PI), which differs between viable and non-viable cells. This procedure made it possible to localize cell types in a flow cytometry dot plot and to differentiate between viable and non-viable somatic cells. Also, in order to show the percentage of cell apoptosis in milk samples, we used Annexin V-fluorescein isothiocyanate.

Result.

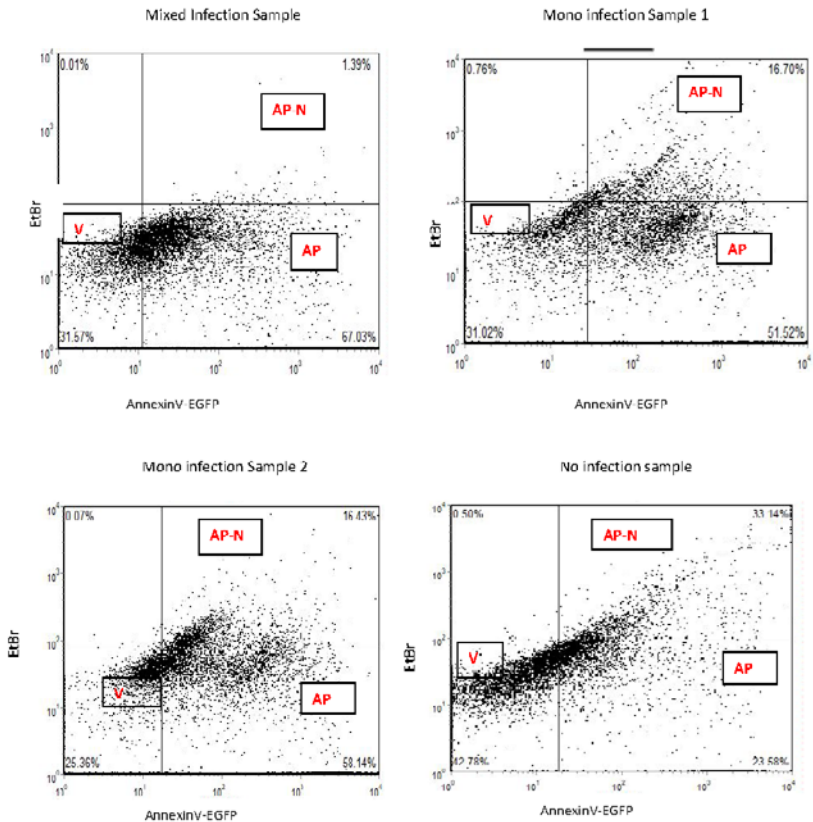


Figure 1. Graphs of a typical experiment for determining apoptosis and necrosis of milk cells with subclinical mastitis of cows, where V-living cells (EGFP- / EtBr -) are presented, V-AP cells (EGFP + / EtBr -) in early apoptosis; AP-N in late apoptosis (EGFP + / EtBr +) and cells during necrosis (EGFP- / EtBr +)

Necrotic cells SCC in a cow without mastitis was 24% compared to 67% in cows with mastitis. The number of apoptotic SCC in a cow without mastitis was 33% compared to 12% in cows with mastitis (Figure 1).

As a result, we can conclude that apoptosis is closely related to the functionality of neutrophils and macrophages [Van Oostveldt et al., 2002; Piepers et al., 2009] and plays a key role in the defense against invading pathogens and physiology of the inflammatory response [Boutet et al., 2004; Mehrzad et al., 2004]. The viability of milk cells only slightly and reproducibly decreased at various stages of washing and centrifugation, which clearly excludes the possibility that the wide range of somatic cell viability observed in this study is caused by sample processing. These various studies suggest that the viability of milk cells varies and depends on the status of the infection [Boutet et al., 2004; Mehrzad et al., 2004], the start time of infection [Sládek et al., 2005], as well as ratios and lactation [Van Oostveldt et al., 2001; Mehrzad et al., 2002].

Conclusions.

1. Apoptosis and necrosis of somatic cells make it possible to better assess the state of immune homeostasis, and that should be considered when diagnosing diseases.

2. The determination of the number of events of phagocytes by the mammary gland of cows allows a more objective assessment of the state of local immunity of the udder in the norm and in the event of a pathology.

3. Cell populations present in milk change during the development of inflammation and mastitis.

4. A heterogeneous population of cells may also be present in cows with a subclinical course of mastitis.

5. Flow cytometry of milk screening can serve as a diagnostic and prognostic criterion in monthly studies of all cows in the dairy herd to diagnose inflammation and predict the development of subclinical mastitis.

6. Correct cell monitoring can reduce the percentage of clinical outbreaks of mastitis, and help to avoid the culling of milk and cows.

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АДВЕНТИВНА ФРАКЦІЯ ФЛОРИ М. ІВАНО-ФРАНКІВСЬК

Останнім часом зростання кількості адвентивних видів є великою проблемою розвитку урбанофлор. Знання видового складу адвентивної фракції, біологічні та ценотичні особливості цих видів, міграційні можливості та здатність до натуралізації в даних умовах допоможуть визначити тенденції розвитку урбанофлори, а також зв'язок її з іншими флорами та наслідки загального процесу антропогенної трансформації