Comparison of cell viability in fresh and frozen porcine colostrum

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Introduction

The ingestion of colostrum by piglet in the first hours of life is crucial for its health status, since the transfer of maternal immunity occurs passively through colostrum (3). Colostrum contains lymphocytes (T and B cells), phagocytes (neutrophils and macrophages), and epithelial cells (1). Colostrum can be storage at 4°C or freezing, however, the freezing and thawing process can damage the cells (2). As little is known about the cell viability and immunogenicity of colostrum, this study aimed to evaluate fresh and frozen porcine colostrum and the effect on immune cells viability.

Materials and Methods

Colostrum was manually collected from 20 sows (multiparous and primiparous) after farrowing and was diluted in PBS containing 5% fetal calf serum. Then, it was centrifuged and the fat upper layer was discarded. Colostrum evaluation was carried out in fresh and in frozen colostrum after 7 and 15 days of storage at -20°C. The viability of cells was evaluated by trypan blue exclusion test. The flow cytometry was performed with Accuri® flow cytometer (Becton Dickinson) and 50.000 events were analyzed. For this, colostrum samples were incubated with the following antibodies: isotypes controls; mouse anti-pig CD3, CD4, CD8, granulocytes, macrophages, CD27, CD45RA, CD79a, CD5, CD14, CD16, IgM, CD45RA/B220, and CD335. The data were pre analyzed by the K-S test. The Friedmann test was used for longitudinal analyses, with the Student-Newman-Keuls posttest, whereas transversal analyses were performed using the Kruskal-Wallis test, with the Student-Newman-Keuls posttest.

Results

Table 1 shows the evolution of granulocyte, NK cells ($CD3^-CD8^{low}CD335^+$), $macrophages^+$, monocyte/ macrophage ($macrophages^+CD14^+CD16^+$), lymphocytes ($CD3^+$, $CD5^+$, $CD79a^+IgM^+$, $CD79a^+$ $CD45R/B220^+$, $CD5^+$) percentage in fresh and frozen colostrum after 7 and 15-days period. The colostrum freezing resulted in a significant decrease of $CD4^+$ $CD3^+$ T cells and its subsets, such as naive $CD4^+$ T cells ($CD4^+CD27^+CD45RA^+$), central memory $CD4^+$T ($CD4^+CD27^+CD45RA^-$), and effector memory $CD4^+$T cells ($CD4^+CD27^-CD45RA^-$). Besides, $CD8^+$ $CD3^+$ T cells and a subset of central memory $CD8^+$T cells ($CD8^+CD27^+CD45RA^+$) were also decreased with colostrum freezing. Moreover, B-lymphocyte ($CD79a^+$) showed a decrease after freezing, but B-lymphocyte subsets did not differ. No variation was observed on the immune cells ($p>0.05$) after frozen for 7 up to 15 days, although the number of them were decreased after 15 days at -20°C.

Table 1. Comparison of cell viability in fresh and frozen porcine colostrum.

Immune cells	Storage conditions		
	Fresh	Frozen for 7 days	Frozen for 15 days
GRANULOCYTES	9.282±1.549	7.140±1.215	8.645±1.890
MACROPHAGES$^+$	40.61±1.265	40.56±1.142	37.42±1.356
CD14$^+$CD16$^+$	14.15±1.441	13.20±0.878	10.80±1.000
CD335$^+$	14.93±1.117	14.39±1.549	15.85±1.850
CD79a$^+$	15.00±1.807^a	5.384±0.937^b	6.132±1.485^b
CD19$^+$, IGM$^+$	22.65±2.372	20.18±3.314	19.53±1.970
CD19$^+$, CD45R/B220$^+$	3.399±0.366	2.406 ±0.834	1.974±0.556
CD5$^+$	38.45±1285^a	34.93±0.972^b	32.15±1.225^b
CD3$^+$CD4$^+$	10.72±1.535^a	6.121±1.542^{ab}	5.219±1.251^b
CD4$^+$CD27$^+$CD45RA$^-$	13.90±2.132^a	10.53±1.091^a	4.835±1.415^b
CD4$^+$CD27$^+$CD4RA$^+$	4.790±0.861^b	3.944±0.634^b	1.94±1.999^a
CD4$^+$CD27$^-$CD45RA$^-$	25.77±2.265^a	22.50±2.002^a	9.575±1.534^b
CD3$^+$CD8$^+$	7.580±0.954^a	4.190±0.982^b	3.976±0.895^b
CD8$^+$CD27$^+$CD45RA$^-$	14.66±1.772	11.32±1.138	9.893±1,339
CD8$^+$CD27$^+$CD45RA$^+$	5.836±1.278	4.468±0.647	5.394±1.229
CD8$^+$CD27$^-$CD45RA$^-$	22.10±1.977^a	20.30±1.948^{ab}	14.96±1.591^b

$^{a, b}$ $p<0.05$ *vs other treatment.*

Conclusions and Discussion

Taking into account only the stability of the immunological components studied, it seems reasonable to recommend freezing storage for 15 days for −20°C. Under this condition, the contents of almost all the bioactive factors evaluated were maintained, except for subsets of T-lymphocytes and B-lymphocytes cells. A longstanding porcine colostrum storage study is underway. The understanding of the effect of storage on immune cells in colostrum is of great importance, mainly for the acquisition of passive immunity by the newborn piglet fed with frozen colostrum.

References

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