Acta Veterinaria (Beograd), Vol. 47. No. 4, 185–194, 1997.

UDK 619:639.2.082.454:612.617

SOME QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF SERUM LIPOPROTEINS IN BOVINE SEMINAL PLASMA

JELKA STEVANOVIĆ,* V. STOJIĆ*, GORDANA GAĐANSKI–OMEROVIĆ* and JELENA VITIĆ**

*Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, Belgrade **Institute for Medical Research, Belgrade

(Received, 5. May 1997.)

The total lipoprotein, cholesterol and phospholipid concentrations were investigated in this paper in blood sera and seminal plasma from bulls. The relationship between the lipoproteins in these two body fluids was evaluated. The results obtained demonstrated that bovine seminal plasma has a significantly lower concentration of total lipoproteins, cholesterol and phospholipids than blood serum. As lipoproteins are serum macromolecules, this finding suggests that a physiological barrier may exist interposed between blood and testis tissue, the function of which is to control the transportof macromolecules from blood to seminal fluid.

During PAGE bull serum and seminal plasma lipoproteins were separated into six fractions. The electrophoretic mobility of each separated lipoprotein fraction, in both body fluids was similar. According to the highest electrophoretic mobility and relative contribution the dominant VI-th fraction, in both fluids consisted of alpha-lipoproteins, also known as HDL-s. They are cholesterol rich lipoprotein fractions. As they are the dominant lipoprotein fraction in bull seminal plasma, we suppose that they probably serve as a source of cholesterol, necessary for spermatozoal maturation and membrane stability, prior to touching the ovum, in the female reproductive tract.

Key words: bull seminal plasma, lipoproteins, cholesterol, phospholipids.

INTRODUCTION

The first examination of Larson and Salisbury (1954) suggested that lipoproteins do not exist in bull seminal plasma, or are present only in traces. However, later many data cogently showed that plasma lipoproteins are an important consituent of bull seminal plasma. According to Pursel and Graham (1967) and Jain and Anan (1976-a, b) their concentration in bull seminal plasma varies widely (1.04-9.18 g/l). In bovine sera the total liporptein concentration is between 6-8 g/l (Chapman, 1980; Vitić, 1993), and alpha lipoproteins,also known as HDLs, are the dominant fraction (Nikolić et al., 1959; Dimopoullos, 1971; Chapman, 1980; Vitić, 1993). The mean value is between 1-3,4 g/l. HDLs are

NEKE KVANTITATIVNE I KVALITATIVNE KARAKTERISTIKE SERUMSKIH LIPOPROTEINA PRISUTNIH U SEMENOJ PLAZMI BIKA

JELKA STEVANOVIĆ, V. STOJIĆ, GORDANA GAĐANSKI-OMEROVIĆ i JELENA VITIĆ*

SADRŽAJ

U ovom radu su ispitivane koncentracije i međusobni odnosi ukupnih lipoproteina, holesterola i fosfolipida u uzorcima krvnog seruma (7), i semenih plasmi (45) dobijenih od kvalitetnih ejakulata istih bikova frizijske rase. Ustanovljeno je da semena plazma bika ima statistički značajno nižu koncentraciju ukupnih lipoproteina, holesterola i fosfolipida od krvnog seruma istih bikova. Smatramo da ovaj nalaz potkrepljuje, u literaturi prisutnu tezu o postojanju barijere "krv-testis", koja kontroliše prolaz makromolekula krvi u tkivo testisa.

Pri elektroforezi na filter hartiji hipoproteini obe ispitivane telesne tečnosti razdvojeni su na po tri frakcije: neutralne lipide, beta i alfa lipoproteine. Daleko bolje razdvajanje lipoproteina obe telesne tečnosti postignuto je pri PAGE (na 7.5% gelu). Tako su lipoproteini krvnog seruma i seme plazme bika razdvojeni na po šest frakcija, približno iste elektroforetske pokretljivosti. Elektroforetski najbrža i kvantitativno najzastupljenija, u obe telesne tečnosti bika, bila je VI frakcija. Na osnovu ovih podataka smo zaključili da ona pripada alfa lipoproteinima (označavaju se još i kao HDL). Poznato je već, da su oni dominantna lipoproteinska frakcija u goveđem krvnom serumu. Alfa lipoproteini su bogat izvor holesterola, pa možemo predpostaviti da oni i u semenoj plazmi služe kao donori holesterola, potrebnog u toku sazrevanja spermatozoida, i neophodnog za očuvanje stabilnosti njihove membrane do eventualnog kontakt sa jajnom ćelijom.

Sera and seminal plasma lipoproteins were electrophoretically separated by paper electrophoresis (Nikolić et al. 1958), and by PAGE (a mixture of 3% and 7,5% gels), using TRIS buffer (pH 8.33), as described by Devis (1964). The relative contribution of each separated lipoprotein fraction were estimated photometrically, at 588 nm.

RESULTS AND DISCUSSION

The results obtained (Table 1) demonstrated that the total lipoprotein concentration in bull seminal plasma was 6.44± 0.33 g/l, which is about 12% lower than in blood sera (7.34± 0.30 g/l). The this difference is highly significant ($p<0.01$) and is in agreement with the results of other authors (Pursel and Graham, 1967; Jain and Anan, 1976-a, b). The total cholesterol concentration in the examined bull seminal plasma samples was 1.66± 0.14 mmol/l, and the total phospholipid concentration was 2.96± 0.32 mmol/l. They were also significantly lower ($p<0.001$) in seminal plasma than in the blood serum (Table 2.). That is why we suppose that the pronounced disparity in the total lipoprotein, cholesterol and phospholipid concentrations between these two bovine body fluids may confirm the existence of on interposed physiological barrier between blood and testis tissue, which controls the passage of molecules from blood to seminal fluid (Knoll and Neil, 1988).

Table 1. The concentration of total lipoproteins in bull sera (BS) and seminal plasma

Bull	BS (g/l) (n=7)	Seminal plasma (g/l) (n=45)	
1.	7.56	6 samples	6.36
2.	7.00	7 samples	6.19
3.	7.56	7 samples	6.02
4.	7.00	6 samples	6.56
5.	7.66	7 samples	6.63
6.	7.06	6 samples	6.62
7.	7.56	6 samples	6.85
$\bar{x}$	7.34		6.44
SD	0.30		0.33
SE	0.11		0.05
CV (%)	4.09		5.12

Bull seminal plasma and sera lipoproteins were separated by paper electrophoresis into three fractions: alpha, beta and neutral lipids (Figure 1.).In both examined body fluids the alpha lipoprotein fraction was the dominant one.

Better separation of serum and seminal plasma lipoproteins was obtained by PAGE electrophoresis (Figure 2). In thay way, lipoproteins of both fluids were separated into six fractions. The electrophoretic mobility of each serum and seminal plasma fraction was similar.

Table 2. The concentration of total cholesterol, phospholipids, and the relative contribution of blood serum (BS) and seminal plasma (Spl) lipoprotein fractions separated by PAGE,together with the statistical significance of differences.

	BS (=7)	Spl (n=45)	Statistical significance
Total cholesterol (mmol/l)	5.91 ± 0.26	1.66 ± 0.14	$p < 0.001$
Total phospholipids (mmol/l)	3.89 ± 0.11	2.96 ± 0.32	$p < 0.001$
Lipoprotein fractions (%)			
I	22.29 ± 1.00	22.37 ± 0.65	ns
II	17.02 ± 0.81	17.48 ± 0.63	ns
II	2.18 ± 0.13	2.78 ± 0.29	$p < 0.001$
IV	4.04 ± 0.26	2.47 ± 0.26	$p < 0.001$
V	3.00 ± 0.11	2.75 ± 0.37	ns
VI	51.47 ± 1.26	52.09 ± 0.56	ns

In both examined fluids the relative contribution (Table 2.) and elektrophoretic mobility (Figure 2.) of the VI-th lipoprotein fraction was the highest. These findings suggest that the VI-th fraction of serum and seminal plasma consists of α-lipoproteins (HDL). This is in agreement with many data (Nikolić, 1959; Dimopoullos, 1971; Chapman, 1980; Vitić and Stevanović, 1993). Alpha lipoproteins are known as a cholesterol-rich lipoprotein fraction,and serve as cholesterol transporters in body fluids. Manjunath et al. (1988) concluded that through their apoA-I, they adhere to the extracellular surface of spermatozoal membranes, in order to provide cholesterol efflux. This is a very important process which occurs during the capacitation of spermatozoa in the female reproductive tract. Leoung (1987) suggested that bull seminal plasma has many floating vesicles with cholesterol, which adhere to the acrosomal membrane in order to stabilize it and prevent any interruption before connecting to the ovum.

In bull seminal plasma, the relative amount of the III-th lipoprotein fraction was significantly higher ($p<0.001$), but the relative contribution of the IV-th fraction was significantly lower($p<0.001$) than in the blood sera (Table 2.). The other separated fractions did not differ significantly in relative amount of lipoprotein fractions, between the two fluids.

Acknowledgement:

This work was supported by a Grant from the Medical Research Foundation of Serbia.

REFERENCES

1. *Chapman, M. J.* 1980. Animal lipoproteins: Chemistry, structure, comparative aspects. *(Review). J. Lipid. Res. 21*, 798-854.

2. *Davis, B. J.* 1964. Disc electrophoresis-II Methods and applications at human serum proteins. *Ann. New. York. Acad. Sci. 121*, 404-428.

Table 2. The concentration of total cholesterol, phospholipids, and the relative contribution of blood serum (BS) and seminal plasma (Spl) lipoprotein fractions separated by PAGE,together with the statistical significance of differences.

	BS (=7)	Spl (n=45)	Statistical significance
Total cholesterol (mmol/l)	5.91 ± 0.26	1.66 ± 0.14	$p < 0.001$
Total phospholipids (mmol/l)	3.89 ± 0.11	2.96 ± 0.32	$p < 0.001$
Lipoprotein fractions (%)			
I	22.29 ± 1.00	22.37 ± 0.65	ns
II	17.02 ± 0.81	17.48 ± 0.63	ns
II	2.18 ± 0.13	2.78 ± 0.29	$p < 0.001$
IV	4.04 ± 0.26	2.47 ± 0.26	$p < 0.001$
V	3.00 ± 0.11	2.75 ± 0.37	ns
VI	51.47 ± 1.26	52.09 ± 0.56	ns

In both examined fluids the relative contribution (Table 2.) and elektrophoretic mobility (Figure 2.) of the VI-th lipoprotein fraction was the highest. These findings suggest that the VI-th fraction of serum and seminal plasma consists of α-lipoproteins (HDL). This is in agreement with many data (Nikolić, 1959; Dimopoullos, 1971; Chapman, 1980; Vitić and Stevanović, 1993). Alpha lipoproteins are known as a cholesterol-rich lipoprotein fraction,and serve as cholesterol transporters in body fluids. Manjunath et al. (1988) concluded that through their apoA-I, they adhere to the extracellular surface of spermatozoal membranes, in order to provide cholesterol efflux. This is a very important process which occurs during the capacitation of spermatozoa in the female reproductive tract. Leoung (1987) suggested that bull seminal plasma has many floating vesicles with cholesterol, which adhere to the acrosomal membrane in order to stabilize it and prevent any interruption before connecting to the ovum.

In bull seminal plasma, the relative amount of the III-th lipoprotein fraction was significantly higher ($p<0.001$), but the relative contribution of the IV-th fraction was significantly lower($p<0.001$) than in the blood sera (Table 2.). The other separated fractions did not differ significantly in relative amount of lipoprotein fractions, between the two fluids.

Acknowledgement:

This work was supported by a Grant from the Medical Research Foundation of Serbia.

REFERENCES

1. *Chapman, M. J.* 1980. Animal lipoproteins: Chemistry, structure, comparative aspects. *(Review). J. Lipid. Res. 21*, 798-854.

2. *Davis, B. J.* 1964. Disc electrophoresis-II Methods and applications at human serum proteins. *Ann. New. York. Acad. Sci. 121*, 404-428.

Sera and seminal plasma lipoproteins were electrophoretically separated by paper electrophoresis (Nikolić et al. 1958), and by PAGE (a mixture of 3% and 7,5% gels), using TRIS buffer (pH 8.33), as described by Devis (1964). The relative contribution of each separated lipoprotein fraction were estimated photometrically, at 588 nm.

RESULTS AND DISCUSSION

The results obtained (Table 1) demonstrated that the total lipoprotein concentration in bull seminal plasma was 6.44± 0.33 g/l, which is about 12% lower than in blood sera (7.34± 0.30 g/l). The this difference is highly significant ($p<0.01$) and is in agreement with the results of other authors (Pursel and Graham, 1967; Jain and Anan, 1976-a, b). The total cholesterol concentration in the examined bull seminal plasma samples was 1.66± 0.14 mmol/l, and the total phospholipid concentration was 2.96± 0.32 mmol/l. They were also significantly lower ($p<0.001$) in seminal plasma than in the blood serum (Table 2.). That is why we suppose that the pronounced disparity in the total lipoprotein, cholesterol and phospholipid concentrations between these two bovine body fluids may confirm the existence of on interposed physiological barrier between blood and testis tissue, which controls the passage of molecules from blood to seminal fluid (Knoll and Neil, 1988).

Table 1. The concentration of total lipoproteins in bull sera (BS) and seminal plasma

Bull	BS (g/l) (n=7)	Seminal plasma (g/l) (n=45)	
1.	7.56	6 samples	6.36
2.	7.00	7 samples	6.19
3.	7.56	7 samples	6.02
4.	7.00	6 samples	6.56
5.	7.66	7 samples	6.63
6.	7.06	6 samples	6.62
7.	7.56	6 samples	6.85
$\bar{x}$	7.34		6.44
SD	0.30		0.33
SE	0.11		0.05
CV (%)	4.09		5.12

Bull seminal plasma and sera lipoproteins were separated by paper electrophoresis into three fractions: alpha, beta and neutral lipids (Figure 1.).In both examined body fluids the alpha lipoprotein fraction was the dominant one.

Better separation of serum and seminal plasma lipoproteins was obtained by PAGE electrophoresis (Figure 2). In thay way, lipoproteins of both fluids were separated into six fractions. The electrophoretic mobility of each serum and seminal plasma fraction was similar.

NEKE KVANTITATIVNE I KVALITATIVNE KARAKTERISTIKE SERUMSKIH LIPOPROTEINA PRISUTNIH U SEMENOJ PLAZMI BIKA

JELKA STEVANOVIĆ, V. STOJIĆ, GORDANA GAĐANSKI-OMEROVIĆ i JELENA VITIĆ*

SADRŽAJ

U ovom radu su ispitivane koncentracije i međusobni odnosi ukupnih lipoproteina, holesterola i fosfolipida u uzorcima krvnog seruma (7), i semenih plasmi (45) dobijenih od kvalitetnih ejakulata istih bikova frizijske rase. Ustanovljeno je da semena plazma bika ima statistički značajno nižu koncentraciju ukupnih lipoproteina, holesterola i fosfolipida od krvnog seruma istih bikova. Smatramo da ovaj nalaz potkrepljuje, u literaturi prisutnu tezu o postojanju barijere "krv-testis", koja kontroliše prolaz makromolekula krvi u tkivo testisa.

Pri elektroforezi na filter hartiji hipoproteini obe ispitivane telesne tečnosti razdvojeni su na po tri frakcije: neutralne lipide, beta i alfa lipoproteine. Daleko bolje razdvajanje lipoproteina obe telesne tečnosti postignuto je pri PAGE (na 7.5% gelu). Tako su lipoproteini krvnog seruma i seme plazme bika razdvojeni na po šest frakcija, približno iste elektroforetske pokretljivosti. Elektroforetski najbrža i kvantitativno najzastupljenija, u obe telesne tečnosti bika, bila je VI frakcija. Na osnovu ovih podataka smo zaključili da ona pripada alfa lipoproteinima (označavaju se još i kao HDL). Poznato je već, da su oni dominantna lipoproteinska frakcija u govedem krvnom serumu. Alfa lipoproteini su bogat izvor holesterola, pa možemo predpostaviti da oni i u semenoj plazmi služe kao donori holesterola, potrebnog u toku sazrevanja spermatozoida, i neophodnog za očuvanje stabilnosti njihove membrane do eventualnog kontakt sa jajnom ćelijom.

Acta Veterinaria (Beograd), Vol. 47. No. 4, 185–194, 1997.

UDK 619:639.2.082.454:612.617

SOME QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF SERUM LIPOPROTEINS IN BOVINE SEMINAL PLASMA

JELKA STEVANOVIĆ,* V. STOJIĆ*, GORDANA GAĐANSKI–OMEROVIĆ* and JELENA VITIĆ**

*Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, Belgrade **Institute for Medical Research, Belgrade

(Received, 5. May 1997.)

The total lipoprotein, cholesterol and phospholipid concentrations were investigated in this paper in blood sera and seminal plasma from bulls. The relationship between the lipoproteins in these two body fluids was evaluated. The results obtained demonstrated that bovine seminal plasma has a significantly lower concentration of total lipoproteins, cholesterol and phospholipids than blood serum. As lipoproteins are serum macromolecules, this finding suggests that a physiological barrier may exist interposed between blood and testis tissue, the function of which is to control the transportof macromolecules from blood to seminal fluid.

During PAGE bull serum and seminal plasma lipoproteins were separated into six fractions. The electrophoretic mobility of each separated lipoprotein fraction, in both body fluids was similar. According to the highest electrophoretic mobility and relative contribution the dominant VI-th fraction, in both fluids consisted of alpha-lipoproteins, also known as HDL-s. They are cholesterol rich lipoprotein fractions. As they are the dominant lipoprotein fraction in bull seminal plasma, we suppose that they probably serve as a source of cholesterol, necessary for spermatozoal maturation and membrane stability, prior to touching the ovum, in the female reproductive tract.

Key words: bull seminal plasma, lipoproteins, cholesterol, phospholipids.

INTRODUCTION

The first examination of Larson and Salisbury (1954) suggested that lipoproteins do not exist in bull seminal plasma, or are present only in traces. However, later many data cogently showed that plasma lipoproteins are an important consituent of bull seminal plasma. According to Pursel and Graham (1967) and Jain and Anan (1976-a, b) their concentration in bull seminal plasma varies widely (1.04-9.18 g/l). In bovine sera the total liporptein concentration is between 6-8 g/l (Chapman, 1980; Vitić, 1993), and alpha lipoproteins,also known as HDLs, are the dominant fraction (Nikolić et al., 1959; Dimopoullos, 1971; Chapman, 1980; Vitić, 1993). The mean value is between 1-3,4 g/l. HDLs are