BOVINE SERUM ALBUMIN AS A POTENTIAL PROTEIN SUPPLEMENT FOR *IN VIRO* CULTIVATION OF SPERMATOZOA

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ABSTRACT

Bovine serum albumin (BSA) appears to be a good protein substrate for spermatozoa cultivation because of its stability, good amino acid profile and protective functions. Therefore, the aim of our study was to analyse the influence of different BSA concentrations used in culure medias on the viability and motility of bull spermatozoa during a 24-hour *in vitro* cultivation. We compared native samples (N) with samples to which a commercial egg yolk medium and medias with different BSA concentrations were added. The analysis was carried out during three time periods (time 0, time 1 hour and time 24 hours) using Sperm Vision™ CASA system. Our experimental study shows an obvious time-dependent decrease of the spermatozoa motility in all experimental groups cultured for 24 hours. The highest inhibition of spermatozoa motility was detected in the native group (28.86±9.087%), followed by groups B (42.89±13.17%) and A (52.28±16.12%) containing a.o. 10% BSA. The highest motility value was found in group D (20% BSA and 5% glucose; 66.46±9.986%) and group R (egg yolk medium; 62.81±8.044%). Based on our experiments we may assume that BSA is a good protein alternative for a long-term *in vitro* spermatozoa cultivation.

Key words: bovine serum albumin, culture media, bovine spermatozoa, motility

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INTRODUCTION

Artificial insemination has become one of the most important pillars in animal biotechnology. Especially in the cattle artificial insemination (AI) industry, bull semen quality is highly important to ensure a good biological matherial for breeding as well as a certain biodiversity protection (Ibrahim et al., 2000; Lukáč et al., 2007). Before preservation and distribution for use in AI, several laboratory tests are routinely conducted in an attempt to predict the fertility of the processed semen. Such tests include but are not limited to sperm motility (Ibrahim et al., 2000). Working with semen samples in laboratory environment recquires a provision of optimal conditions for their in vitro cultivation.

Sperm in vitro cultivation is a relatively complicated process, since the sperm cells are extremely sensible to ex vivo conditions. Semen cultivation media ususally contain glucose or fructose as an energetic substrate, egg yolk as a protein supply and glycerol (McPhie et al., 2000; Matsuoka et al., 2006). However, preparation of a uniform semen cultivation media may vary because of the quality of the egg yolk. Therefore, investigations have been conducted to find an alternative protein substrate for the sperm cultivation (Muller-Schlosser et al., 1995).

Bovine serum albumin (BSA) has been used recently as a protein alternative to egg yolk. Most previous studies have reported that the role of albumin appears to be protective as a result of its "general capacity and ability to trap toxic substances in the culture media" (Yamane et al., 1976) and lipid binding properties (Fox and Flynn, 2003). Investigations using semen from various mammalian species have indicated that bovine serum albumin stimulates sperm motility by an unknown mechanism (Harrison et al., 1982; Klem et al., 1986).

However, there is a lack of information about the influence of BSA as a culture medium component on the general spermatozoa *in vitro* viability. Therefore, this study was designed to test the effects of two levels of BSA concentration on the bovine spermatozoa motility during a 24 hour *in vitro* cultivation.

MATERIAL AND METHODS

Bull semen samples were obtained from 15 adult breeding bulls (Slovak Biological Services, Nita, Slovak Republic). The samples had to accomplish the basic criteria given for the corresponding breed.

After collecting the samples were stored in the laboratory at room temperature (22-25 °C) and basic measurements were performed – volume (ml), pH and concentration (x 10^9 ml^{-1}) were determined. Each sample was diluted in physiological saline solution (sodium chloride 0,9% w/v, Bieffe

Medital, Italia), using a dilution ratio of 1:39, 1:50 and 1:60 respectively, depending on the original spermatozoa concentration. We used heterospermy for the experimental cultivation.

Six cultivation medias were prepared with a different composition (Table 1), five of them containing BSA (Fluka, Sigma-Aldrich, USA). Heterospermy was added to the medias and cultivated in an incubator (37°C) for 24 hours.

Motility analysis was carried out using a CASA system – SpermVision™ (MiniTüb, Tiefenbach, Germany) with Olympus BX 51 phase microscope (Olympus, Japan) at cultivation time 0 hour, 1 hour and 24 hours. Each sample was placed into Makler Counting Chamber (deph 10 µm, Sefi-Medical Instruments, Izrael) and percentage of motile spermatozoa (motility > 5µm/s) was evaluated.

Obtained data were statistically analyzed with the help of PC program GraphPad Prism 3.02 (GraphPad Software Incorporated, San Diego California USA). Descriptive statistical caracteristics were evaluated (mean, minimum, maximum, standard deviation and coefficient of variation).

Tab. 1 Culture media used for the experiment

GROUP	COMPOSITION
N - Native group	Native sample with physiological saline solution,
R - Commercial medium	Triladyl, egg yolk and redistilled water,
A	Triladyl, 10% BSA, 5% glucose (D-glukosa monohydrat p.a, Penta, Chrudim) and distilled water,
B	Triladyl, 10% BSA, 5% glucose (D-glukosa monohydrat p.a, Penta, Chrudim), 1% trehalose (D(+)-trehalose, Fluka, Sigma-Aldrich, USA) and distilled water,
C	Triladyl, 20% BSA and distilled water,
D	Triladyl, 20% BSA, 5% glucose (D-glukosa monohydrat p.a, Penta, Chrudim) and distilled water,
E	Triladyl, 20% BSA, 5% glucose (D-glukosa monohydrat p.a, Penta, Chrudim), 1% trehalose (D(+)-trehalose, Fluka, Sigma-Aldrich, USA) and distilled water.

RESULTS AND DISCUSSION

In vitro effects of different BSA concentrations on the bovine spermatozoa motility were analysed in three time periods (time 0 hours, 1 hour, 24 hours). We especially focused on comparing the motility results of every used medium containig different BSA concentrations with the native sample and the sample containing egg yolk medium (Table 2).

Initial (time 0) spermatozoa motility in the native N group was 87.31±5.162%, however, the lowest motility was detected in group R containing the egg yolk commercial medium, 83.86±6.567%. The motility was significantly ($P<0,001$) high in group D containing 20% BSA, and 5% glucose, compared to group R. Apart from group D, a relatively high motility was detected in group C (containing 20% BSA), 96.15±1.491%. This observation could be explained by an originally high concentration of energetic and protein substrate in the medium.

After 1 hour of cultivation, we observed a slight decrease of the speramtozoa motility in the native sample, 85.66±2.350%. Similar tendence was detected in groups B (containing 10% BSA, 5% glucose and 1% trehalose), C, and D. However, the highest motility rate was found again in group D; 91.29±3.767%. On the other hand an increase of spermatozoa vitality was detected in groups A (with 10% BSA and 5% glucose), E (with 20% BSA, 5% glucose and 1% trehalose) and R containing the egg yolk medium. Therefore we may assume that 10% BSA and egg yolk may stimulate the spermatozoa metabolism for a short period and therefore may be used for a short time cultivation.

Evaluation of the percentage of motile spermatozoa showed decreased values in all experimental groups cultured for 24 hours. The highest inhibition of spermatozoa motility after 24 hours was detected in group N (28.86±9.087%), followed by group B (42.89±13.17%). The highest motility value was found in group D (66.46±9.986%) and group R (62.81±8.044%). However, no statistical significance was found.

Tab. 2 Spermatozoa motility (%) in groups and time periods

Group	N/MOT	R/MOT	A/MOT	B/MOT	C/MOT	D/MOT	E/MOT
0 h							
x	87.31	83.86	89.99	92.62	96.15	97.39[A]	87.46
minimum	82.22	70.83	85.85	85.84	93.85	93.54	82.35
maximum	97.22	92.85	94.44	95.45	98.12	100.0	92.59
S.D.	5.162	6.567	2.960	3.373	1.491	2.030	3.666
CV (%)	5.91	7.83	3.29	3.64	1.55	2.08	4.19
1 h							
x	85.66	87.23	91.07	83.25	84.43	91.29	89.17
minimum	82.35	79.34	81.57	75.00	62.50	83.33	82.22
maximum	89.01	97.46	94.73	92.10	100.0	95.23	95.00
S.D.	2.350	5.278	4.123	5.810	13.15	3.767	4.390
CV (%)	2.74	6.05	4.53	6.98	15.57	4.13	4.92
24 h							
x	28.86	62.81	52.28	42.89	55.41	66.46	57.75
minimum	21.15	51.85	22.22	20.00	31.25	53.12	36.84
maximum	44.00	78.43	78.57	60.00	72.00	78.94	82.14
S.D.	9.087	8.044	16.12	13.17	13.96	9.986	12.87
CV (%)	31.49	12.81	30.84	30.72	25.19	15.03	22.30

x – mean, S.D. – standard deviation, CV (%) – coefficient of variation
[A] P<0.001, [B] P<0.01, [C] P<0.05

The use of a protein source has been extensively employed as a supplement of culture media for handling and culture of spermatozoa, gametes and embryos. Protein molecules provide nutrients and protection to the cell, behave as colloids in solution and contribute to the osmotic pressure of fluids (Correa-Pérez et al., 2003; Gebauer et al., 1970).

The discovery that egg yolk has a beneficial effect on fertility led to its widespread use in bull semen extenders (Pace and Graham, 1974). However, it is difficult to produce semen diluents of uniform quality, because of individual quality differences inherent in egg yolk. Egg yolk is also relatively unstable for extended periods of time because of high content of fatty acids sensitive to degradation. Therefore, it seems that removal of chicken egg yolk from semen diluents produces several advantages, such as improvement of consistency in the components of semen diluents and elimination of various patogens (Matsuoka et al., 2006; Muller-Schlosser et al., 1995).

BSA could be a good protein alternative because of its stability, good amino acid profile and protective functions. There are several authors who had been studying the possible effects of BSA on the sperm viability of different animal species (Bakst and Cecil, 1992).

Matsuoka et al. (2006) studied the effects of different BSA concentrations on the post-thaw viability of ram spermatozoa. The rates of progressive motility were significantly highet ($P<0,05$) in the 10% and 15% BSA groups than in the tris-fructose-egg yolk control group. Also, the viability of post-thaw spermatozoa with BSA was significantly improved and the rates of swollen tails and intact acrosomes were also higher.

The aim of the investigation of Serniene et al. (2001) was to study the effect on semen quality caused by the addition of bovine serum albumin (BSA) to boar semen and to determine the optimal dose of the BSA. The analysis revealed that addition of BSA, sperm storage time and their interaction had significant effect only on aggliutination rate. The effect of addition of BSA and sperm storage time was significant for sperm motility, vigor rate and a number of viable/nondamaged spermatozoa per ejaculation.

Our experiment indicates similar results agreeing with the stimulating function of BSA towards several measurable characteristics describing bull sperm motility. Based on our results we can assume that the optimal concentration of BSA for sperm cultivation is 20%, since medias containing 20% BSA presented overall best sperm viability results. 10% BSA medias had moreless average quality. There are questions about the maximum concentration of BSA a sperm cultivation, since hight concentrations of any substance may be toxic.

CONCLUSION

This study demonstrates that BSA could be a good protein suplement for a long-term bull spermatozoa cultivation. There are still questions about the optimal BSA concentration and whether BSA could be used also for further processing and cryoconservation of bull spermatozoa.

We also have to be aware on the fact that a proper protein supplementation is not the only factor crucial for a good *in vitro* sperm motility and viability. An appropriate energetic substrate and minerals, as well as optimal laboratory conditions are equally important for a successfull *in vitro* spermatozoa cultivation.

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