COMPARISON OF FOUR DILUENTS FOR CRYCONSERVATION OF BULL SEMEN AND THEIR EFFECT ON SPERM SURVIVAL

Beran J., Stádník L., Ducháček J., Louda F.

Department of Animal Husbandry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Suchdol 165 21, Praha Suchdol, Czech Republic

E-mail: beranj@af.czu.cz

ABSTRACT

The addition of components of animal origin (egg yolk, milk) to most commercial diluents used to freeze bull semen represents a potential risk of contamination of the doses with bacteria or mycoplasma. For this reason these components are substituted by soja lecithin. In this work we compared four extenders: two without egg yolk (AndroMed®, Bioxcell®) and two egg yolk based diluents (Triladyl®, Optidyl®). We used 80 samples of ejaculate, collected from 4 bulls of the same age, frequency of collecting and reared on one Artificial Insemination Centre (A. I. Centre). Semen with demand quality were used for producing A. I. doses by standard methodology of A.I. Centre. We monitored sperm activity of these A. I. doses after thawing by a heat test (38°C, 120 minutes). The results were evaluated by the SAS GLM procedure. Higher activity of sperm after thawing (48.4-51.6%, $P<0.05$-0.001) was detected in extenders with egg yolk than in extenders without egg yolk (41.5-47.4%). Sperm activity declined unevenly to (25.5%-31.4%) in egg yolk extenders compared to ones without egg yolk (18.9-23.3%) after 120 minutes of the survival test. Significant differences in sperm survival among individual sires were determined. We can recomended the use of diluents based on egg yolk.

Key words: bull semen, cryopreservation, sperm survival, diluent, egg yolk, bacterial contamination.

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INTRODUCTION

Extenders with egg yolk are routinely used for the cryopreservation of bull semen. The main benefit of egg yolk is that it adheres to sperm cell membranes during the freeze-thaw process and resists it against cold shock (AMIRAT et al., 2004; MUINO et al., 2007).

In recent years, however, there has been a trend against the use of egg yolk or milk in cryoprotective media because of the risks associated with the use of animal products and the presence of steroid hormones, which may reduce the fertilizing capacity of spermatozoa. Furthermore, egg yolk and milk introduce a risk of bacterial or mycoplasmatic infections. Such contamination is a possible source of endotoxins capable of damaging the fertilizing capacity of spermatozoa. To solving this problem, a steriled diluent for bull semen has recently been marketed. These commercial extenders are based on glycerol and contains soja lecithin as a replacement for egg yolk or egg yolk+milk and thus has no products of animal origin. (BOUSSEAU et al., 1998; THUN et al., 2002).

The aim of this paper was to compare and assess the impact of different types of bull semen diluents on the sperm survival.

MATERIALS AND METHODS

Four bulls of the same age, frequency of collecting and reared on one A.I. centre were chosen as donors of semen. Twenty ejaculates were obtained from each bull using an artificial vagina. The semen samples were assessed immediately after collecting by experts of A. I. centre by their standard methodology. Only semen with demanded quality (minimum progressive motility 70% and sperm concentration 0.7 x 106/mm3) was used for following processing on samples.

Semen was then divided into four equal fractions; the first one was diluted with AndroMed® (Minitübe, Tiefenbach, Germany), an extender containing soybean lecithin extract. The second one was Bioxcell® (IMV, L'Aigle, France), an extender which is not contain any product of animal origin. The third fraction was diluted with Triladyl® (Minitübe, Tiefenbach, Germany), containing 20% (w/v) of fresh egg yolk. The fourth aliquot was diluted with Optidyl® (IMV, L'Aigle, France), an extender which contains ionized egg yolk. There were produced insemination doses from that diluted aliqouts and stored in liquid nitrogen.

We evaluated in vitro parameters after thawing with two pooled straws from each diluted sample of semen. Thus, considering that we had four bulls, 20 samples of ejaculate from each bull divided into four aliquots and diluted with different extender, we analyzed 160 straws per bull and extender, 640 straws altogether.

We assessed sperm motility subjectively using phase contrast microscopy set at a magnification of x200 (LP 3000, Arsenal®). Straws were thawed in water bath at a temperature of 39±1°C for 45sec. and input to the preheated sterile tubes with the physiological solution. The motility values were detected in the beginning of the test and then after 30, 60, 90 and 120 minutes of the test duration in a Thermo-block FALC at a temperature of 38±1°C.

The dataset was analyzed by statistical program SAS STAT 8.0 – GLM (SAS, 2001).

RESULTS AND DISCUSSION

Results of heat test of sperm survival in separate extenders are shown on the table 1.

Tab 1. Results of heat test of sperm survival in separate extenders

Time	Andromed		Bioxcell		Triladyl		Optidyl		P
[min.]	μ+α	SE	μ+α	SE	μ+α	SE	μ+α	SE	
0	47,4	2,1	41,5	2,1	48,4	2,1	51,6	2,1	B – T *; B – O **
30	44,1	2	38,5	2	44,9	2	48,1	2	B – O ***
60	37,2	2	33,4	2	38,7	2	43	2	A – O *; B – O ***
90	30,3	1,9	26,5	1,9	32,6	1,9	36,3	1,9	A - O, B - T*; B - O***
120	23,3	1,9	18,9	1,9	25,5	1,9	31,4	1,9	B - T*; A - O**; B - O***

P ≤ 0,05 * – 95 %; P ≤ 0,01 ** – 99 %; P ≤ 0,001 *** – 99,9 %

Higher activity of sperm after thawing (48.4-51.6%, P<0.05-0.001) was detected in extenders with egg yolk than in extenders without egg yolk (41.5-47.4%). Sperm activity declined unevenly during the survival test. Sperm activity was significantly higher (P<0.05-0.001) in egg yolk extenders (25.5-31.4%) compared to ones without egg yolk (18.9-23.3%) after 120 minutes of the survival test.

Significant differences in sperm survival among individual sires were determined. That is shown on the figures 1 - 4.

Fig. 1. Sperm survival of bull 1

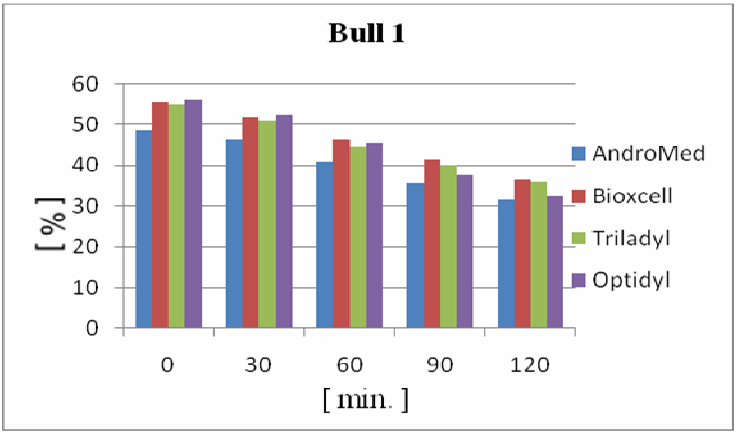


Fig. 2. Sperm survival of bull 2

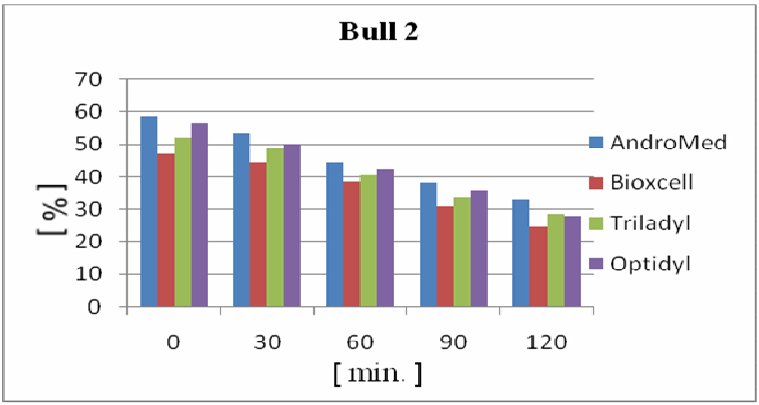


Fig. 3. Sperm survival of bull 3

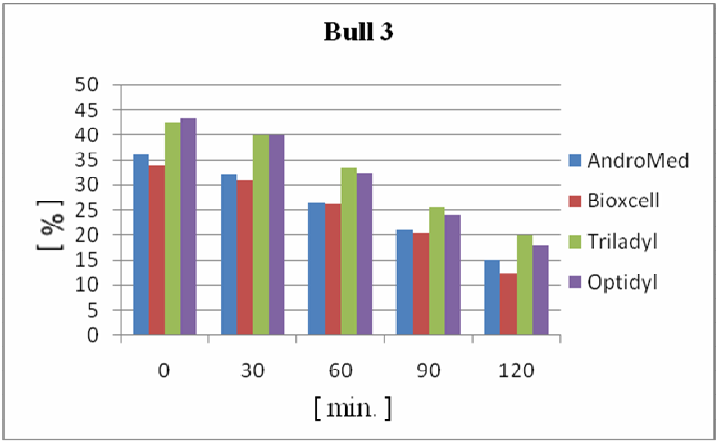
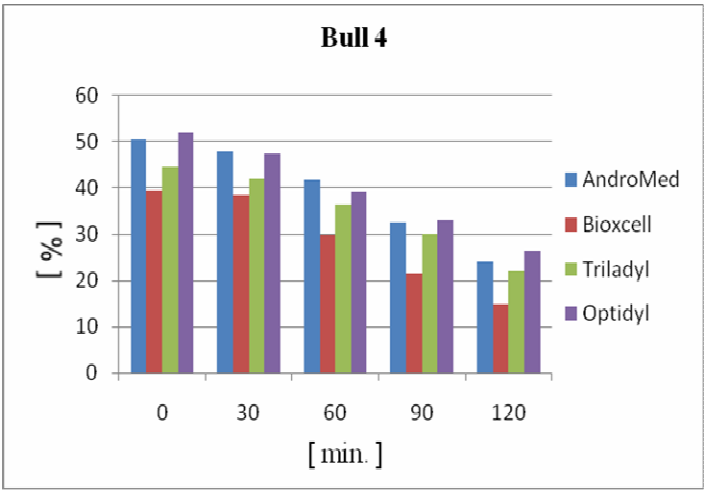


Fig. 4. Sperm survival of bull 4



Conclusions of MUINO et al. (2007), that egg yolk extenders have better cryoprotective effects and have reached better results than extenders without egg yolk, were confirmed. Conversely, the findings of JANNET et al. (2005), that AndroMed® is the best suited for long-term preservation of bull semen, did not confirm.

We can ask why the yolk extenders are still popular. The answer is LDL ("low-density lipoprotein"), component of egg yolk. The cryoprotective effect of LDL were found first by PACE and GRAHAM (1974). This results were confirmed by further works, such as AMIRAT et al., (2004). That facts were also cornfirm by our monitoring.

CONCLUSION

The quality of insemination doses have a major impact on the success pregnancy of cows, which are currently deteriorate. Farmers and agricultural scientists are looking for all ways to improve it. One possible way is improve the quality of insemination doses, which are distributed to farmers.

Our results indicate that the influence of used extender on selected indicators of quality of bull's insemination doses is considerable. To selection a suitable diluent is necessary give attention.

Cvurrently, the use of extenders based on egg yolk declines due to the risk of bacterial contamination. However, egg yolk still shows as the best cryoprotectivum for long-term preservation of bull semen. Based on our results, we can recomended the use of diluents based on egg yolk.

REFERENCES

AMIRAT, L. – TAINTURIER, D. – JEANNEAU, L. – THORIN, C. – GÉRARD, O. – COURTENS, J. L. – ANTON, M. (2004). Bull semen in vitro fertility after cryopreservation using egg yolk LDL: a comparison with Optidyl®, a commercial egg yolk extender. Theriogenology, 61 (2004): 895 – 907.

BOUSSEAU, S. – BRILLARD, J. P. – MARQUANT, B. – GUÉRIN, B. – CAMUS, A. – LECHAT, M. (1998). Comparison of bacteriological qualities of various egg yolk sources and the in vitro and in vivo fertilizing potential of bovine semen frozen in egg yolk or lecitin based diluents. Theriogenology, 50 (1998): 699 – 706.

JANETT, F. – KEO, S. – BOLLWEIN, H. – HÄSSIG, M. – THUN, R. (2005). Comparison of AnfroMed®, Bioxcell® and Triladyl® extender for cryopreservation of bull semen. Schweiz Arch. Tierheilk, 147, 62.

MUINO, R. – FERNANDEZ, M. – PENA, A. I. (2007). Post-thaw survival and longevity of bull spermatozoa frozen with an egg yolk-based or two egg yolk-free extenders after an equilibration period of 18 h. Reprod. in Dom. Anim., 42 (3), JUN 2007: 305 – 311.

PACE, M. M. – GRAHAM, E. F. (1974). Components in egg yolk which protect bovine spermatozoa during freezing. Journal of Anim, Sci., 39 (1974): 1144 – 1149. In: AMIRAT et al.,

(2004). Bull semen in vitro fertility after cryopreservation using egg yolk LDL: a comparison with Optidyl®, a commercial egg yolk extender. Theriogenology, 61 (2004): 895 – 907.

THUN, R. – HURTADO, M. – JANETT, F. (2002). Comparison of Biociphos-Plus® and TRIS-egg yolk extender for cryopreservation of bull semen. Theriogenology, 57 (2002): 1087 – 1094.