

RESEARCH CONCERNING THE INFLUENCE OF CAPACITATION CONDITIONS ON THE QUALITY OF SPERMATOZOA USED FOR *IN VITRO* FERTILIZATION

CERCETĂRI PRIVIND INFLUENȚA CONDIȚIILOR DE CAPACITARE ASUPRA CALITĂȚII SPERMATOZOIZILOR DESTINAȚI FECUNDAȚIEI *IN VITRO*

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The optimization of capacitation technique is based on the study of the influence factors of this process. Studying the influence of capacitation conditions on boar sperm quality we concentrated our research upon capacitation technique and method. We used a dilution and washing method (centrifugation), a migration method (swim-up) and a selective washing method (Percoll). The capacitation medium acts and interacts with the capacitation technique and in order to study these factors we used: Tyrode's, TALP-HEPES and TCM 199 media. The results showed that the capacitation technique influences the parameters of the sperm used for embryo in vitro production and the technique depends on the quality of the raw semen.

Key words: spermatozoa, capacitation, technique, media

Introduction

At *in vitro* production (IVP) of embryos the factors of paternal origin have at least the same importance as those of maternal origin. They have an influence on the success of this process due to the biological quality of the gametes. So the decisions concerning providing and preparation of sperm are obvious because they influence the forming and development of embryos by cellular and molecular mechanisms (HARRISON R.A.P., 2003). The molecular mechanisms and the signal transduction pathways mediating the processes of capacitation are only partially defined (BALDI ELISABETTA et al, 1996). Considering these the study of some influence factors of sperm capacitation may contribute to the optimization of this step of IVP biotechnology due to a better knowledge concerning the impact of these factors.

Due to the little knowledge we have about capacitation methods and due to the lack of efficient and facile result evaluation methods, the optimization of

capacitation technique is still based on the study of influence factors of this process. By the researches concerning the influence of capacitation conditions on boar sperm quality used for IVP, we studied the capacitation medium and technique.

Materials and Methods

The *biologic material* came from Great White (GW), PIC and Bazna (B) boars from SDE Cluj-Napoca and from Great White (GW), Landrace (L) and Pietrain (P) boars belonged to Semtest BVN S.A. Târgu-Mureş. The boars used for semen collection are trained for manual collection with the artificial mannequin and they presented a normal sexual libido.

The *semen collection* was made on an artificial mannequin by the gloved-hand method (MICLEA V., 2003). The spermatic fraction was collected in an isotherm sterile cup with filter (sterile text gauze) in order to separate the gelatinous fractions.

The *media used* were BTS (Beltsville Thawing Solution) extender with an 50 IU/ml addition of Penicillin and 50 µg/ml of Streptomycin sulphate (7.4 pH), Tyrode's medium (7.8 pH), TALP-Hepes medium (7.8 pH) and TCM 199 medium (with Hepes, Earle's salt and L-Glutamine, 7.8 pH).

The *evaluation of the raw sperm* for making common analysis required the determination of concentration (using SDM 5 photometer), motility and agglutination (at the microscope with phase contrast Karl-Zeiss, Peraval) and sperm morphology.

Table 1.

Experimental trials depending on capacitating technique and medium

<i>Experimental trial</i>	<i>Capacitation technique</i>	<i>Capacitating medium</i>
S1	<i>Swim-up</i>	Tyrode
S2		TALP-HEPES
S3		TCM 199
C1	Centrifugation	Tyrode
C2		TALP-HEPES
C3		TCM 199
P1	Percoll	Tyrode
P2		TALP-HEPES
P3		TCM 199

Capacitation method by washing required the centrifugation of sperm at 600 g (Sigma 3-18K, with 12154H rotor) for 10 minutes in the washing medium (according to experimental plan from table 1). After capacitation, the sperm was washed twice (for 10 minutes at 600g). The *swim up method* required the introduction of sperm under the average column of capacitation. For migration the samples were put to incubate for 60 minutes at 37 °C, at an angle of 45°. After this period the sperm suspension from the surface of the medium was taken with a Pasteur pipette and put it in Eppendorf tubes. The *Percoll capacitation method*

required a sperm centrifugation in a Percoll column (90% and 40%). After centrifugation, 15 minutes at 900g (Sigma 3-18K, with 11180 rotor) the supernatant was eliminated and the sperm pellet washed twice (10 minutes at 600g).

In order to *evaluate sperm capacitation* we analyzed the concentration of recovered sperm, their motility, hyperactivity (with a faster swimming model and in circle, after SUAREZ S.S. and H-C HO, 2003) and agglutination. The results were statistically analyzed using one-way ANOVA. When ANOVA revealed significant differences we made the Turkey-Kramer Multiple Comparisons test. All the analyses were made using GraphPad InStat soft.

Results and Discussions

Determination of some parameters of raw semen (table 2) was required in order to establish the general quality of boar sperm used for this paper.

The results of the influence of technique and medium on the quality of capacitated sperm in the two experimental trials are shown in table 3.

Evaluated the capacity of sperm the four parameters that were determined have concurred to form a useful pattern to a proper interpretation of the capacitation technique and the culture medium influence.

Regarding the post-capacitation sperm recovery rate we can notice huge differences between the centrifugation technique and the other two (swim-up and Percoll).

Table 2.

Common analysis of raw semen

Unit	race / hybrid	Evaluated parameters ($\bar{X} \pm s \bar{X}$)					
		conc. (1×10^9)	motility (%)	agglutination (%)	abnormal (%)		immature (%)
					primary	secondary	
SDE	MA	0.346±0.11	72±1.00	8.0±1.22	3.3±0.25	9.8±2.04	3.4±0.24
	PIC	0.541±0.08	74±0.81	7.0±1.22	2.1±0.18	7.6±1.11	2.0±0.31
	B	0.263±0.22	75±1.29	5.0±0.00	3.1±0.64	8.9±1.60	5.4±0.50
Total		0.383±0.32	73,66±2.96	6.66±2.44	2.83±0.26	8.76±0.90	3.6±0.42
Semtest	MA	0.221±0.09	71 ± 1.00	6.0±1.00	4.0±0.89	6.2±1.49	4.8±0.48
	L	0.253±0.01	71 ± 1.00	5.0±0.00	4.6±1.07	8.4±1.50	6.0±0.44
	P	0.385±0.05	74 ± 1.00	8.0±1.22	3.8±1.11	7.2±0.91	3.8±0.73
Total		0.280±0.03	72 ± 0.65	6.33±0.59	4.13±0.55	7.26±0.75	4.86±0.38

The multiple comparison Tukey-Kramer test showed very important differences between these techniques (C vs. S and C vs. P). By comparing by the technical from the point of view of recovered sperm centrifugation (75.37%) is definitely better than the passive technique (3.55%) and the washing in gradients of

different concentration (7.19%), centrifugation being much more simple and faster (fig. 1).

Motility had similar average values to those in the case of selective techniques: 90% consecutive swim-up and respectively 90.66% consecutively Percoll. Due to the recovery of a greater number of spermatozoa, the centrifugation washing technique helps to obtain spermatozoa with a smaller average motility (76.33%). The results showed a positive influence of TALP-Hepes medium on sperm quality at every technical trials, followed by those of Tyrode's medium.

Table 3.

Analysis of boar semen capacitating

Experimental trial	Evaluated parameters ($\bar{x} \pm s_{\bar{x}}$)*			
	recovery rate (%)	motility (%)	hyperactivation (%)	agglutination (%)
S1	4.50 \pm 0.64	91 \pm 1.00 ^a	12 \pm 1.22 ^{abc}	2 \pm 1.22
S2	3.13 \pm 0.50	92 \pm 1.22 ^a	11 \pm 1.00 ^b	3 \pm 1.22
S3	2.99 \pm 0.47	87 \pm 3.00 ^{ab}	14 \pm 1.00 ^{abc}	3 \pm 1.22
C1	74.00 \pm 3.06	78 \pm 2.00 ^c	15 \pm 0.00 ^{ab}	6 \pm 1.00
C2	72.99 \pm 3.64	79 \pm 2.91 ^{bc}	18 \pm 1.22 ^a	5 \pm 1.58
C3	79.10 \pm 2.01	72 \pm 1.22 ^c	15 \pm 1.58 ^{ab}	8 \pm 1.22
P1	5.78 \pm 0.48	89 \pm 1.00 ^a	12 \pm 1.22 ^{bc}	1 \pm 1.00
P2	8.23 \pm 1.18	93 \pm 1.22 ^a	8 \pm 1.22 ^c	1 \pm 1.00
P3	7.55 \pm 0.49	90 \pm 1.58 ^a	10 \pm 1.58 ^{bc}	2 \pm 1.22

*The differences between any trials followed by at least one common letter are insignificant

The analysis of hyperactive motility is very important because it offers certainty to the sperm capacitating mechanisms. The greatest hyperactive motility appears in the case of sperm that was capacitated by centrifugation. With a single exception (P2 vs. S3) important differences were registered while comparing the experimental trials of centrifugation with some experimental trials of Percoll and swim-up techniques.

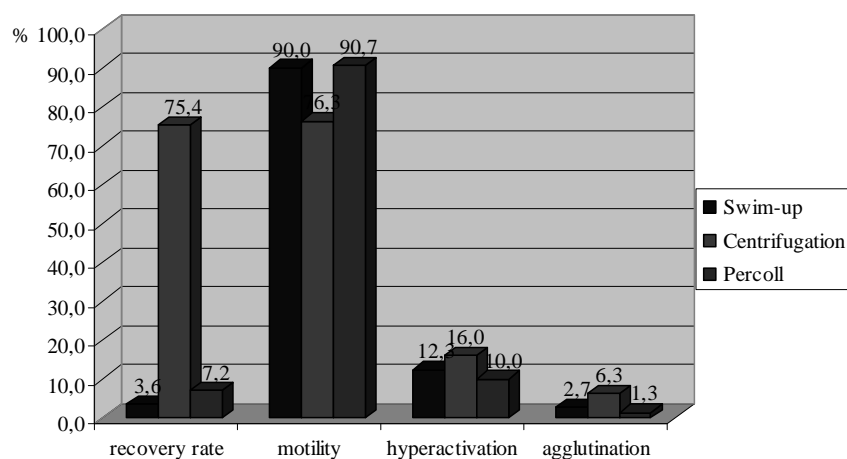


Figure 1. Sperm parameters depending on the capacitation technique

The lowest percent of agglutination was determined at the samples of sperm capacitated with Percoll technique (1.33%). A small percent was identified in the case of swim-up technical trial (2.66%) while the average agglutination value was the highest in the case of centrifugation (6.33%). One can observe the influence of the two sperm selection techniques (passive and active) without any differences in the case of centrifugation in comparison with the raw semen.

Conclusions

After semen collection, capacitation and *in vitro* development of early swine embryos, the main conclusions concerning their preferences for energetic substrate are:

- the choice of sperm capacitation technique for *in vitro* fertilization depends on the quantity and the quality of the semen, taking into consideration the fact that the centrifugation technique leads to a higher rate of post-capacitation sperm recovery and to better percents of hyperactivity, while *swim-up* and Percoll we get a better motility and lower percents of agglutination, due to selective particularities of these techniques;
- simple media like TAPL-Hepes or Tyrode's have a positive influence on sperm motility without influencing the other parameters verified after post-capacitation evaluation of sperm.

Bibliography

1. **Baldi Elisabetta, Michaela Luconi, Lorella Bonaccorsi, Csilla Krausz and Gianni Forti** (1996)- *Human sperm activation during capacitation and acrosome reaction: role of calcium, protein phosphorylation and lipid remodelling pathways*. *Frontiers in bioscience* 1, 189-205
2. **Bollendorf A., J.H. Check, Diane Katsoff, D. Lurie** (1994)- *Comparison of direct swim-up, mini-Percoll and Sephadex G10 separation procedures*. *Archives of Andrology*, 32, 157-162
3. **Harrison R.A.P.** (2003)- *Cyclic AMP signalling during mammalian sperm capacitation – still largely Terra Incognita*. *Reprod. Dom. Anim.*, 38, 102-110
4. **Miclea V.** (2003)- *Însămânțarea artificială la animalele de fermă*. Ed. Argonaut, Cluj-Napoca
5. **Ng L.H. Florence, De Yi Liu, H.W. Gordon Baker** (1992)- *Comparison of Percoll, mini-Percoll and swim-up methods for sperm preparation from abnormal semen samples*. *Human Reproduction*, 7 (2) , 261-266
6. **Suarez S.Susan and C-H. Ho** (2003)- *Hyperactivated motility in sperm*. *Reprod. Dom. Anim.*, 38, 119-124