ELECTRICAL PROPERTIES OF THE RABBIT SEMEN IN DIFFERENT DILUTION MEDIA

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Abstract - The biological quality of rabbit semen in a natural environment, in Tris buffer and in three diluants was studied by plotting of current-voltage characteristic in d.c. between 0 V and 1 V in direct and opposite sense.
The analysis of the current-voltage characteristics and the statistical analysis of the data, indicated that the electric current intensity of the seminal cell suspensions depends upon the cellular viability and upon the dilution medium and varies between 97.85 μA for the living spermatozoa and 37.43 μA for the dead spermatozoa. The Student's t-test made evident distinct significant differences between the electrical properties of the raw sperm and the diluted sperm ($P<0.01$) and nonsignificant differences between the raw sperm with the dead spermatozoa and the sperm in Tris buffer($P>0.05$).

INTRODUCTION

The artificial inseminations carried out in the last period on a large scale on almost all species of animals, the rabbits included, raised the problem of the structure preserving and the function of the spermatozoa which are in another environment than the natural one.
Thus, physical methods for the study of the seminal cells as the photonic correlation spectroscopy (FINSEY *et al..*, 1979), the spin electronic resonance, the protonic electronic resonance (RUSTIGI *et al..*, 1978), the depolarised laser light (SHIMIZU and MATSUMOTO, 1980), laser light (BEEK, 1995; SEEGER, 1995; CLEGG, 1995) and electric measurements (ESPINOZA and DARSZON, 1995; MICU, 1994) were used.
These techniques directed to the knowledge of the properties of the seminal cells on a submolecular and molecular level demonstrated that the living cells respond to the exogenous factors, starting with the subatomic and molecular levels.
Within this work, taking into account that the electric properties of the living cells are those which dominate the cellular activity, we have drawn the current-voltage characteristic curve in d.c. in a natural environment and in an environment with various diluants.
The electrical parameters and the electric field interaction with the living cells were used in the rabbit in order to study unfertilised oocytes and to induce electrofusion of the embryos (ARNOLD *et al..*, 1989 ; GARCIA XIMENEZ *et al.*, 1994).
The aim of this study is to test three diluants by appreciating the biophysical quality of the diluted rabbit semen in order to obtain information concerning the modifications in the spermatozoon metabolism.

MATERIALS AND METHODS

The biological material was represented by 25 rabbit males. The collection method of the seminal material was that proposed by P. BUNACIU (1985).The sampled sperm was diluted in a 1:1 rate in three diluants Dl, D2, D3 and in 10mM Trisbuffer, pH 7.31.
The quality of the seminal material was assessed by morphological determination (motility, concentration) and by microscopic observations.

Chemical composition of the diluants

Specification	Diluant 1 (Dl) g %	Diluant 2 (D2) g %	Diluant 3 (D3) gr. %
Tris	3	2.5	0.002
Citric acid	1 .675	1.78	-
Fructose	-	1 .25	-
Glucose	1 .250	-	3. 9
Sodium sulphate	-	-	0.5
Peptone	-	-	0.2
pH	6.5	6.8	7.2
Osmotic pressure (mOsm/k)	3 1 7	3 00	290

The current-voltage characteristic in d.c. was traced in the interval 0-1V in a direct and in an opposite sense, for the spermatozoon suspension placed between two nickel electrodes, in a natural environment, in the three diluants and in the 10 mM Trisbuffer.

The dead spermatozoa were obtained by maintaining the sample at the room temperature 15°C for 3 hours. Statistical analysis of the results was done using Excel 5.0 Program.

RESULTS AND DISCUSSION

The microscopic observations effectuated over the rabbit seminal cells in various diluants showed that the viability, respectively the cellular motility is not affected by the electric field used for the plotting of the current - voltage characteristic with values comprised between 0-3 V/cm. In the Trisbuffer (10 mM), the spermatozoa were dead.

The results concerning the statistical analyse of the morphological and electric properties of the rabbit seminal material in various diluants are given in table 1.

Table 1 : Morphologic and electric properties of the raw sperm in different media of dilution

Specifications	Concentrations (mil/ml)	Motility % (1)	Current intensity (μA) (2)	Correlation coefficient r (1/2)
Raw sperm	156.81 ± 31,1	89.8± 6.29	97.85 ± 32.87	0.598**
Dead spermatozoa	143 ± 27	0	61.25 ± 11.39	0.6185***
Dl	98.18 ±20.66	76.5 ± 18.14	91.33 ± 4.04	0.667***
D2	87.27 ± 18.48	73 ± 20.51	96 ± 23.51	0.708* **
D3	96.28 ± 19.71	63.005±21.61	51.26 ± 5.4	0.582**
Trisbuffer	97.20 ±17.5	5±0.6	37.43 ± 16.55	0.627***

Note: Values shown are mean ± S.D.; P>0.05 ns; *P<0.05; * *P<0.01; ***P<0.001

The t-Student test showed that between the motility of the raw sperm and the motility of the diluted one in D3 diluant there are significant distinct differences P< 0.01 and between the other variants there are no significant differences.

In the Table 1, we can observe that the maximum intensity of the electric current and the cellular motility are very significantly correlated. The intensity of the electric current and the cellular concentration are not correlated r=0.218. We can say that the intensity of the electric current of the seminal cell suspensions depends only of the intrinsic properties of the spermatozoa.

Figure 1. The current - voltage characteristic in d.c. in the direct ▶ and in the opposite ◀ sense for the living raw sperm

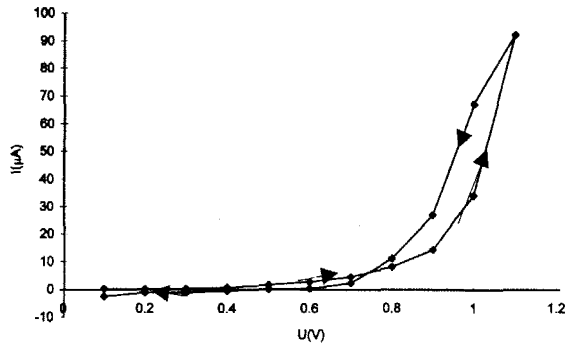
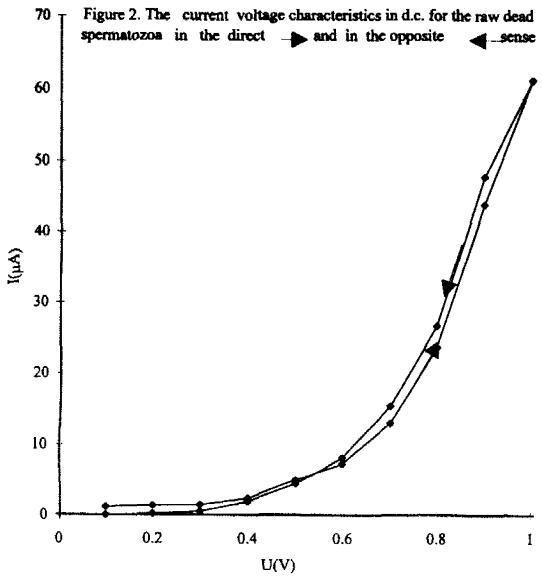


Figure 2. The current voltage characteristics in d.c. for the raw dead spermatozoa in the direct ▶ and in the opposite ◀ sense



The current-voltage characteristic for the raw sperm is given in Figure 1. It can be observed that the intensity of the electric current of the seminal suspensions by the applied electric tension of 1 V is 97.85 μA. An accentuated hysteresis cycle was also noticed. The regression equation in a direct sense is exponential, $y = 0.179e^{0.66x}$ with $R^2 = 0.973$, and in an opposite sense is a three-degree polynom

$y = -0.3573x3 + 8.0129x^2 - 59.175x + 145.47$ with $R^2 = 0.889$.

The current-voltage characteristic for the raw sperm with the dead spermatozoa (Figure 2) is similar to a characteristic for a crystalline net with order at small distance. The current intensity was 61.25 μA and the regression equation in the both senses were a two degree polynom : $y = 1.3001x^2 - 8.3487x + 11.85$ with $R^2=.9776$ and $y = 1.261x^2 - 20.28x + 79.57$ with $R^2 = 0.986$.

Figure 3 shows the current-voltage characteristic for the spermatozoa in Trisbuffer. The maximum of the current intensity was 37.43 μA and the regression equations were two degree polynoms in the both directions: $y= .6939x^2 - 3.5189x + 3.6865$ with $R^2 = 0.9934$ and $y = 0.6923x^2 - 11.928x + 50.069$ with $R^2 = 0.9792$. The absence of the

hysteresis is observed.

The results regarding the behaviour of the sperm in the three diluting media are in Figure 4(a, b, c). The regression equations for sperm in Dl were $y = 1.634x^2 - 7.8549x + 8.84$ with $R^2 = 0.994$ in the direct sense and $y = 7741x^2 - 20.498x + 125.37$ with $R^2 = 0.9689$ in the opposite sense. For sperm in D2, Figure 4b, the equation was exponential for the direct sense $y=1.0626e^{0.4289x}$ with $R^2=0.982$ and in the opposite sense a two-degree polynom $y = 1.7235x^2 - 27.68x + 111.79$ with $R^2 = 0.9848$.

For the sperm in the diluant D3 (Figure 4c), the regression equations were $y = 0.256x^3 - 3.9223x^2 + 18.99$ Ix - 6.269 ($R^2 = 0.9781$) for the direct sense and $y = 1.021x^2 - 16.263x + 62.681$ ($R^2 = 0.9833$) for the opposite sense.

From the living raw sperm raw sperm in the three diluants Dl, D2, D3, it is observed the presence of the hysteresis cycle.

Figure 3. The current -voltage characteristic in d.c. for the sperm in 10 mM Tris buffer, pH 7.31. in the direct ▶ and opposite ◀ sense

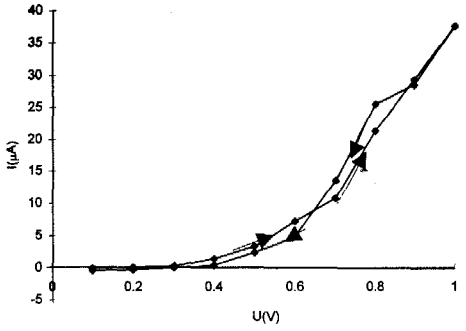


Figure 4a. The current- voltage characteristics in d.c. for the rabbit sperm in Dl diluant pH 6.5 in the direct ▶ and in the opposite ◀ sense

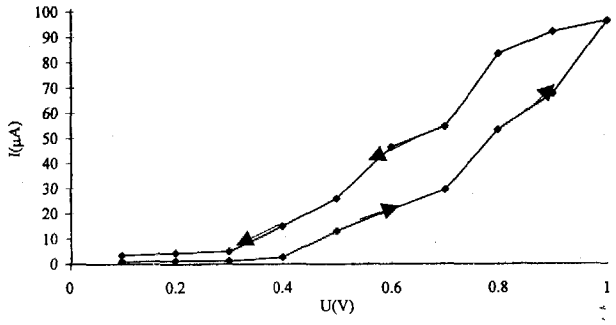


Figure 4 b, The current-voltage characteristic in d.c. for the rabbit sperm in D2, diluant, pH 6.8, in the direct ▶ sense and in the opposite ◀ sense

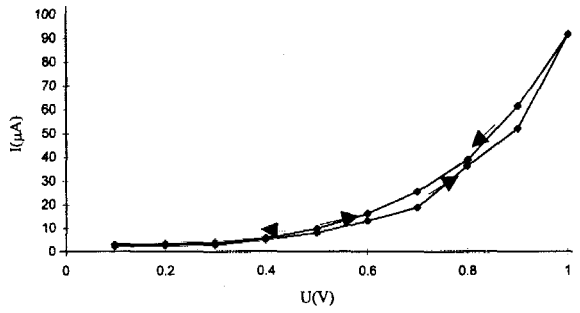
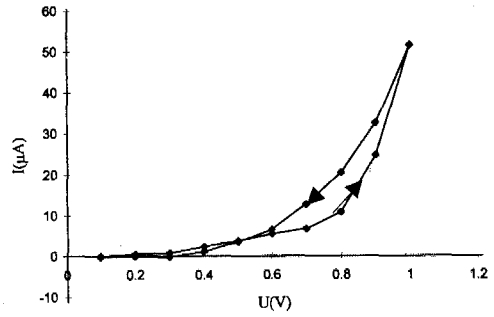


Figure 4 c. The current - voltage characteristic for the rabbit sperm in D3, diluant pH 7.2 , in the direct —▶ and in the opposite ◀ sense



In the table 2 are given the significance's of the differences between the current voltage characteristics of the raw sperm in various diluants, calculated by the t-Student test.

Table 2 : The significance of the differences between the current - voltage characteristics in d.c. of the semen in different diluted media.

Specifications	Diluant Dl	Diluant D2	Diluant D3	10 mM Tris	Dead sperm
Raw sperm	0.06 n.s.	$8.67\ 10^{-5}$ ***	0.01**	0.0061**	0.00318**
Dead sperm	0.00012***	0.00023 ***	0.0021 ***	0.014 *	-
Dl	-	0.096 n.s	0.001***	0.00023***	0.00012***
D2	0.096 ns	-	0.0001 ***	0.0001 ***	0.00023 ***
D3	0.001***	0.0001 ***	-	0.045 *	0.0021 ***

P>0.05 n.s.; *P<0.05; **P<0.01; ***P<0.001

From the table it can be observed that the differences depend of the viability respective of the cellular motility, and of the chemical compositions of the suspension medium.
The Student's t-test made evident distinct significant differences between the electrical properties of the raw and the diluted sperm ($P<0.01$) and nonsignificant differences between the raw sperm with the dead spermatozoa and the sperm in Trisbuffer($P>0.05$).
The results obtained indicated that for the living spermatozoa, the current - voltage characteristics have an accentuated hysteresis and an exponential increase of the current intensity with the applied voltage. That behaviour may be due to the semiconducting properties of the lipid membranes (BHATTACHARYYA *et al.*, 1993) and to the negative electrical charge that interacts with the electric field and stimulates the sperm motility (ROFFEY , 1994).
For the dead spermatozoa, the current intensity may decrease because the spermatozoa lose their negative electric charge or some of them change their type of conductivity.

CONCLUSION

The study shows that the electrical parameters of the raw sperm and of the sperm in different dilution media are correlated with the metabolical activity of the sperm and they may furnish information regarding the use of the better dilution medium for the preserving of the spermatozoa viability.

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