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Correlation of lactate dehydrogenase isoenzyme C_4 activity with the count and motility of human spermatozoa

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Summary. The activity of lactate dehydrogenase isoenzyme C_4 was determined on 90 human semen samples. The correlation between the isoenzyme activity and sperm count and motility was good ($r = 0{\cdot}74$ for values of U/ml semen against sperm count).

Introduction

Sperm count and motility are the most widely used characteristics for assessment of sperm quality, but although they show a good correlation with fertilizing capacity of semen (Santomauro, Sciarra & Varma, 1972), they are not always reliable. Some investigations of enzyme activities and metabolite concentrations have failed to demonstrate consistent correlations with sperm density or fertilizing ability (Nun, Musachio & Epstein, 1972; Beck, Schonhofer, Rodermund, Dinnendahl & Peters, 1976), but Crabbe (1977) found a direct relationship between fumarase activity and sperm count and motility, while diamine oxidase presented an inverse relation.

It appears logical to assume that activity of sperm-specific enzymes, associated with metabolic processes unique to spermatozoa, should be a direct and reliable index of sperm normality. One such enzyme is the lactate dehydrogenase (EC 1.1.1.27) isoenzyme C_4 (LDH C_4) which is exclusive to germ cells (Blanco, Zinkham & Walker, 1975).

The development of an assay method for determination of LDH C_4 in sperm extracts (Burgos, Gerez de Burgos, Coronel & Blanco, 1979) led to the present investigation of correlation between LDH C_4 activity and sperm count and motility.

Materials and Methods

Sperm samples

Semen was obtained from 90 patients sent for sperm counts to the Central Laboratory of the University Hospital (Hospital de Clinicas, National University of Córdoba). The sperm count was performed with a Neubauer haemocytometer. An aliquot of the semen sample was frozen at −20°C for 2 h and thawed at room temperature. Freezing and thawing was repeated once and then the samples were centrifuged for 20 min at 20 000 ***g*** and 4°C. Supernatants were used for assays. In some samples, another aliquot of semen was treated as described for washed spermatozoa by Burgos *et al.* (1979).

Lactate dehydrogenase C_4 assay

The activity of lactate dehydrogenase isoenzyme C_4 was determined at 37°C as indicated by Blanco, Burgos, Gerez de Burgos & Montamat (1976) by using 5·0 mM-2-oxohexanoate as substrate. Total lactate dehydrogenase activity was also determined in the samples with 0·5 mM-pyruvate as substrate.

Protein determination

Total protein in the samples was determined by measuring optical densities (D) of diluted semen samples at 260 and 280 nm and applying the following formula (Layne, 1957):

$$(1{\cdot}55 \times D_{280}) - (0{\cdot}76 \times D_{260}) = \text{mg protein/ml.}$$

Correlations

The regression lines and coefficients of correlation were calculated as indicated by Dunn (1967).

Results

LDH C_4 activity in semen

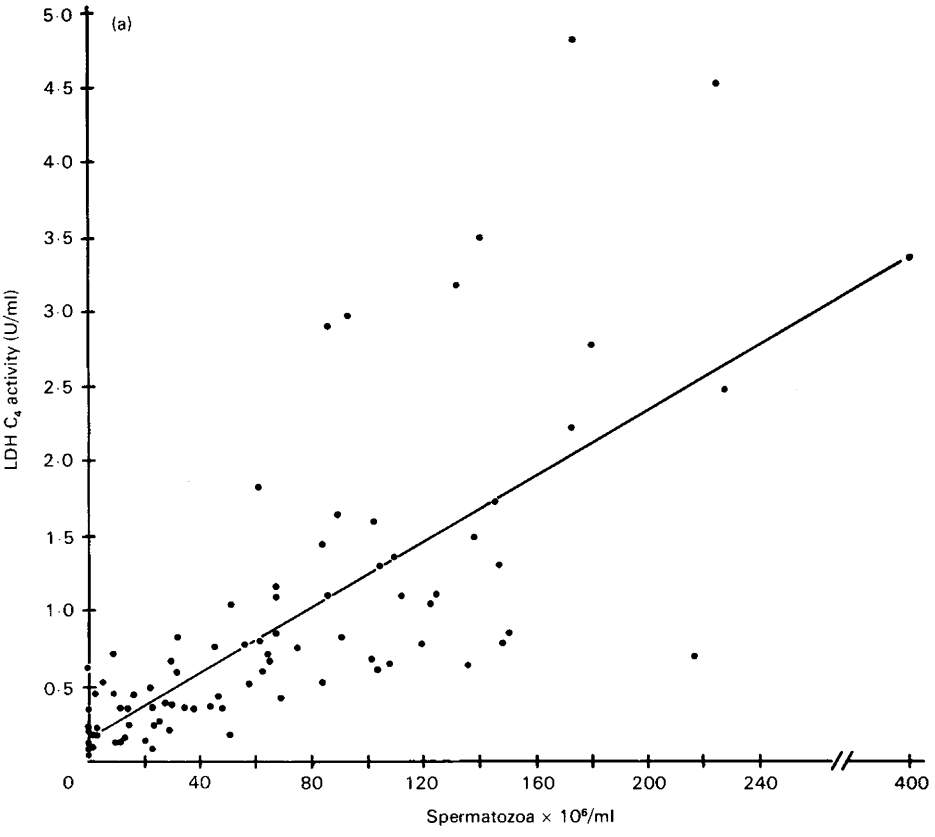
Zinkham, Blanco & Clowry (1964) demonstrated that, despite gentle handling of semen samples, there was always some leakage of LDH C_4 from spermatozoa. This observation suggested that determinations of enzyme activity in washed spermatozoa probably do not reflect the actual amount of LDH C_4 present in the original sample. To study the magnitude of that leakage, comparative determinations of LDH C_4 were performed on a given volume of semen and on washed spermatozoa obtained from an identical volume of the same semen sample as indicated by Burgos *et al.* (1979), except that the sperm pellet was suspended in a volume of distilled water identical to that of the original aliquot. The samples of semen and of spermatozoa were submitted to the same treatment of freezing and thawing and sonication used by Burgos *et al.* (1979).

Table 1 presents results of determinations carried out on 5 different samples. Activity against 5·0 mM-2-oxohexanoate was much higher in semen: the loss of enzyme from washed spermatozoa was about two-thirds of the total found in the original semen.

Table 1. Lactate dehydrogenase C_4 activity in semen and washed spermatozoa

Sample	Semen (U/ml)	Washed spermatozoa	
		U/ml	%
1	0·237	0·076	(32·07)
2	0·120	0·04	(33·3)
3	0·13	0·044	(33·9)
4	0·19	0·068	(35·8)
5	0·21	0·072	(34·3)
		Mean	(33·87 ± 1·37)

Values represent the mean of duplicate determinations on each sample using 5·0 mM-2-oxohexanoate as substrate. Numbers in parentheses represent the % activity in washed spermatozoa, taking as 100% the activity in semen.



Text-fig. 1a. Correlation between LDH C_4 activity in semen and the total sperm count.

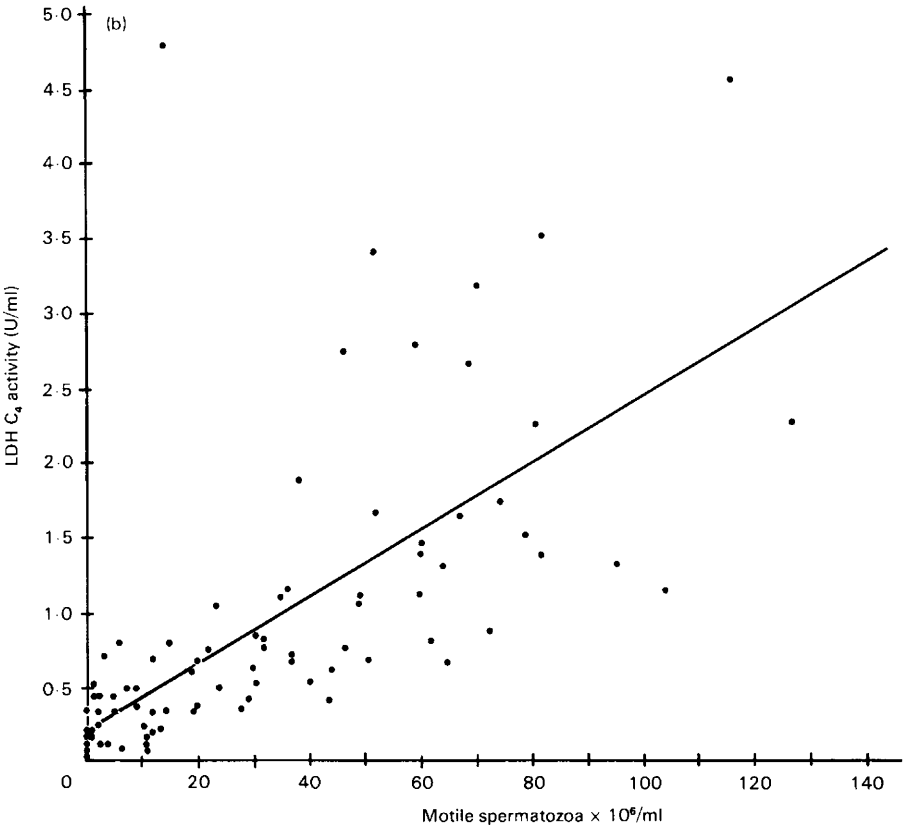
The effectiveness of the method used for disruption of spermatozoa was also studied from the point of view of amount of LDH C_4 liberated. Two methods for the lysis of germ cells in semen were compared: one comprised two cycles of freezing and thawing as described in 'Materials and Methods', and the other was that utilized by Burgos *et al.* (1979), i.e. freezing and thawing plus sonication in one cycle only. The enzymic activity liberated was about the same for both procedures. The activity of 2 aliquots of the same sample was determined in 10 samples. Taking as 100% the activity of the sonicated aliquot, the differences between the two methods ranged between +11·82% and −7·75%, the mean being +0·49% (s.d. ± 7·1).

Correlation between LDH C_4 activity and sperm count and motility

The results presented above led us to utilize semen instead of washed spermatozoa for the assays and to perform freezing and thawing as the lysing procedure.

Values of LDH C_4 activity were expressed in Units of enzyme per ml semen and Units per mg protein and showed correlation with sperm count (Text-fig. 1a). When correlated with the number of spermatozoa/ml, $r = 0{\cdot}74$ for values of U/ml semen and $r = 0{\cdot}64$ for U/mg protein.

When the activity was correlated with the number of motile spermatozoa (Text-fig. 1b), $r = 0{\cdot}57$ for U/ml semen and $r = 0{\cdot}66$ for U/mg protein.



Text-fig. 1b. Correlation between LDH C_4 activity in semen and the count of motile spermatozoa.

Discussion

The utilization of 2-oxohexanoate as substrate for the assay of isoenzyme C_4 activity in crude preparations (Burgos *et al.*, 1979) gives a selective estimation of this molecular form of lactate dehydrogenase which is specific to spermatozoa. The present results show that the correlation between LDH C_4 activity and sperm count and motility is good whether expressed as activity per ml semen or per mg protein.

The activity measured in semen after lysis of spermatozoa by freezing and thawing gives a more accurate estimate of the amount of isoenzyme present in spermatozoa than determinations on separated sperm cells. LDH C_4 is a very soluble enzyme which leaks into the seminal fluid even when previous manipulations are reduced to a minimum (Zinkham *et al.*, 1964). For this reason, assays with washed spermatozoa give values well below the actual enzyme concentration. On the other hand, the assay with semen is much simpler, because the separation and washing steps are avoided.

The C_4 isoenzyme of lactate dehydrogenase is believed to be a very specialized enzyme, associated with metabolic processes providing energy to the germ cell (Blanco *et al.*, 1975, 1976; Gerez de Burgos, Burgos, Montamat, Moreno & Blanco, 1978). Such a functional role of LDH C_4 would suggest that determination of its activity in semen could be a very useful criterion for assessing the ability of spermatozoa to promote conception. Whether this index affords a greater degree of reliability than those usually employed in the clinical laboratory remains to be

established. Sperm counts are not dependable as an index of fertilizing capacity when sperm density is low (Zukerman, Rodriguez-Rigau, Smith & Steinberger, 1977). Further study is needed to show whether the activity of LDH C_4 is a reliable index of fertility.

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