

Validation of Transcriptomic Data by the Principal Component Analysis Methodology

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ABSTRACT

Selecting embryos with the highest implantation potential using data mining and decision tools based on classical embryo morphology and morpho kinetics. Several studies suggest that genes involved in intercellular communication in ovocyte cells crosstalk, could serve as biomarkers for selecting these embryos. Embryo selection is a crucial step in assisted reproductive technologies (ART), and more specifically *in vitro* fertilization (IVF). It relies on identifying the most viable embryos for transfer through rigorous selection to optimize the chances of a successful pregnancy. This process involves evaluating embryos based on their morphological characteristics, genetic viability, and developmental potential, with the aim of increasing the chances of implantation and the birth of a healthy child. Reproductive experiments can introduce variability of different sources (biological, technical, etc.), which can lead to poor embryo selection. Therefore, it is necessary to verify the validity of this data using appropriate diagnostic tools such as Principal Component Analysis to avoid wasting time on data analysis. Hence the need to analyze these data by the PCA to see the path to follow in terms of experimental feasibility. In summary, the main objective of this study is to validate or not these transcriptomic data by the Principal Component Analysis (PCA) method. The PCA will make it possible to give up the complete analysis in the case where the data are biased and thus save time. The author can repeat the experiment if the data is biased by minimizing the correlation of the variables.

Keywords: Embryos; Ovocyte; Reproductive; Pregnancy; Variability; Diagnostic; PCA; Biased; Correlation

Abbreviations: ART: Assisted Reproductive Technologies; IVF: *In Vitro* Fertilization; PCA: Principal Component Analysis; PC: Principal Component

Introduction

The transcriptomic data used correspond to 21 genes as genomic signature and 102 sample- cumulus of patients previously treated. And our goal is to optimize the time of data analysis and their interpretations in terms of correlation (redundancy). The factorial tool that will be used for pretreatment is the main component decomposition. It is recalled that the principal component analysis is used to extract and visualize the important information contained in a multivariate data table. The PCA synthesizes this information into just a

few new variables called main components. These new variables correspond to a linear combination of the original variables. The number of principal components is less than or equal to the number of original variables [1-4]. The information contained in a dataset corresponds to the variance or total inertia it contains. The objective of the PCA is to identify the directions (main axes or principal components) along which the data variation is maximum. In other words, the PCA reduces the dimensions of a multivariate data to two or three main components, which can be visualized graphically, losing as little information as possible [5].

Background

Basics of the PCA

Note that the PCA is particularly useful when the variables in the dataset are highly correlated. The correlation indicates that there is redundancy in the data. Because of this redundancy, the PCA can be used to reduce the original variables to a smaller number of new variables, the PCA accounting for most of the variance contained in the original variables [5,6].

Data Standardization

In principal component analysis, variables are often standardized. This is particularly recommended when variables are measured in different units (for example: kilograms, kilometers, centimeters, ...); otherwise, the result of the PCA obtained will be strongly affected. The goal is to make the variables comparable. Typically, the variables are normalized so that they ultimately have

- i. a standard deviation of one and
- ii. an average of zero.

Technically, the approach is to transform the data by subtracting a reference value (the average of the variable) from each value and dividing it by the standard deviation. At the end of this transformation, the data obtained are called centric-reduced data. The PCA applied to these transformed data is called the standard PCA. Data standardization is a widely used approach in the context of analyzing gene expression data prior to PCA and Clustering Analysis. When normalizing variables, the data can be transformed as follows:

$$[X - \text{mean}(x)] / \text{sd}(x)$$

Where $\text{mean}(x)$ is the average of the values of x , and $\text{sd}(x)$ is the standard deviation. The scale function can be used to normalize the data under R.

Eigenvalues / Variances

As described in previous sections, eigenvalues measure the amount of variance explained by each major axis. The eigenvalues are large for the first axes and small for the following axes. In other words, the first axes correspond to the directions carrying the maximum amount of variation contained in the dataset [1-4]. We examine eigenvalues to determine the number of principal components to consider. The eigenvalues and the proportion of variances (i.e. information) retained by the main components can be extracted using the function `get_eigenvalue` [package `factoextra` under R].

Formulation of the Problem

The variable to explain Y that takes only two modalities: the positive or negative pregnancy is a categorical and dependent binary random process that follows a binomial probability distribution with the parameters (N, p) . This random variable is formulated as follows:

$$Y = X * \beta T + \epsilon$$

- X is the descriptive matrix.
- β is the predictor vector.
- ϵ is the residual error following a normal distribution defined on the space $\Omega(0, \sigma^2)$ of zero mean and of variance $= \sigma^2$.

Y is the vector to best predict by maximizing the likelihood criterion. The knowledge of the probability $P(Y = 1 | X = x)$ implies that $P(Y = 0 | X = x)$. It is therefore enough to model the probability by: $p(x) = P(Y = 1 | X = x)$.

The explanatory variable X represents the gene expression data. In our case, it is the following matrix:

$$X = \begin{bmatrix} 1x_{11} \dots x_{1P} \\ \dots \dots \dots \dots \\ \dots \dots \dots \dots \\ 1x_{N1} \dots x_{NP} \end{bmatrix}$$

It is an explicative matrix of N rows (cumulative), and P genes. The goal is to predict the Y vector. To do this, we perform a standardization and a PCA to explore the quality of the data and see if we go far in the analysis.

Results

Eigenvalues and Cumulative Variances

Below, we give the results of the PCA of our dataset: (Figure 1)

From the graph above, we might want to stop at the first main component. 98% of the information (variances) contained in the data is retained by the first main component.

Correlation Circle

The correlation between a variable and a Principal Component (PC) is used as the coordinates of the variable on the principal component. The representation of the variables differs from that of the observations: the observations are represented by their projections, but the variables are represented by their correlations [1,5] (Figure 2).

	eigenvalue	percentage of variance	cumulative percentage of variance
comp 1	2.058519e+01	9.802473e+01	98.02473
comp 2	3.635247e-01	1.731070e+00	99.75580
comp 3	5.124489e-02	2.440233e-01	99.99982
comp 4	2.898433e-05	1.380206e-04	99.99996
comp 5	7.279691e-06	3.466520e-05	99.99999
comp 6	8.174810e-07	3.892766e-06	100.00000
comp 7	3.722871e-07	1.772796e-06	100.00000
comp 8	4.206170e-08	2.002938e-07	100.00000
comp 9	3.532653e-08	1.682216e-07	100.00000
comp 10	1.207326e-08	5.749172e-08	100.00000
comp 11	1.347210e-09	6.415287e-09	100.00000
comp 12	8.610187e-10	4.100089e-09	100.00000
comp 13	5.812445e-10	2.767831e-09	100.00000
comp 14	3.920804e-10	1.867050e-09	100.00000
comp 15	2.492393e-10	1.186854e-09	100.00000
comp 16	1.998953e-10	9.518824e-10	100.00000
comp 17	8.968748e-11	4.270832e-10	100.00000
comp 18	5.192826e-11	2.472774e-10	100.00000
comp 19	2.185256e-11	1.040598e-10	100.00000
comp 20	7.257646e-12	3.456022e-11	100.00000
comp 21	4.983582e-12	2.373134e-11	100.00000

Note: The large variation is held by the main component 1: 98.024 %

Figure 1: Percentage of variance explained by dimensions.

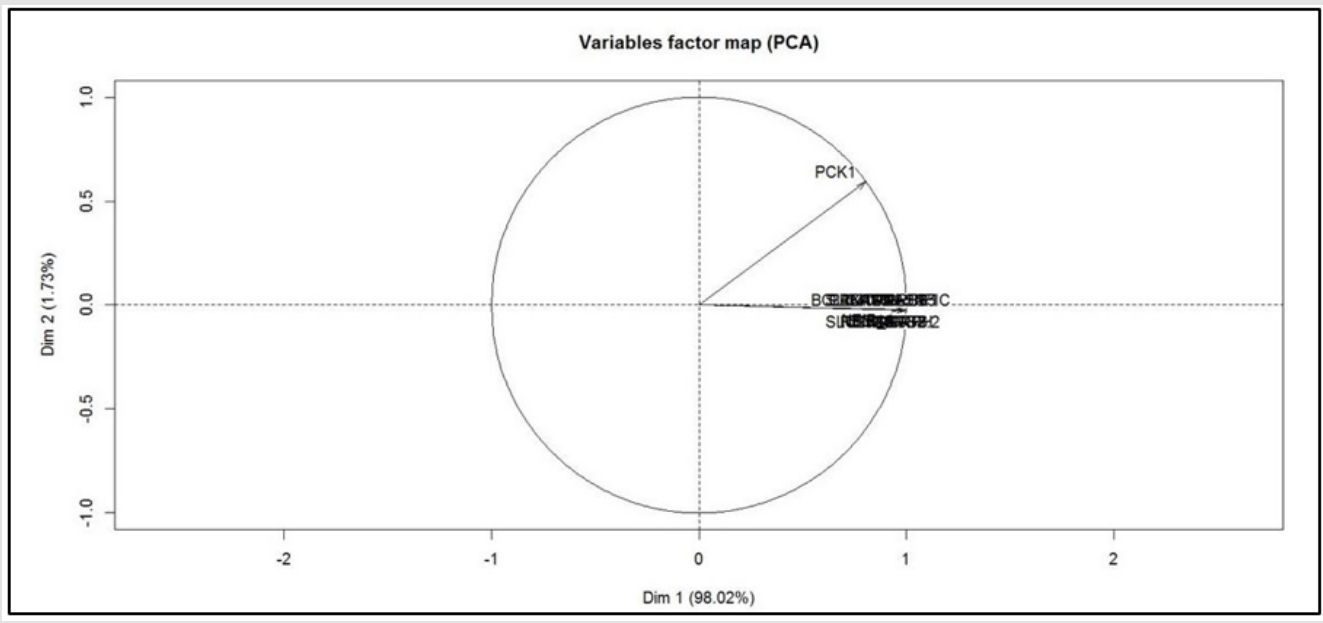


Figure 2: The representation quality of the variables on the PCA map is called \cos^2 depending on the genes. The number of axes can be limited to one axis.

The graph above is also known as a correlation graph of variables. It shows the relationships between all the variables. It can be interpreted as follows:

- Positively correlated variables are grouped together.
- Negatively correlated variables are positioned on opposite sides of the chart origin (opposite quadrants).
- The distance between the variables and the origin measures the quality of representation of the variables.
- Variables that are far from origin are well represented by the PCA.

Note that almost all variables are correlated and contained in axis 1.

- A low \cos^2 indicates that the variable is not perfectly represented by the main axes. In this case, the variable is close to the center of the circle.

It can therefore be deduced from the obtained results that these

transcriptomic data do not allow obtaining a model that discriminates in the absolute sense, despite the relatively near-acceptable performance. Consequently, these data are not exploitable. It therefore appears that these data are highly correlated indicating a single group of individuals whose variance is practically collinear with axis 1 and more or less representative since \cos^2 is less than or equal to 1 (Figure 3).

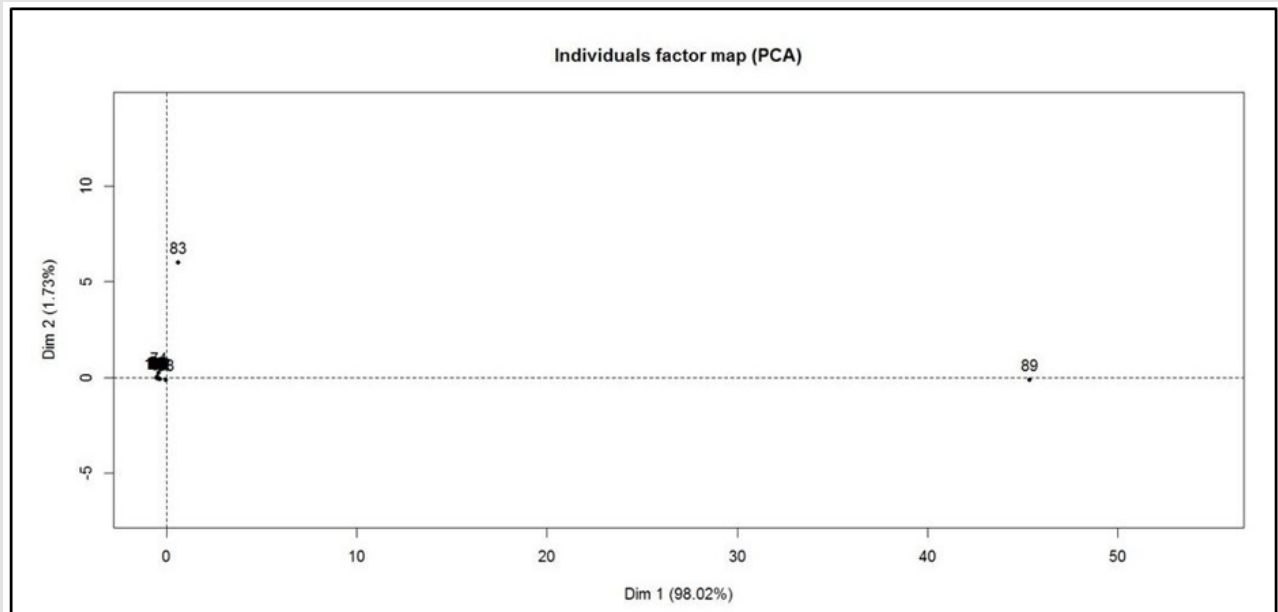


Figure 3: Note that individuals who are similar are grouped on the graph. There is strong redundancy between the variables and therefore strong correlation.

Conclusion

PCA was calculated using the PCA function [FactorMineR]. Then we used the R factoextra package to produce a ggplot2 visualization of PCA results. The data is strongly correlated, and the principal component number can be reduced to a single component (axis_1). The result is the impossibility of defining a mathematical model, because all the inertia (variability) is carried by a single principal component (component_1). The complete analysis of this data stops there, no need to continue the treatment, thus saving time for the user. We can visualize the \cos^2 variables on all dimensions using the package corrplot. It can therefore be deduced from the obtained results that these transcriptomic data do not allow obtaining a model that discriminates in the absolute sense, despite the relatively near- acceptable performance. Consequently, these data are not exploitable. It therefore appears that these data are highly correlated indicating a single group of individuals whose variance is practically collinear with axis 1 and more or less representative since \cos^2 is less than or equal to 1.

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