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Assessment of the relationship of number of sertoli cells with number of germ cells, spermatogenic activity and germ cell apoptosis rates in bulls using different regression models

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ABSTRACT

This study aimed to model the quantities of germ cells, Leydig cells, and spermatogenic activity in relation to Sertoli cell numbers in bulls. Histological and histometric methods were used to process left testes of 36 bulls. Prediction models were constructed using both linear and non-linear regression. Standard error (SE), coefficient of correlation (r), R-squared (R^2), mean squared error (MSE) and root mean squared error (RMSE) statistics were used to identify the optimal model. Statistical analysis revealed that, except for Leydig cell counts, Sertoli cell numbers may predict the overall composition of testicular cells. Spermatogenic activity and Sertoli cell count are most accurately correlated using nonlinear models. Total Sertoli cell numbers contributed to modest variance in germ cell apoptosis rates during meiosis (13.6%) and post-meiotic germ cell loss rates (7.90%), but these associations were not statistically significant. Cubic models were the best fit for describing the relationship between testicular morphometric parameters and Sertoli cells numbers (X). However, the diameter of seminiferous tubules (Y) was more accurately described by a growth model, represented by the equation $Y=e^{(5.428+(0.022*X)}$. It can be recommended that the non-linear model using the Sertoli cells numbers as the predictive biomarkers can be used for predicting bull's spermatogenetic activity.

Keywords: Bull, Nonlinear regression, Spermatogenetic activity, Sertoli cells

Sertoli cells are crucial for the development and maturation of testis throughout the fetal and early postnatal phases, as well as for the regulation of sperm production at maturity (Hutchison *et al*. 2008). These cells are derived from the coelomic epithelium, where they induce the appearance of seminiferous tubules and growth of fetal Leydig cells (O'Donnell *et al*. 2022).

Various statistical methods are used to evaluate the precision and accuracy of mathematical models. However, no single method can fully assess model's performance by itself (Hussain *et al.* 2019). The coefficient of determination (R^2) indicates the percentage of the variation in the cell population of seminiferous epithelium, and spermatogenic activity that might be explained by Sertoli cell numbers. However, using R^2 solely does not offer a comprehensive view of the model's quality, since it reflects precision but not accuracy (Onyutha 2020). The mean square error (MSE) evaluates a regression equation by measuring the accuracy of the fitted model using the differences between observed and predicted values. Lower values of standard error (SE), MSE, and root mean square error (RMSE) indicate more accurate estimates. RMSE is more suitable when the error distribution is expected to be Gaussian (Hodson 2022).

Simple regression analysis has been extensively used to describe the quantitative relationship between the number of various germ cell populations (dependent variable) and the number of Sertoli cells (independent variable) in different species. These studies primarily relied on linear models and correlation coefficients (r) or the coefficient of determination (R^2). Nevertheless, it is important to recognize that a high R^2 does not automatically suggest a linear relationship between predicted and observed values; the relationship may actually be curvilinear (Gurgel *et al.* 2023).

The objective of this study was to examine the relationship between Sertoli cell numbers and the counts of germ cells, spermatogenic activity, and germ cell apoptosis rates in bulls, using metrics such as r, R^2, SE, MSE, and RMSE.

MATERIALS AND METHODS

Location: The experiment occurred in the Batna region of Algeria, situated at 35°33′21″ North latitude and 6°10′26″ East longitude. The average elevation is 968 meters above sea level. The climate of Batna is semi-arid and is characterized by four different seasons. The average annual rainfall is 329 mm, and the mean temperature varies

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from 4°C in January to 35°C in July. Relative humidity varies between 36% and 82% throughout the year, with only a few days falling below this range.

General procedures: A total of 36 bulls were randomly chosen from a population of bulls intended for slaughter. During the antemortem examination, each animal's scrotal circumference (SC, cm) and body weight (BW, kg) were measured and noted. Immediately after slaughter, the testicular weights (TW, g) were measured.

Testis stereology: Fragments of the left testis, approximately 5 mm^3 in thickness, were collected from various segments of the testis both transversally and longitudinally using a razor blade. The fragments were immersed in 10% formalin for 24 hours for fixation, followed by dehydration in a series of increasing ethanol concentrations, clarification in xylene, and embedding in paraffin wax. Serial paraffin sections, each 5 µm thick, were deparaffinized in xylene and then rehydrated with progressively decreasing ethanol concentrations (Karmore *et al.* 2003).

These slices were stained with eosin and hematoxylin procedures for light microscopic analysis using a microscope (ZEISS, Germany, Axioplan) fitted with camera (MICROCAM MA88-500) and AxioVision Rel 4.6 software (Carl Zeiss, Thornwood, NY). The morphometric parameters, along with the counts of germ cell populations, Sertoli cells (SCN), Leydig cells (LEY), and the efficiency of spermatogenesis were determined using the methods described by Belkhiri *et al.* (2021).

Table 1. Models and corresponding syntax

Regression model	Equation
Linear	$y=b_0+(b_1*x)$
Logarithmic	$y=b_0+(b_1*\ln(x))$
Inverse	$y=b_0+(b_1/x)$
Quadratic	$y=b_0+(b_1*x)+(b_2*x^2)$
Cubic	$y=b_0+(b_1*x)+(b_2*x^2)+(b3*x^3)$
Power	$y=b_0*(x^{b}{}_{1})$ or $\ln(y)=\text{In}(b_0)+(b_1*\ln(x))$
Compound	$y=b_0*(b_1^{x})$ or $\ln(y)=\ln(b_0)+(\ln(b_1)*x)$
S-curve	$y=e^{(b0+b1/x)}$ or $\ln(y)=b_0+(b_1/x)$
Growth	$y=e^{(b0+(b1*x))}$ or $\ln(y)=b_0+(b_1*x)$
Exponential	$y=b_0*(e^{(b1/x)})$ or $\ln(y)=\ln(b_0)+(b_1*x)$

y: dependent variable (body weight, testicular weight, circumference scrotal, germ cells number, Leydig cells numbers, spermatogenetic activity and morphometric parameters), b_0: regression equation's constant term, b_1: regression coefficient, x: value of independent variable (sertoli cells number)

Statistical Analysis: The statistical analysis was performed using SPSS version 25.0 (IBM Corp. 2017). The significance level was established at alpha = 5%. Initially, the Shapiro-Wilk test was used to determine if continuous variables had a normal distribution.

One simple linear model and nine non-linear models were employed to analyse the evolution of germ cells numbers, LEY and spermatogenic activity of the 27 bulls in the study sample. Table 1 shows the functions for each fitted model, as well as the model syntax to be copied and pasted into the non-linear regression task from SPSS's Regression method. The best-fit model was determined from Pearson's coefficient of correlation (r), the R squared (R^2), the standard error (SE), Mean Squared Error (MSE) and the root mean squared error (RMSE).

RESULTS AND DISCUSSION

This study is the first to evaluate the relationship between the number of Sertoli cells and germ cell counts, spermatogenic activity, and germ cell apoptosis rates in bulls, employing statistical metrics such as R, R^2, SE, MSE, and RMSE.

Regression between Sertoli cells number and body weight and testicular measurements: Table 2 presents estimators for models that relate body weight and testicular measures to Sertoli cell counts in bulls. According to the estimators used to choose the best-fit model, the cubic model had the highest correlation coefficient (r) and determination coefficient (R^2), as well as the lowest SE. Consequently, the MSE and RMSE of the cubic model never exceeded 0.008 and 0.089, respectively. These results indicate that the cubic model, $BW = 506.151 - 258.92\ SCN + 81.346\ SCN^2 - 7.710\ SCN^3$, offers the best fit for predicting body weight (BW) based on the number of Sertoli cells (SCN). Additionally, the cubic model is the most suitable regression model for describing the relationship between testicular weight (TW) and SCN. The parameters are represented by the equation $TW = 975.331 - 913.41\ SCN + 309.408\ SCN^2 - 28.58\ SCN^3$. However, the linear function model demonstrated the best-fitting properties for the relationship between scrotal circumference (SC) and SCN, with the regression equation $SC=0.639SCN+30.945$. Similarly, Berndtson *et al.* (1987), reported that total SCN explained just 36.5% (R^2) of the variability in testis size, suggesting that Sertoli cell number is not the sole factor regulating mature testicular size. Petersen *et al.* (2015) also reported no significant correlation between testicular weight and Sertoli cell numbers ($r = 0.27$, $p= 0.19$) in

Table 2. Estimators of the models relating body weight and testicular measurements to Sertoli cell numbers in bulls.

Trait	Equation	Model Estimate		Variability			*p* value
		r	R^2	SE	MSE	RMSE	
BW	Cubic	0.503	0.253	0.096	0.008	0.089	*
TW	Cubic	0.458	0.209	0.197	0.039	0.197	*
CC	Linear	0.481	0.231	0.046	0.002	0.044	*

BW: body weight (Kg), TW: testicular weight (g), SC: scrotal circumference (cm), *: Significant at $p<0.05$.

Table 3. Estimators of the models relating germ cell numbers, Leydig cell numbers and spermatogenic activity to Sertoli cell numbers in bulls.

Trait	Equation	Model Estimate		Variability			*p* value
		r	R^2	SE	MSE	RMSE	
SG	Linear	0.998	0.997	0.055	0.003	0.054	***
SI	S	0.413	0.168	0.267	0.071	0.266	**
SII	S	0.532	0.283	0.245	0.060	0.244	***
SD	Power	0.669	0.447	0.150	0.022	0.148	***
LEY	Cubic	0.378	0.143	0.755	0.513	0.716	ns
CESM	Power	0.539	0.290	0.270	0.073	0.270	***
OSY	Growth	0.700	0.490	0.228	0.052	0.228	***
MI	Cubic	0.369	0.136	0.520	0.270	0.519	ns
PMGL	Cubic	0.281	0.079	0.252	0.063	0.250	ns
SCE	Exponential	0.689	0.474	0.228	0.052	0.228	***

SG: spermatogonia; SI: primary spermatocytes, SII: secondary spermatocytes, SD: spermatids, LEY: leydig cells, CESM: spermatogonial mitosis efficiency coefficient, OSY: overall spermatogenesis yield, MI: Meiotic Index, PMGL: post-meiotic germ loss, SCE: Sertoli cell efficiency, ns: No significant, **: Significant at $p<0.01$, ***: Significant at $p<0.001$.

human males. These conflicting findings may be attributed to species-specific differences, but are more likely to be due to variations in the counting methods used. Their studies predominantly utilized linear models and relied solely on correlation coefficients (r). Other factors, in addition to SCN, which are likely to influence TW in bulls. These factors may include precise age, environmental influences such as nutrition, the rate of testicular growth and development, and body weight (Latimer *et al*. 1982).

Regression between the number of Sertoli cells and the counts of germ cells and LEY: Table 3 summarize the results for the determination coefficients (R^2), standard error (SE), mean squared error (MSE) a root mean squared error (RMSE), for the best-fitting models that describe the relationships between germ cell numbers and LEY numbers in relation to Sertoli cell numbers.

In estimating the number of spermatogonia (SG) using the SCN, the linear regression equation ($SG=-0.073+0.987SCN$) provides accurate estimations with 75.0% explained variance. The estimation of the number of primary (SI) and secondary spermatocytes (SII) from SCN was more accurate using the S-curve models, as indicated by higher R^2 values and lower SE, MSE and RMSE (Table 2). The parameters are described by the equations $SI=e^{(2.460-1.275/SCN)}$ and $SII=e^{(2.533-1.275/SCN)}$. This is consistent with the results of Berndtson and Jones (1989) in horses, and those of Hochereau-de Reviers *et al*. (1978) in rats, rams, and bulls. Tripathi *et al.* (2015) proposed that having more Sertoli cells per spermatogenic cell would give sufficient support and nutrition for efficient spermatogenesis and the production of good quality sperm.

The power model provided the best fit between the number of spermatid (SD) and Sertoli cells (SCN), exhibiting the highest correlation and determination coefficients, the lowest standard error, and mean squared error (Table 3). The equation used is $SD=44.387\times(SCN^{0.452})$. This finding is consistent with the findings of Orth *et al.* (1988), who reported that reducing Sertoli cell division in neonatal rats resulted in a similar change in spermatid counts in the adult.

In contrast to these findings, the number of LEY were not related to the SCN ($p>0.05$). There are only a few studies that investigate this relationship. On the other hand, Rebourcet *et al.* (2014) demonstrated that when Sertoli cells are completely destroyed in a neonate, the tubule structure is lost, peritubular myoid cells dedifferentiate, and the ability of the LEY population to differentiate and develop is severely limited. Additionally, 70% of the adult LEY is lost when the adult Sertoli cells are ablationated, ablated which is associated with the loss of germ cells. Nevertheless, it has been shown that LEY apoptosis occurs in BCLW-deficient animals subsequent to Sertoli cell loss (Russell *et al.* 2001).

Regression between Sertoli cells number and spermatogenic activity: The determination coefficients (R^2), standard error (SE), mean squared error (MSE), and

Table 4. Estimators of the models relating morphometric data of testes to Sertoli cell numbers in bulls.

Trait	Equation	Model Estimate		Variability			*p* value
		r	R^2	SE	MSE	RMSE	
VTS	Cubic	0.480	0.230	0.048	0.048	0.219	*
STD	Growth	0.455	0.207	0.113	0.013	0.114	*
LST	Cubic	0.901	0.811	0.203	0.041	0.202	***
GEH	Cubic	0.449	0.201	0.132	0.017	0.130	*

VTS: Total seminiferous tubule volume per testis (%), STD: Seminiferous tubuli diameter (μm), LST: Length of seminiferous tubules (m), GEH: Germinal epithelium height of testes (μm), *: Significant at $p<0.05$; ***: Significant at $p<0.001$.

root mean squared error (RMSE) for the best accurate models that describe the correlations between Sertoli cell counts and spermatogenic activity are presented in Table 4.

According to the current study, nonlinear models work best for establishing a relationship between spermatogenic activity and Sertoli cell count. The power law provides a very good description of the present relationship between the quantity of Sertoli cells and the spermatogonial mitotic efficiency coefficient. Therefore, in order to forecast the efficiency of spermatogonial mitosis (CESM) based on SCN, we advise adopting the model $CESM=5{,}040\times SCN^{-0.60}$. However, no comparable studies are available in the current literature to evaluate these results. Knowing the number of spermatogonial generations is critical for understanding spermatogenesis' regulating processes (De Rooij and Russell 2000). In mammals, primary spermatocytes are normally produced after two to six mitotic divisions.

According to our research, about half of the difference in total spermatogenic yields (OSY) may be explained by the quantity of Sertoli cells (SCN) (R^2 = 49%). The parameters are described by the equation $OSY=e^{(2{,}683+(-0.235\times SCN))}$. However, there are no comparable reports in the existing literature to assess these results against.

However, SCN explained some of the variations in the rate of germ cell apoptosis during meiosis (MI) (13.6%) and in the rate of post-meiotic germ cell loss (PMGL) (7.90%), but these rates were not significantly related ($p>0.05$) to SCN. Therefore, the rates of cellular apoptosis must be caused by variables other than the quantity of Sertoli cells. Nakanishi and Shiratsuchi (2004) examined the function of Sertoli cells in spermatogenic cell survival and differentiation and found that removing Sertoli cells from primary co-cultures of rat testicular cells enhanced the ratio of apoptotic spermatogenic cells. Sertoli cells, on the other hand, appeared to decrease the quantity of apoptotic cells rather than stop spermatogenic cells from going through apoptosis. This suggests that the presence of Sertoli cells causes apoptotic spermatogenic cells to vanish from cultures (Mizuno *et al.* 1996).

The goodness-of-fit parameters indicated that the exponential model provided the most appropriate fit for the observed data when describing Sertoli cell efficiency (SCE) based on the number of Sertoli cells (SCN). The parameters are described by the equation $SCE=13{,}796*e^{(-0.228/SCN)}$). The spermatid-to-Sertoli cell ratio serves as a key indicator of spermatogenesis, as studies have shown that fluctuations in daily sperm production directly influence the number of spermatids sustained by the Sertoli cell population (Russell and Peterson 1984). Our results indicated that the total number of Sertoli cells explained a moderate portion of the variability in Sertoli cell efficiency among bulls (R^2 = 47.40%). As age advances, spermatogenic efficiency has progressively declined across different vertebrate species. This reduction is closely related to the Sertoli cell's ability to support spermatids, which decreases to about 3 spermatids per Sertoli cell in humans (França *et al.* 2015).

Regression between SCN and morphometric data of testes: Our findings revealed cubic relationships between total seminiferous tubule volume (VTS) and SCN (r = 0.480, $p< 0.05$) (Table 4). Only a small portion of the overall variation in seminiferous tubule volume was explained by the quantity of Sertoli cells (R^2 = 23%). Based on these findings, we may conclude that the cubic model is $VTS = 506.151 - 258.92\ SCN + 81.346\ SCN^2 - 7.710\ SCN^3$.

As a result, the growth model, $STD = STD=e^{(5.428+(0.022*SCN))}$, gives the best match for the association between Sertoli cell numbers (SCN) and seminiferous tubule diameter (STD).

The cubic equation: $LTS= 695.176+103.264SCN + 8.170SCN^2 +0.001SCN^3$, describes the association between the average number of Sertoli cells (SCN) and the length of seminiferous tubules (LST), with $p<0.001$ and R^2=81.1%. Additionally, the relationship between SCN and the height of the germinal epithelium (GEH) is given by the cubic equation: $GEH= 17.054+42.624SCN -8.791SCN^2 +0.498SCN^3$ with $p<0.05$ and R^2=20.1%. Increases in total tubular length, tubule diameter and germinal epithelium height, which indicate the enlargement of the seminiferous tubule compartment, were linked to an increase in Sertoli cell counts.

The relative mass of the tubular compartment in the testis influences the amount of space available for sperm production (Hess and De Franca 2008). In adult testes, Sertoli cells account for approximately 25% of the volume of the seminiferous tubules (de Kretser *et al.* 2016). Likewise, the overall count of Sertoli cells per testis is considered to be an important determinant in influencing sperm production levels (Lara *et al.* 2018).

It may be concluded from the study that there is a notable correlation between Sertoli cell numbers and testicular size and testicular histomorphometric parameters. These findings lend credence to the concept that Sertoli cell count is crucial role in defining testicular dimensions and developing a bull's spermatogenic activity. These criteria can thus be used to evaluate the semen production capacity of adult bulls and to choose superior bull sires.

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