EFFECT OF THE NUMBER OF SPERMATOZOA AND SPERMATOZOAL QUALITY ON FERTILITY IN RABBITS

PIZZI F.[1], GUAITA N.[2], LUZI F.[2], BIFFI B.[2], BRIVIO R.[3], CRIMELLA C.[2]

[1] Istituto per la Difesa e Valorizzazione del Germoplasma Animale, CNR, Via Celoria, 10, 20133 Milano, Italia
[2] Istituto di Zootecnica, Facoltà di Medicina Veterinaria, Via Celoria, 10, 20133 Milano, Italia
[3] Medico Veterinario, L. P. Piano Ipofecondità Regione Lombardia, Via Monfalcone, 26 - 20099 Sesto San Giovanni (Mi), Italia

Abstract - The importance of a correct evaluation of semen quality lies in the relationship between quality and fertility. To identify, among the qualitative parameters, the best indicators of male reproductive efficiency, relationship between the characteristics of the seminal material evaluated using a computerised system of analysis of images and fertility were studied. Further, a predictive exponential model of fertility was used, which allowed the stabilisation of the number of motile spermatozoa per dose necessary to obtain optimum fertility.
The average values of the variables analysed are as follows: cells concentration in the dose 161.41±42.19 (10^6/ml), motility 39.15±16.92 (%), concentration of motile spermatozoa 64.05±34.47 (10^6/ml), forward progression 1.73±0.62, pregnancy percentage 80.87±12.25, total number born 9.08±0.84 and number born alive 9±0.88. As regards statistical analysis, the effect on fertility of the qualitative characteristics of the semen evaluated using the automatic analyser was seen to be significant. In particular, the motility percentage and the spermatozoa concentration per ml seem to be the best parameters for the fertility (% of pregnancy, number of total born). The repeatability of semen characteristics evaluated with the automatic analyser are: 0.11 for the spermatozoa concentration, 0.06 for the concentration of motile cells and 0.12 for forward progression rate. Results obtained relating to prediction of fertility through the exponential model indicate that $15x10^6$ motile spermatozoa/ml seem to be enough to obtain a high fertility (78%) and further increasing of this parameter does not correspond to higher fertility.

INTRODUCTION

The relationship between semen quality and fertility is not yet known for most animal species. Moreover the effect of the number of spermatozoa on fertility has a direct impact on the breeding management of the rabbits. To consider the impact of this relationship on economic efficiency, many researchers have worked in this field. SALISBURY and VAN DEMARK (1978) indicated an asymptotic approach to semen maximum fertility level, consistent with the optimum fertility of the cow population, with increasing values for certain individual semen characteristics. Once an optimum semen evaluation value is reached, further increased values of the characteristic do not result in corresponding increases in fertility (CHEN and FOOTE, 1994).
The best fit was obtained on the field data by the following non-linear regression equation:

Non Return Rate=$ae^{-b/n}$ (DEN DAAS, 1992).

Also PACE *et al.* (1981) found that the relationship between seminal viability and fertility was best explained by an exponential model.
The aim of this study was to estimate some environmental and genetic effects on fertility in rabbits. Trying to define the minimum number of spermatozoa necessary to reach the highest level of fertility, the relationship between seminal characteristics and fertility was investigated.

MATERIAL AND METHODS

This trial was performed in a rabbit farm at Mandello del Lario (Italy), at an altitude of 600 metres. The farm was divided into several sections: one housed the reproductive males, one the pregnant female, one the fattening unit. All the structures were built from expanded clay blocks, covered in fibro-cement on metal frames. The animals were reared into flat-deck cages, positioned between a shed with pressure ventilation and with natural photoperiod. All rabbits utilised during the trial, were fed with an integrated commercial feed.

After some weeks preparation, semen from 31 hybrid Charles River "New Zealand" rabbits was collected weekly, two collections per day from September 1994 until January 1995. The rabbit age ranged from 5 months to 2 years. The age has the following distribution : 3.3% of the males had 5 months of age, 30% from 7 to 9 months, 23.3 had 10 months, 13.3% 11 months, 10% 12 months, 3.3% 16 months and 16.7% 25 months of age. Ejaculates were diluted 1:1 using a M III (Minitüb®) and then evaluated giving a subjective judgement to sperm concentration, motility and viability firstly. Then the final dilution was made to obtain a fertilisation dose (ml 0.5) containing no less than $10x10^6$ cells. 50 µl of diluted semen was kept from the pool of the two collections per buck then evaluated using a computerised semen analyser system (CellSoft®). The samples were brought to the laboratory in a thermostat at 17°-18° C. The computer permits the evaluation of the percentage of motility, the velocity and the forward progression of spermatozoa. Furthermore, it allows to know the concentration in million/ml, and concentration of motile cells making out spermatozoa from other components of the ejaculate. A total of 1531 females were treated by PMSG 20-25 UI about 60-72 hours before insemination and by 0.8 µg GnRH (Buserelin) at the moment of insemination. The ejaculate of a single male was utilised for artificial insemination of an average of 13 females. Does were inseminated 18-25 days after parturition. The females management in this rabbitry was the following: 7 delivery by doe per year, usually the females were kept for a maximum of 2 years, the average herd replacement was less then 100%, the highest culling occur between the first and second delivery. Concerning physiological status, 7.7% of the females inseminated were nulliparous.
Concerning fertility traits, the percentage of pregnancy was evaluated as a ratio between the total number of pregnant females and the total number of inseminated females. Moreover, the number of live born and still born was recorded. The percentage of pregnancy, number of total born and number of born alive were analysed by a mixed model (Proc Mixed SAS®) including the month of collection as fixed effect, days from the previous collection and semen qualitative characteristics (concentration, % motility, concentration of motile cells, forward progression rate) as covariates and the buck as random effect. The repeatability of the semen characteristics were calculated from variance components using a mixed model (Proc Varcomp SAS®) including sire (random), month of collection and days from previous collection.
The predicted fertility was evaluated by a linear regression equation as a function of some qualitative semen characteristics. Furthermore, the relationships between the number of motile spermatozoa/ml and fertility were studied by an exponential model (Proc Nlin SAS®)

$$\% \text{ pregnancy} = ae^{-b/x}$$

x=n. of motile spermatozoa (millions/ml)
a=asymptotic limiting value
b=rate at which the asymptote is approached

RESULTS AND DISCUSSION

The mean values of the variables were: 161.41±42.19 (10^6/ml) concentration of spermatozoa in the dose, 39.15 ±16.92 (%) motility, 64.05±34.47 (10^6/ml) concentration of motile spermatozoa, 1.73±0.62 forward progression, 80.87±12.25 percentage of pregnancy, 9.08±0.84 number of total born, 9±0.88 number of alive born.
Although the percentage of motility was not particularly high, probably because motility was estimated 3 hours after collection, the mean fertility rate (80.87%) indicates a good level of reproductive efficiency. Significant was the effect of qualitative characteristics evaluated by an automatic analyser on fertility. In particular, the percentage of motility and spermatozoa concentration seem to be the best indicators of pregnancy, with a slope of 0.71 for the % of motility and 0.15 for the number of spermatozoa/ml (figures 1-2) and of the number of total born. Repeatability for semen characteristics were 0.11 spermatozoa concentration, 0.16 percentage of motility, 0.06 concentration of motile cells, 0.12 forward progression rate. In conclusion, the objective evaluation of the percentage of motility was found to be associated with the percentage of pregnancy in rabbits. Repeatability for this trait resulted higher than for the other sperm characteristics. Considering our results percentage of motility can be used as a reliable and constant indicator of fertility in rabbits.
The asymptotic model predicts a limiting percentage of pregnancy (asymptotic value) as semen characteristics increase in value (figure 3). This limit seems to be near $15x10^6$ motile spermatozoa/ml, that are enough to obtain a fertility of 78%; at this value a plateau is reached and further increase of the number of motile spermatozoa has a little influence on percentage of pregnancy. The results of this study indicate that the increase in fertility did not result solely from increases in the number of motile spermatozoa/ml.

Figure 1: Linear regression equation between % of motility and pregnancy

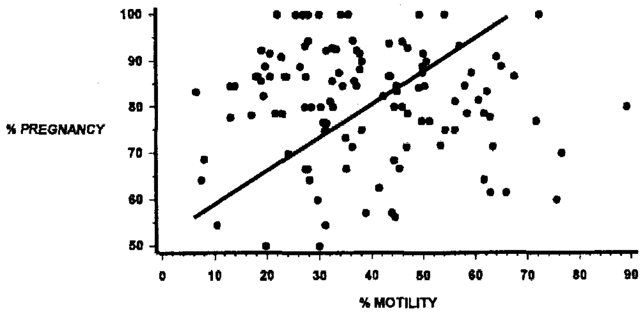
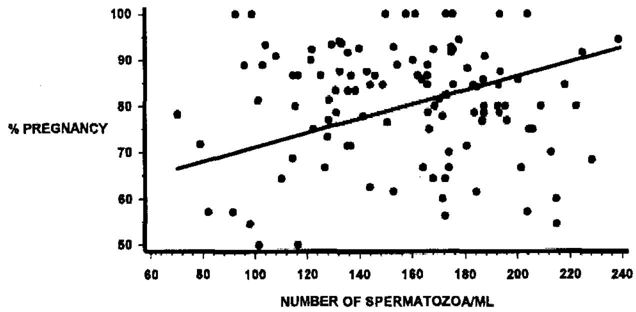


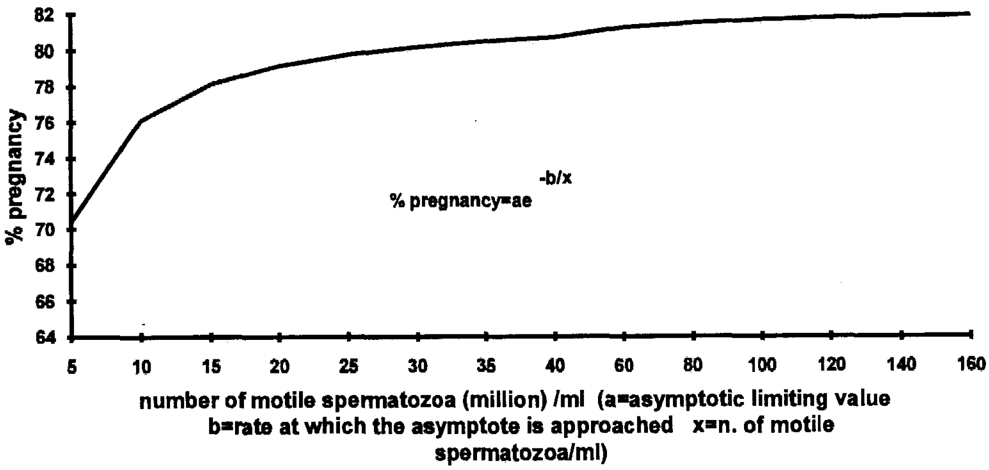
Figure 2: Linear regression equation between number of spermatozoa/ml and % of pregnancy



Semen was diluted to obtain a dose containing no less than 10×10^6 cells, while the following analysis showed a concentration of motile spermatozoa of 32.03×10^6 per dose indicating that the visual evaluation of the cells concentration on which the dilution factor is based, does not correspond to the real number of sperm per ml.

Considering that in our experience 15×10^6 motile spermatozoa/ml (7.5×10^6 per dose) seem to be enough to reach high fertility level, the use of doses containing a lower number of motile spermatozoa could allow to obtain a more efficient utilisation of the best buck and to keep a lower number of bucks in the rabbitry; thus being of clear economical and practical utility.

Figure 3: Relationship between % of pregnancy and number of motile spermatozoa/ml



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Riassunto - L'importanza di una corretta valutazione della qualità del seme risiede nella relazione tra qualità e fertilità. In questo lavoro si è studiata la relazione tra le caratteristiche del materiale seminale, valutate con un sistema computerizzato di analisi di *immagini* e la fertilità nella specie cunicola al fine di identificare, tra i parametri qualitativi, i migliori indicatori del'efficienza riproduttiva maschile intesa come percentuale di gravidanza ottenuta e numero di nati. Si è inoltre utilizzato un modello predittivo della fertilità, con una forma esponenziale, che consente di stabilire il numero minimo di spermatozoi motili per dose necessario per ottenere l'optimum di fertilità.
I valori medi delle variabili analizzate sono risultati i seguenti: concentrazione di cellule nella dose 161,41±42,19 (10^6/ml), motilità 39,15±16,92 (%); concentrazione degli spermatozoi motili 64,05±34,47(10^6/ml), tipo di movimento 1,73±0,62; percentuale di gravidanza 80,87±12,25; numero nati totali 9,08±0,84; numero nati vivi 9±0,88. Per quanto riguarda l'analisi statistica significativo è risultato l'effetto sulla fertilità delle caratteristiche qualitative del seme valutate con l'analizzatore. In particolare, la percentuale di motilità e la concentrazione degli spermatozoi per ml, sembrano essere i parametri migliori indicatori della fertilità (% gravidanza e n°nati totali). La ripetibilità delle caratteristiche del seme di coniglio valutate oggettivamente è risultata rispettivamente di 0,11 per la concentrazione di spermatozoi, 0,16 per la percentuale di motilità, 0,06 per la concentrazione di cellule mobili e di 0,12 per il punteggio di motilità. I risultati ottenuti relativamente alla previsione della fertilità tramite il modello esponenziale hanno indicato, che $15x10^6$ spermatozoi motili/ml sembrano sufficienti per ottenere una fertilità del 78%, questa infatti rimane pressoché costante ad un ulteriore aumento della concentrazione di spermatozoi motili/ml.