VELOCITY CHARACTERISTICS AND NUMBERS OF BULL SPERMATOZOA IN RELATION TO AGEING, DETERMINED BY PHOTO-ELECTRIC METHODS*

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Summary. With Rikmenspoel's photo-electric method, the initial mean velocity and number of normally moving bull spermatozoa per unit volume, the velocity frequency distributions and the rates of decrease of numbers and velocity with time have been studied, using a standard egg-yolk-citrate diluent at pH 6.75 (= 6.80 at 37°C). The distribution of mean velocities between ejaculates differs significantly from a Gaussian one, but conforms within measuring error with a logarithmic distribution. No significant differences in initial velocity of the spermatozoa between bulls were found on random sampling with respect to feeding and keeping conditions and season.

A positive correlation was found between the number of normally moving spermatozoa \( N_{\text{mov}} \) per unit volume and the total sperm concentration \( r = +0.43 \), and a negative correlation between \( N_{\text{mov}} \) and the mean velocity \( \bar{v} \) \( r = -0.54 \). There was no correlation between the mean velocity and total sperm concentration \( r = 0.06 \).

Ageing was studied on storage for 20 hr or longer in a Dewar vessel with ice. The velocity decrease with time \(-\partial \bar{v}/\partial t\) was \( 0.66 \pm 0.39 \) (\( \mu \)sec)/hr \( (s.e. = \pm 0.06) \), mode \( 0.30 \) (\( \mu \)sec)/hr, and the half-life period of the number of normally moving spermatozoa \( t_i(N) \) was \( 60 \pm 44 \) (s.d.) \( (s.e. = \pm 7.6) \) with a mode of 30 hr. Significant correlations were found between \(-\partial \bar{v}/\partial t\) and the initial mean velocity \( \bar{v}_o \) \( r = +0.57 \) and between \( \log (-\partial \bar{v}/\partial t) \) and \( \log t_i(N) \) \( r = -0.40 \).

There was no correlation between the initial number of normally moving spermatozoa \( N_o \) per unit volume and \( t_i(N) \) \( r = 0.114; 0.50 < P < 0.60 \).

It is concluded that although high initial swimming velocity of bull spermatozoa might still be thought to be advantageous for fertilization in natural mating, this will probably not be so when the semen is used for artificial insemination, since the higher the initial velocity is, the shorter the period during which it is maintained and the shorter the period of survival of normally moving spermatozoa. It appears that the rates


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of velocity-decrease with time, and the half-life period of the number of spermatozoa moving normally, are more important for evaluating the quality of semen samples with respect to motility characteristics than the initial swimming velocity of the spermatozoa.

INTRODUCTION

Data on the mean swimming velocity and velocity frequency distributions of bull spermatozoa, determined by objective physical measurements with photo-electric equipment, have been published by Rikmenspoel (1957b, 1960) Van Duijn & Rikmenspoel (1960) and Rikmenspoel & Van Duijn (1960). The present study is an evaluation of data obtained in the course of several years in continuation of previous investigations.

MATERIAL AND METHODS

The material included ejaculates from bulls of Dutch-Friesian and Meuse-Rhine-Yssel breeds. The bulls were not kept under standard conditions, but differed with respect to rations, methods of semen collection, and eventually other environmental conditions. Sampling included (in an unplanned way) first and second and some third ejaculates, obtained during all seasons. The results, therefore, apply to a ‘random bull’. The total number of ejaculates differs with the parameter studied because the material as a whole was not collected especially for the present purpose.

The mean velocities and numbers of spermatozoa moving normally (i.e. excluding spermatozoa that swim in circles and do not flash in the dark-field) were determined from the recordings obtained with Rikmenspoel’s (1957b) photo-electric equipment.

For investigating ageing effects, the diluted semen was stored in a Dewar vessel with ice. The tube with semen was protected from direct contact with the ice by an air mantle. At intervals drops of the diluted semen were taken for investigation. Rates of velocity decrease with time and half-life periods of numbers were calculated only from measurements extending over periods of at least 20 hr.

Fresh ejaculates were diluted ten times with standard egg-yolk-citrate buffer medium at pH 6·75±0·05 (McInnes pH scale; determined at 22 to 23°C, corresponding to pH 6·80±0·05 at 37°C — Van Duijn & Rikmenspoel, 1960), which had been made ultramicroscopically clear by ultracentrifugation, followed by ultrafiltration (Rikmenspoel, 1957a). About an hour later, a further dilution was made, varying between four and ten times, depending on actual spermatozoan concentration. Drops of the diluted semen were brought into a microchamber and warmed for 5 min to 37°C; the sample was then brought under the microscope. The dark-field image of individual swimming spermatozoa was projected onto a small aperture ‘viewed’ by a photomultiplier through an auxiliary lens. Every time a spermatozoon passed by the area ‘seen’ by the photomultiplier the latter received light impulses and emitted a corresponding number of photo-electrons. The resulting photocurrent was amplified
and recorded. From the recordings, the velocities of individual spermatozoa can be measured (the length of the recorded track being proportional to the velocity of the specimen), discrimination between normal and abnormal movements (cells swimming in circles) is possible (from the shape of the recorded track) and the method allows also determination of the total number of normally moving spermatozoa, which is proportional to the number of specimens passing by per unit time, divided by their mean velocity. More detailed descriptions of the method are to be found elsewhere (Rikmenspoel, 1957b; Rikmenspoel & Van Herpen, 1957; Rikmenspoel, Van Herpen & Van Dam, 1956; Rikmenspoel, Van Herpen, Van Dam & Eijkhout, 1960; Rikmenspoel & Van Duijn, 1960; Van Duijn & Rikmenspoel, 1960; Van Duijn, 1961b).

RESULTS

DISTRIBUTION OF MEAN VELOCITIES OF DIFFERENT EJACULATES

Text-fig. 1 gives the distribution of the initial mean velocities of 190 ejaculates obtained from thirty-nine different bulls. The distribution is skew and differs significantly from the Gaussian one \( P = 1/128; \) binomial test) as demonstrated by the probability plot represented in Text-fig. 2. It conforms within ordinary limits of error with a logarithmic distribution, which can be expressed as:

\[
N_{\bar{v}} = N_{\text{max}} \cdot e^{-\frac{1}{\text{s.d.}}(\bar{v}/\bar{v}_m)^2}
\]

where \( N_{\bar{v}} = \) number of ejaculates with mean velocity \( \bar{v} \), \( N_{\text{max}} = \) number of ejaculates with the modal mean velocity (top value of distribution) \( \bar{v}_m \). The mode is 97.5 µ/sec and the mean 100.2 ±9.9 µ/sec (s.d.) (s.e. = 0.7).

The hypothesis that no significant difference in initial velocity of the spermatozoa from different bulls should exist (Rikmenspoel, 1957b, 1960) has been tested by means of an analysis of variance and has not been refuted, the result
being $F_{11, 46} = 1.91 \rightarrow P \approx 0.07$. This analysis was performed before it became evident that the distribution was logarithmic. The effect of this neglect on the outcome of the analysis of variance leads to a $P$ value which is lower than the really best estimate. Since the result even with this bias is not significant, it was not necessary to repeat the procedure with the logarithmic figures. Of course, this result applies only to bulls sampled under random environmental conditions. There are indications that there may be bull-specific differences, but the present conclusion is that, if these do exist, they will have a smaller effect than the environmental conditions.

Table 1 gives the initial mean velocities of the spermatozoa from different bulls, from each of which at least four ejaculates obtained at different dates have been investigated.

**Table 1**

<table>
<thead>
<tr>
<th>Bull</th>
<th>Mean velocity $\bar{v}$ of different ejaculates (u/sec)</th>
<th>Average mean velocity over ejaculates, $\bar{v} \pm$ S.D. (u/sec)</th>
<th>S.E. of $\bar{v}$ (u/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alem 1</td>
<td>113 117</td>
<td>106 ± 10.8</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>94 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alem 2</td>
<td>100 103</td>
<td>108 ± 8.6</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>94 103</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>115 109</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>110 118</td>
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<td></td>
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<td></td>
<td>119</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Velocity characteristics of spermatozoa

Table 1—cont.

<table>
<thead>
<tr>
<th>Bull</th>
<th>Mean velocity $\bar{v}$ of different ejaculates (u/sec)</th>
<th>Average mean velocity over ejaculates, $\bar{v} \pm \text{s.d.}$ (u/sec)</th>
<th>S.E. of $\bar{v}$ (u/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anton 10</td>
<td>96 95 104 95 94 100 96 102</td>
<td>97 ± 5-8</td>
<td>1-6</td>
</tr>
<tr>
<td>Beers 1</td>
<td>119 103 115</td>
<td>110 ± 10-4</td>
<td>4-6</td>
</tr>
<tr>
<td>Beers 2</td>
<td>102 107 112</td>
<td>103 ± 10-0</td>
<td>4-1</td>
</tr>
<tr>
<td>Co-1</td>
<td>94 89 88</td>
<td>88 ± 6-9</td>
<td>2-8</td>
</tr>
<tr>
<td>D-23</td>
<td>100 89 95</td>
<td>95 ± 3-4</td>
<td>1-3</td>
</tr>
<tr>
<td>Lunteren</td>
<td>100 93 122</td>
<td>104 ± 12-6</td>
<td>6-3</td>
</tr>
<tr>
<td>Marie 1</td>
<td>130 98 107</td>
<td>102 ± 15-6</td>
<td>6-4</td>
</tr>
<tr>
<td>Marie 2</td>
<td>108 85 92</td>
<td>93 ± 14-2</td>
<td>5-8</td>
</tr>
<tr>
<td>Noord-Beemster 1</td>
<td>94 113 80</td>
<td>94 ± 14-4</td>
<td>7-2</td>
</tr>
<tr>
<td>Rudolf 1</td>
<td>95 96 104</td>
<td>102 ± 7-0</td>
<td>2-8</td>
</tr>
<tr>
<td>Rudolf 2</td>
<td>94 102 98</td>
<td>99 ± 7-8</td>
<td>2-5</td>
</tr>
<tr>
<td>Victor 2</td>
<td>115 89 112</td>
<td>102 ± 12-1</td>
<td>5-4</td>
</tr>
<tr>
<td>Willem 1</td>
<td>107 103 109 103 99 98</td>
<td>98 ± 8-5</td>
<td>2-7</td>
</tr>
<tr>
<td>Willem 2</td>
<td>100 86 94</td>
<td>95 ± 7-2</td>
<td>3-6</td>
</tr>
<tr>
<td>Zuna 1</td>
<td>111 104 102 92</td>
<td>99 ± 8-2</td>
<td>3-1</td>
</tr>
</tbody>
</table>
VELOCITY FREQUENCY DISTRIBUTION

Since no significant differences between random ejaculates from different bulls were found, it is permissible to pool the velocities measured for individual ejaculates. Text-fig. 3 represents the normalized velocity frequency distribution obtained by pooling the individual velocities of 14,781 spermatozoa of 105 ejaculates from thirty-three different bulls, measured at pH 6·75 ±0·05 (22 to 23° C measuring temperature) corresponding to pH 6·80 ±0·05 at the measuring temperature of 37° C. Mode 110 µ/sec; mean 98·2 µ/sec.

Text-fig. 3. Normalized velocity frequency distribution of 14,781 normal bull spermatozoa. Pooled data of 105 ejaculates from thirty-three different bulls, measured at pH 6·75 ±0·05 (22 to 23° C measuring temperature) corresponding to pH 6·80 ±0·05 at the measuring temperature of 37° C. Mode 110 µ/sec; mean 98·2 µ/sec.

This distribution also differs from a Gaussian one, the mode being 110 µ/sec and the mean 98·2 ±31·5 (s.d.) µ/sec (s.e. ±0·26).

The shape and spread of this distribution function is in agreement with previous findings (Van Duijn & Rikmenspoel, 1960), where the mean standard deviation of the velocity frequency-distributions obtained for twenty-four ejaculates at pH 6·75 was 30 ±1·1 (s.e.) µ/sec and in another series (Van Duijn, 1961b) of five ejaculates from five different bulls, where the result was 30 ±2·6 (s.e.) µ/sec. The difference between these values of 30 and the standard deviation of 31·5 µ/sec of the present distribution is not significant.

The shape and spread of the velocity frequency-distributions are changed by environmental conditions. The mean standard deviations $\sigma$ of the distributions were found to be a linear function of pH, according to:

$$\sigma = 2·64\ \text{pH} + 12 \ (\mu/\text{sec})$$

(Van Duijn, 1959; Van Duijn & Rikmenspoel, 1960). There are also definite effects of illumination and photosensitization by dyes or fluorochromes (Van Duijn, 1960, 1961a, b, 1962).

A more detailed analysis of the velocity frequency-distributions will be published separately.

CORRELATIONS BETWEEN INITIAL MEAN VELOCITIES AND NUMBERS OF SPERMATOZOA

There was no correlation between the mean velocity and total sperm count (determined with a haemocytometer) compared in a group of sixty-seven ejaculates ($r = -0·06$).

A highly significant negative correlation was found between the initial mean
velocity $\bar{v}_o$ and the number $N_{\text{mov}}$ of normally moving spermatozoa per unit volume of forty-nine ejaculates from twenty-eight bulls ($r = -0.54; P < 0.001$) (Text-fig. 4). This confirms the previous findings by Rikmenspoel (1957b, 1960) obtained with twenty-two ejaculates ($r = -0.43; P = 0.04$).

In a group of thirty ejaculates a significant positive correlation was found between $N_{\text{mov}}$ and total sperm count $N_{\text{tot}}$ ($r = +0.43; 0.01 < P < 0.02$) (Text-fig. 5).

AGEING EFFECTS

Ageing of spermatozoa can be characterized with respect to movement by the rate of velocity decrease with time ($-\frac{\partial \bar{v}}{\partial t}$) and by the half-life period of the number per unit volume of spermatozoa moving normally, $t_\frac{1}{2}(N)$.

Velocity decrease with time can be described most generally by equation (3):

$$\bar{v}_t = \bar{v}_o \left(1 - Ae^{kt}\ln t\right) = \bar{v}_o \left(1 - Ae^{kt}\right)$$

(3)

where $\bar{v}_t$ = mean velocity at time $t$, and $\bar{v}_o$ = initial mean velocity, $A$ and $k$ are constants. Differentiation yields:

$$-\frac{\partial \bar{v}}{\partial t} = \bar{v}_o \frac{Ae^{kt}\ln t}{t} = \bar{v}_o Ak. t^{(k-1)}$$

(4)
If the numerical value of $k$ approaches unity, equation (3) reduces to a straight-line equation:

$$\bar{v}_t = \bar{v}_0 (1 - A.t)$$

and $-\partial \bar{v}/\partial t$ becomes a constant. This simple equation covers most of the data obtained in experiments of short duration relative to the rate of decrease of velocity if special adverse conditions are absent. Of course, if it is not known whether this approximation is applicable, owing to lack of a sufficiently large number of measurements during the entire ageing period, a true comparison is still obtainable at constant time intervals, as has been done in the present investigations. Theoretically, evaluation of the constants would be preferable, but the overall accuracy of the data was not so high that this could improve on the simpler procedure actually adopted.

The mean rate of velocity decrease with time determined in a group of forty-two ejaculates from nineteen different bulls, was $0.66 \pm 0.06$ (s.e.) (µ/sec)/hr with a spread of s.d. $= \pm 0.39$ (µ/sec)/hr. Velocity is measured in µ/sec and it would therefore seem logical to express the rate of velocity decrease with time, which is a negative acceleration (retardation), in µ/sec². However, this would suggest a timing accuracy during the ageing period measurable in seconds, which of course would be absurd. Therefore, the dimension velocity decrease in µ/sec per hour of ageing period was chosen. The temperature at which the diluted semen was kept in the Dewar vessel was $1.9 \pm 1.2$ (s.d.) °C.

The frequency distribution of $-\partial \bar{v}/\partial t$ is given in Text-fig. 6. It is definitely skew, the mode being only 0.30 (µ/sec)/hr.

![Text-fig. 6. Frequency distribution of the rates of velocity decrease with time, $-\partial \bar{v}/\partial t$. Mode 0.30 (µ/sec) per hr, mean 0.66 (µ/sec) per hr. (Forty-two ejaculates from nineteen bulls.)](image)

The number $N_t$ per unit volume, of spermatozoa moving normally, has been found to decrease exponentially with time during the limited periods actually investigated (Rikmenspoel, 1957b, 1960; Van Duijn & Rikmenspoel, 1960; Rikmenspoel & Van Duijn, 1960), according to:

$$N_t = N_0 e^{-kt}$$

Whether this relationship would hold over an extended range is doubtful, since according to (6) the number of moving specimens would only become zero at
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infinite time, which is obviously impossible, the last specimen in reality becoming immotile at a very finite time indeed (Van Duijn, 1961a). However, since it was found generally to hold over at least two or more half-life periods (Van Duijn, 1962), the half-life periods \( t_i(N) \) determined over a total period of storage of at least 20 hr can be used as a valuable criterion. The mean half-life period of the number of normally moving spermatozoa in forty-three ejaculates from sixteen bulls was \( 60 \pm 7.6 \) (s.e.) hr with a spread of s.d. = \( \pm 44 \) hr. The frequency distribution, which is also definitely skew, is represented by Text-fig. 7. The mode is only 30 hr, which is only half the mean value, just as is the case with \(-\partial \bar{v}/\partial t\).

**Text-fig. 7.** Frequency distribution of the half-life periods of the number of normally moving bull spermatozoa, \( t_i(N) \). Mode 30 hr, mean 60 hr. (Forty-three ejaculates from sixteen bulls.)

### Correlations between Initial Mean Velocities and Ageing Effects

A highly significant correlation was found between the rate of velocity decrease with time \(-\partial \bar{v}/\partial t\) and the initial mean velocity \( \bar{v}_0 \) \((r = +0.57, f = 45; P<0.001)\) (Text-fig. 8).

The data also permit exponential regression, as indicated by the dotted line, according to the correlation \( \log (\!-\!\frac{\partial \bar{v}}{\partial t}) \times \bar{v}_0 \) \((r = +0.61; P<0.001)\). However, the difference between these correlation coefficients is not significant, whereas there are at present no theoretical arguments available favouring non-linear regression in this special case.

Since a highly significant correlation between \( N_{mov} \) and \( \bar{v}_0 \) was also found, as mentioned previously, a negative correlation may be expected between the half-life period \( t_i(N) \) and \( \bar{v}_0 \). With the available material this correlation was not yet significant, however \((r = -0.29, f = 27; 0.05<P<0.10)\), which is due to non-linearity of the relationship, as will be discussed below. Text-fig. 9 gives the scatter diagram of these data.

There was no correlation between \( t_i(N) \) and the initial number of normally moving spermatozoa \( N_0 \) per unit volume \((r = 0.114, f = 27; 0.50<P<0.60)\).

A correlation between \(-\partial \bar{v}/\partial t\) and \( t_i(N) \) must be expected to be non-linear. From equation (6) it follows that:

\[
- \frac{\partial \ln N}{\partial t} = \frac{\ln 2}{t_i(N)}
\]

(7)
Hence, since there is a linear correlation to be expected between $-\partial \ln N/\partial t$ and $-\partial \bar{v}/\partial t$, at least over the periods of time where equations (6) and (5) hold, the corresponding correlation based on half-life periods becomes:

$$-\frac{\partial \bar{v}}{\partial t} = \left( \frac{\ln 2}{t_{1/2}(N)} \right) K$$

which is of the type $y = k/x$, representing a hyperbola. It follows that a linear correlation may exist between $\log (-\partial \bar{v}/\partial t)$ and $\log [t_{1/2}(N)]$. This correlation was actually found and proved significant ($r = -0.40$, $f = 32$; $0.001 < P < 0.002$).

**Text-fig. 8.** Correlation between the rate of velocity decrease with time, $-\partial \bar{v}/\partial t$, and the initial mean velocity $\bar{v}_0$. $r = +0.57$; $P<0.001$. Since the derivative cannot be an independent variable, regression $x = f(y)$ does not make sense and consequently is not shown. The dotted line indicates exponential regression of the same data. For $\log (-\partial \bar{v}/\partial t) \times \bar{v}_0$, $r = +0.61$, $P<0.001$, but the difference between the correlation coefficients is not significant.

**Text-fig. 9.** Scatter diagram of $t_{1/2}(N) \times \bar{v}_0$. A non-linear correlation does exist, as mathematically proven in the text.
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0.01) (Text-fig. 10; the corresponding hyperbolic correlations are shown in Text-fig. 11).

**Text-fig. 10.** Correlation between log \((-\partial \theta/\partial t)\) and log \([t_f(N)]\). \(r = -0.40; 0.001 < P < 0.01.\)

**Text-fig. 11.** Hyperbolic correlation between the rate of velocity decrease with time, \(-\partial \theta/\partial t\), and half-life period of the number of normally moving spermatozoa. Same data as presented in Text-fig. 10. Erroneous calculation of linear correlation on these data would yield \(r = -0.35\), with a significance only at 5% level.

**DISCUSSION**

The average of the mean velocities of the 190 ejaculates from thirty-nine bulls included in the present investigations, \(100.2 \pm 9.9\) (s.d.) \(\mu/\text{sec}\) (s.e. \(\pm 0.7\)), is higher than the values found in earlier investigations with smaller numbers of ejaculates. For the average \(\bar{v}\) of the mean velocities, \(\bar{v}\) of the spermatozoa of twenty-four ejaculates from six bulls of Friesian and red and white Meuse-Rhine-Yssel breeds Rikmenspoel (1957b) found: \(\bar{v} = 97 \pm 6\) \(\mu/\text{sec}\). In another series of twenty-nine ejaculates from twelve different bulls, the result was: \(\bar{v} = 96 \pm 6\) (s.d.) \(\mu/\text{sec}\) (Van Duijn & Rikmenspoel, 1960). The differences between the means of the latter two series and that of the present one are significant, if the skewness of the distribution is ignored.

However, there is no significant difference between the mode (97.5 \(\mu/\text{sec}\)) of the present distribution and the means of the older series, which points to the reason for the difference being essentially the deviation from normality, and
standard statistics are not applicable with confidence any more. It is concluded that the difference between the means of the distributions is not fundamental, the modal values being in close agreement. In the smaller groups, the range of extreme values that makes the distribution skew is missing owing to their lower incidence, hence mode and mean would not differ significantly, as they do with sufficiently large numbers as have been obtained now. It is not likely that seasonal variation may have contributed to the difference, because Rikmenspoel's (1957b) investigations were mainly performed in the winter season, and they were in perfect agreement with those by Van Duijn & Rikmenspoel (1960) where ejaculates obtained during winter, spring and summer were used, whereas the present investigations extended over all seasons during a period of about 4 years. Since seasonal variations have been demonstrated, this may require further investigation before it can be definitely decided.

There is no significant difference between the mean velocity of all measured spermatozoa of 105 ejaculates of the present series: \( \bar{v} = 98.2 \pm 0.56 \) (s.e.) \( \mu/\sec \) \((N = 14,781)\) and the result obtained previously: \( \bar{v} = 97.6 \pm 0.5 \) (s.e.) \( \mu/\sec \) (4796 spermatozoa of twenty-nine ejaculates from twelve bulls) (Van Duijn & Rikmenspoel, 1960). Whereas this frequency distribution (Text-fig. 3) also differs from a normal (Gaussian) distribution, with a considerable difference between mean (98.2 \( \mu/\sec \)) and mode (110 \( \mu/\sec \)), it still approximates sufficiently to the normal distribution to allow application of standard statistics for most purposes, as can be demonstrated by plotting the data on normal probability paper, where a straight line can still be obtained. That the agreement is not very good in a purely mathematical sense, however, can be shown by other more sensitive transformations, as by plotting \( \sqrt{\log(N_{\max}/N)} \) versus \( |v - v_m| \). However, this distribution is not logarithmic, either, although it is found to change in the direction of a logarithmic distribution at decreasing pH values.

A true comparison between the data presented here and the velocity frequency distribution determined from cinemicrographs published by Rothschild (1953) cannot be given within the scope of this paper, because the conditions of the experiment by Rothschild were too different. A pure phosphate buffer with fructose added was used as the extender instead of our standardized ultramicroscopically clear egg-yolk-citrate. Further, Rothschild's investigations were concerned primarily with development of a technique and did not contain such large numbers of measurements as would be needed to get a reliable comparison. Nevertheless, Rothschild's (1953) distribution with \( \bar{v} = 123 \) \( \mu/\sec \) and \( \sigma = 39 \) \( \mu/\sec \) still fits in the range of our present data.

The finding of a highly significant positive correlation between the rate of velocity decrease with time \(-d\bar{v}/dt\) and the initial mean velocity \( \bar{v}_0 \), coupled with a negative correlation between log \(-d\bar{v}/dt\) and log \([t_{1/2}(N)]\), indicates that high initial velocity of the spermatozoa of a semen sample is coupled with quick deterioration. From these correlations, together with that between \( N_{\text{mov}} \) and \( \bar{v}_0 \), it follows that there must be some kind of negative correlation between \( t_{1/2}(N) \) and \( \bar{v}_0 \), too. That this correlation was not yet significant must therefore be considered as incidental, either being due to insufficient number of data (with \( f \sim 35 \) instead of 27, the same correlation coefficient would be significant) or to
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the correlation in reality not being linear, which would yield a too low value of \( r \). In fact, a non-linear correlation must be expected, as follows from the following argument. Taking the linear correlation between \( -\partial \bar{v}/\partial t \) and \( \bar{v}_o \), whereas for the total number of data equation (8) holds, we may write:

\[
-\frac{\partial \bar{v}}{\partial t} = a.\bar{v}_o + C = K \ln 2 \quad t_1(N)
\]

(9)

where \( a, C \) and \( K \) are constants. Solving this equation for \( \bar{v}_o \) we get:

\[
\bar{v}_o = \frac{K \ln 2}{a \cdot t_1(N)} - \frac{C}{a}
\]

(10)

Hence, a straight-line relationship is only to be expected between \( \log (\bar{v}_o + C/a) \) and \( \log [t_1(N)] \). However, from (9) it follows that:

\[
\frac{C}{a} = -\frac{-\partial \bar{v}/\partial t}{a} - \bar{v}_o
\]

(11)

and

\[
\bar{v}_o + \frac{C}{a} = \bar{v}_o -\frac{-\partial \bar{v}/\partial t}{a} - \bar{v}_o = \frac{1}{a} \cdot -\frac{\partial \bar{v}}{\partial t}
\]

(12)

Consequently, the correlation \( \log (\bar{v}_o + C/a) \times \log [t_1(N)] \) reduces to \( \log (-\partial \bar{v}/\partial t) \times \log [t_1(N)] \), which has already been shown to be highly significant.

If it should be assumed that the true function underlying the correlation between \( \bar{v}_o \) and \( -\partial \bar{v}/\partial t \) would be the exponential one, a similar argument leads to a reduction of the correlation to \( \log (-\partial \bar{v}/\partial t) \times \log [t_1(N)] \), which has the same general properties as the original hyperbolic correlation, i.e. the asymptotes of the regression hyperboles do not differ significantly from the coordinate axes.

All correlations are now consistent with the conclusion that high initial swimming velocity of the spermatozoa in an ejaculate cannot be considered to be a positive mark for semen quality without restriction. Although, in spite of its correlation with quick deterioration, high initial velocity might be still advantageous in natural mating, it must be expected that for artificial insemination a slow rate of decrease of velocity with time and long half-life period of the number of spermatozoa moving normally are the really important quality marks, since the time lapse between the ejaculation and the moment the spermatozoa reach the ovum is much greater in A.I. practice than in natural mating. Furthermore, the effect may be enhanced by the absolute number of spermatozoa inseminated in A.I. being very much lower owing to the use of extenders. However, even in natural mating longevity may be more important than very high swimming velocity, since it has been found that it takes 4 to 12 hr before the spermatozoa reach the ovum (Dauzier, 1958). Consistent with these views are the observations by Rickard, Ludwick, Hess & Ely (1957), who found no significant difference in non-return rates with 1-day-old semen after activation with sodium carbonate (increasing pH means increasing velocity) immediately preceding insemination, while there was a significant difference at 1% level in favour of the higher pH with 2-day-old samples. From the latter observation, it would appear that velocity may become more important if it falls below a certain critical level, reached after a longer period of storage.
Rikmenspoel (1957b, 1960) suggested that the half-life periods of the number of normally moving spermatozoa would probably be bull-specific. No evidence supporting this view could be obtained in the present investigations, but this result is inconclusive owing to the random sampling of ejaculates which increases variance within bulls and decreases variance between them. To decide this question, investigations will have to be directed to specific bulls under standard conditions instead of to random bulls, the randomness being that of all environmental conditions, including season. Definite seasonal rhythms have been discovered in all parameters studied; these will be presented in a separate report.

REFERENCES


