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# A Comparison of Two Computer-Automated Semen Analysis Instruments for the Evaluation of Sperm Motion Characteristics in the Stallion

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**Two commercially available computer-automated semen analysis instruments (CellSoft Automated Semen Analyzer and HTM-2000 Motion Analyzer) were compared for their ability to report similar results based on the analysis of pre-recorded video tapes of extended, motile stallion semen. The determinations of the percentage of motile cells by these instruments were more similar than the comparisons between subjective estimates and either instrument. However, mean values obtained from the same sample may still differ by as much as 30 percentage units between instruments. Instruments varied with regard to the determinations of mean sperm curvilinear velocity and sperm concentration, but mean sperm linearity determinations were similar between the instruments. We concluded that the determinations of sperm motion characteristics by subjective estimation, CellSoft Automated Semen Analyzer, and HTM-2000 Motility Analyzer are often dissimilar, making direct comparisons of results difficult.**

**Key words: sperm motility, curvilinear velocity, sperm concentration**

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The use of computer-automated semen analysis instruments for the evaluation of fertility in men and male domestic animal species has recently received much attention in the literature. One popular instrument is the CellSoft Automated Semen Analyzer (CRYO Resources Ltd, New York, NY), which utilizes an external microscope for sperm imaging. Another commonly used instrument is the HTM-2000 Motility Analyzer (Hamilton Thorn Research, Danver, MA). Although this system is built with its own specimen stage and optics, it will analyze video cassette tapes that have been previously recorded from an external microscope and camera, as well as video images obtained directly from an external microscope.

The CellSoft instrument has been validated for use in the clinical practice of andrology and veterinary medicine. The user has the ability to select, among other parameters, the number of frames analyzed per second (15 or 30 Hz), the total number of frames analyzed, the minimum number of frames successfully tracked for inclusion in the determination of motion characteristics, and the

threshold grey level used to visualize digitized images. The alteration in these set-up parameters has been reported to influence the determinations based on framing rate (Mack et al, 1988), and the total number of frames tracked and the minimum number of frames successfully tracked (Knuth et al, 1987; Budworth et al, 1988; Vantman et al, 1988; Blach et al, 1989). Recommended set-up values for the CellSoft system have been reported for the analysis of human semen (Mack et al, 1988).

The precision of results for a semen sample analyzed with CellSoft under standardized set-up conditions has also been studied. As the number of fields and cells that are analyzed was increased, the precision in results also increased (Budworth et al, 1988; Jasko et al, 1988; Blach et al, 1989). A study reporting similar results with the HTM-2000 Motility Analyzer has also been published (Pedigo et al, 1989).

The usefulness of the CellSoft system in clinical practice has been questioned by Mortimer et al (1988) due to the strong influence of sperm cell concentration on results. However, Working and Hurtt (1987) and Mathur et al (1986) reported good agreement between CellSoft's determinations for sperm cell concentration and the percentage of motile cells with those obtained by conventional methods. Also, Mack et al (1988) reported good agreement between CellSoft's velocity measurements and a manual method of track digitalization.

Recently, results obtained with the CellSoft system were compared to results obtained with the HTM-2000 Motility Analyzer (Mahony et al, 1988). The same samples were analyzed by both instruments. The mean values for the concentration of sperm cells and percentage of motile cells did not differ between the instruments, but the determined values for velocity and linearity did differ. However, instrument settings were not uniform between instruments.

We previously reported the use of the CellSoft instrument for the determination of sperm motion characteristics in extended stallion semen and found that instrument precise enough for this type of analysis (Jasko et al, 1988). These results were verified in a subsequent report by other investigators (Blach et al, 1989). In this report, we compared sperm motion characteristics in extended stallion semen obtained with the CellSoft and HTM-2000 instruments to determine if these instruments provide similar results.

## Materials and Methods

### *Animals and Semen Evaluation*

Single ejaculates of semen were evaluated from 22 Standardbred stallions standing at stud on commercial breeding farms in New York State. Each stallion was reevaluated approximately biweekly from March 20, until June 30, 1989. Semen collections were obtained with the use of an artificial vagina designed for the collection of stallion semen. Gel fractions were removed from the semen with either a filter placed in the artificial vagina or removed directly after collection. Semen evaluations were performed as outlined by the Society of Theriogenology (Kenney et al, 1983), including: the measurement of gel-free volume (semen), the determination of sperm cell concentration in semen with the use of a densimeter (Model 534A Mod 1, Animal Reproduction Systems, Chino, CA), the subjective estimation of the percentages of total motile and progressively motile sperm cells in extended semen, and the determination of the percentage of morphologically normal sperm cells.

### *Preparation of Samples for Computer Automated Semen Analysis*

Following semen collection and determination of sperm cell concentration, an aliquot of semen was extended 1:1 in a nonfat dry skim milk–glucose extender (Kenney et al, 1975). The concentration of sperm in this extended sample was further diluted to approximately $20 \times 10^6$ cells/ml by using serial dilutions of fixed amounts of extended semen (100 μl) with equal amounts of extender. A 6 μl drop of the final dilution of extended semen was placed on a microscope slide and covered with a 18 $mm^2$ coverslip resulting in a theoretical depth of 18 μm. However, observed depths of the suspensions were similar to those obtained with the use of a 10 μm chamber (Horwell Counting Chamber, ARH Laboratory Supplies, London, UK). The slides were viewed using a microscope with a ×10 negative-phase objective (BH-2, Olympus Optical Co, Ltd, Tokyo, Japan). Stage temperature was maintained at 37°C by a stage warmer (Rocky Mountain Microscope Corp, Ft. Collins, CO). A video camera (NC-67M, DAGE-MTI Inc, Michigan City, IN) mounted on the microscope was used to record images on video tape with a videocassette recorder (VO6800, Sony, Paramus, NJ).

Each slide was placed on the temperature controlled stage and allowed 30 seconds for the cessation of nonspecific movement before recording. A slide was viewed by fields, and fields were changed every 5 seconds. A slide was discarded after 1.0 to 1.5 minutes of viewing. For each ejaculate, two slides were used to record the motile sperm for subsequent determination of sperm motion characteristics by computer-automated semen analysis instruments. The same slides were viewed for the subjective evaluation of the percentages of total motile and progressively motile cells. Approximately 2.5 minutes of video tape were recorded for each ejaculate.

### *Optimization of Computer-Automated Semen Analysis Instruments*

Each segment of tape corresponding to individual ejaculates (2.5 minutes), but not the same scenes within segments, were analyzed with two computer-automated semen analysis instruments: CellSoft Automated Semen Analyzer and the HTM-2000 Motility Analyzer. No attempt was made to analyze exact scenes within segments (ejaculates), since the overall results of each ejaculate were to be compared as if analyzed independently by each instrument. Instrument set-up values were selected to optimize the efficiency in sperm tracking of each instrument, while being as uniform between instruments as possible (Tables 1 and 2). For the CellSoft instrument, these settings usually resulted in the analysis of 25 to 30 cells/field. Approximately 85% of the motile cells were tracked for a minimum of five frames, and thus included in the curvilinear velocity and linearity measurements. Less than 50% of the motile cells were successfully tracked for all 20 frames. For the HTM-2000 Motility Analyzer, the selected settings usually resulted in the analysis of 40 to 50 cells/field with almost all motile cells included in the curvilinear velocity and linearity measurements. Mean values for each ejaculate were obtained after the analysis of 300 to 400 cells (individual fields were automatically summated by each instrument); intra-ejaculate coefficients of variation for the CellSoft System results were under 10% (Jasko et al, 1988). This usually resulted in the analysis of 12 to 15 fields for the CellSoft instrument and six to nine fields for the HTM-2000 instrument for each ejaculate.

Direct comparisons, within limitations of the specific computer programs used for data acquisition and analysis, could only be made between specific motion characteristics, since the identical set of characteristics are not determined by both instruments. The determinations compared were: percentage of motile cells, mean curvilinear velocity of motile cells (CellSoft velocity and HTM-2000 track speed), mean linearity of motile cells (the ratio of straight line velocity to curvilinear velocity), and concentration of sperm cells. Linearity values determined from the HTM-2000 Motility Analyzer were divided by 10 to make them equivalent to the values reported with the CellSoft system. Concentration values were those obtained for the extended samples as analyzed by each instrument. Both instruments analyzed 20 frames at 30 frames/second with equivalent conversions of video pixels to μm and specified field depths of 10 μm. In addition, the subjective estimation of the percentage of total motile cells was compared with the estimations obtained with the computer-automated instruments.

Table 1. *Set-up parameters for the CellSoft Automated Semen Analyzer**

| | |
|---|---|
| Number of frames to analyze | 20 |
| Number of frames per second | 30 |
| Minimum number of frames tracked | |
| Motility | 2 |
| Velocity | 5 |
| Maximum velocity (μm/s) | 250 |
| Threshold velocity (μm/s) | 20 |
| Threshold grey level | Variable |
| Pixel scale (μm/pixel) | 0.975 |
| Dilution factor | 1.000 |
| Cell size range (pixels) | |
| Low | 12 |
| High | 50 |

* CellSoft version 3.21.

Table 2. *Set-up parameters for the HTM-2000 Motility Analyzer**

| | |
|---|---|
| Chamber depth (μm) | 10 |
| Image type | Phase-contrast |
| Frames at frame rate | 20 frames at 30/s |
| Minimum contrast | 9 |
| Minimum size | 7 |
| Size gates | |
| Low | 0.5 |
| High | 4.0 |
| Intensity gates | |
| Low | 0.5 |
| High | 4.0 |
| Low Vap value (μm/s) | 20 |
| Magnification | 2.0 |
| Threshold straightness | 70 |

* HTM-2000 Motility Analyzer version 7.0.

### *Statistical Analysis*

A total of 144 ejaculates were available for semen analysis including the determination of motion characteristics by computer-automated semen analysis. The mean of the differences in determinations between instruments was calculated for those determinations suitable for comparison. Ninety-five percent confidence intervals (Snedecor and Cochran, 1980) were constructed around each mean difference to determine if that difference was significantly different from zero. Regression slopes and coefficients of determination ($R^2$ values) were determined for each comparison (Statistical Analysis Systems, SAS institute, Cary, NC). Slopes of regression lines were tested for divergence from a line of direct correspondence (a line having a slope equal to one).

## Results

For the percentage of motile cells in 144 ejaculates of stallion semen, the mean of the differences between the subjective estimation and the CellSoft system was significantly different from zero ($P < 0.05$). Analysis of the regression line ($R^2 = 0.53$, $P < 0.05$) demonstrated a slope (0.78) unequal to a line of correspondence ($P < 0.05$). The mean difference between subjective estimation and the HTM-2000 instrument was not different than zero;

however, the analysis of the regression line ($R^2$ = 0.44, $P < 0.05$) again demonstrated a slope (0.67) unequal to a line of correspondence ($P < 0.05$). The mean difference between computer-automated semen analysis instruments for the percentage of motile cells was significantly different from zero ($P < 0.05$), and the slope of the regression line significantly ($P < 0.05$) differed from a line of correspondence (Table 3, Fig 1).

The mean of the differences in mean sperm curvilinear velocity between the two automated systems significantly differed from zero ($P < 0.05$), and the slope of the regression line significantly differed ($P < 0.05$) from a line of correspondence (Table 3, Fig 2). The mean of the differences in mean sperm linearity between the computer automated systems also differed significantly ($P < 0.05$) from zero (Table 3). However, the slope of the regression line was not significantly different from a line of correspondence (Fig 3).

The mean of the differences in the sperm concentration of the extended samples determined by the two computer-automated systems was significantly different ($P < 0.05$) from zero, and the slope of the regression line was also significantly different ($P < 0.05$) from a line of correspondence (Table 3, Fig 4). These data were replotted to compare the mean of the concentrations determined for both instruments with the difference between concentrations (Fig 5). It is evident that, at higher mean concentrations, the values obtained with the HTM-2000 instrument exceeded those of the CellSoft system.

Table 3. *Comparison of motility characteristics and concentration of sperm cells in extended stallion semen determined by CellSoft Automated Semen Analyzer and HTM-2000 Motility Analyzer*

| Comparison (CellSoft–HTM) | Mean difference ± SD in values | Regression slope | $R^{2*}$ |
|---|---|---|---|
| Percentage of motile cells | 2.8 ± 7.9* | 0.79† | 0.71 |
| Mean sperm velocity (μm/s) | −8.1 ± 13.3* | 0.38† | 0.25 |
| Mean sperm linearity | 0.18 ± 0.5* | 0.91 | 0.71 |
| Concentration of sperm cells ($10^6$/ml) | −12.7 ± 10.6* | 0.33† | 0.45 |

Values shown are based on the evaluation of 144 ejaculates.

Mean values determined by CellSoft Automated Semen Analyzer and HTM-2000 Motility Analyzer are for the percentage of motile cells, 75.9 and 72.5%; mean sperm velocity, 99.6 and 107.7 μm/second; mean sperm linearity, 5.97 and 5.79, and sperm cell concentration, 27.6 and 40.3 × $10^6$/ml, respectively.

* The mean of the differences for the comparison differs significantly from zero ($P < 0.05$).

† Regression slope differs significantly from 1 ($P < 0.05$).

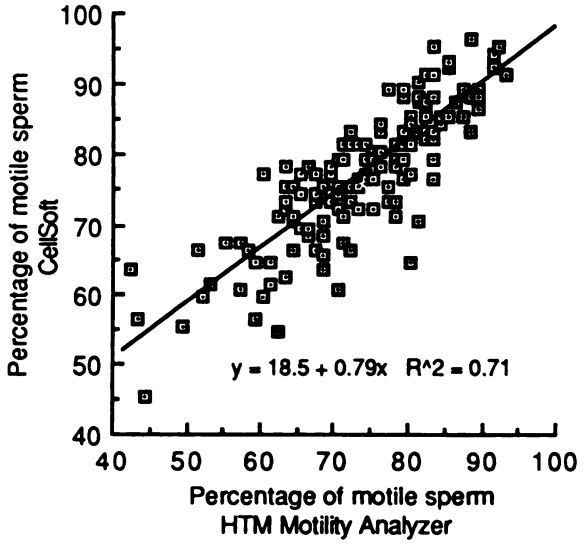


Fig 1.—Scatterplot of the percentage of motile sperm in extended stallion semen determined with the CellSoft Semen Analyzer and the HTM-2000 Motility Analyzer (n = 144).

## Discussion

In this study, segments of video recordings cor-

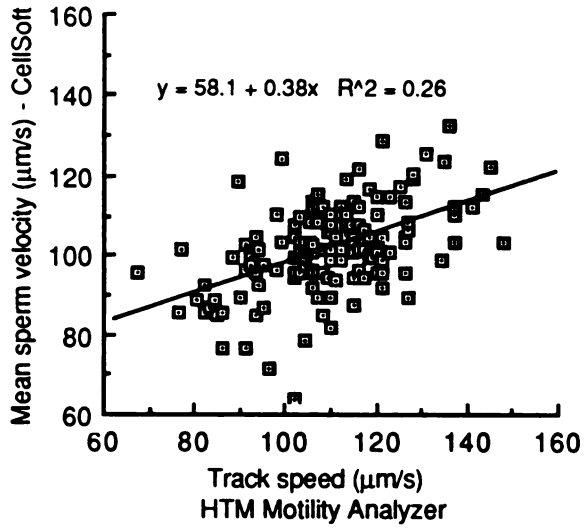


Fig 2.—Scatterplot of the mean sperm velocity (μm/second) in extended stallion semen determined with the CellSoft Semen Analyzer and the HTM-2000 Motility Analyzer (n = 144).

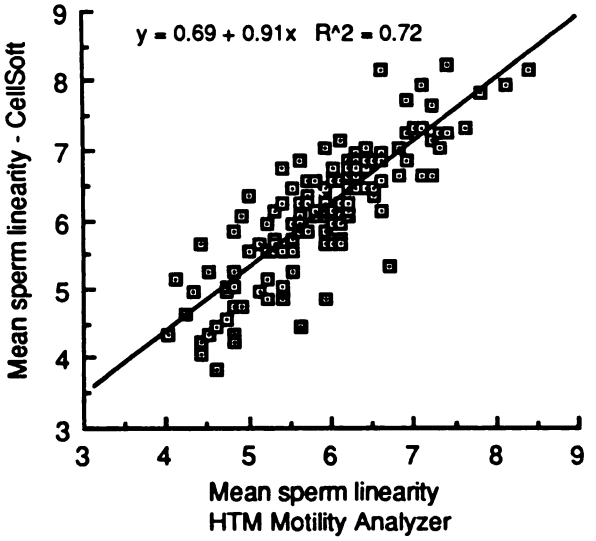


Fig 3.—Scatterplot of mean sperm linearity in extended stallion semen determined with the CellSoft Semen Analyzer and the HTM-2000 Motility Analyzer (n = 144).

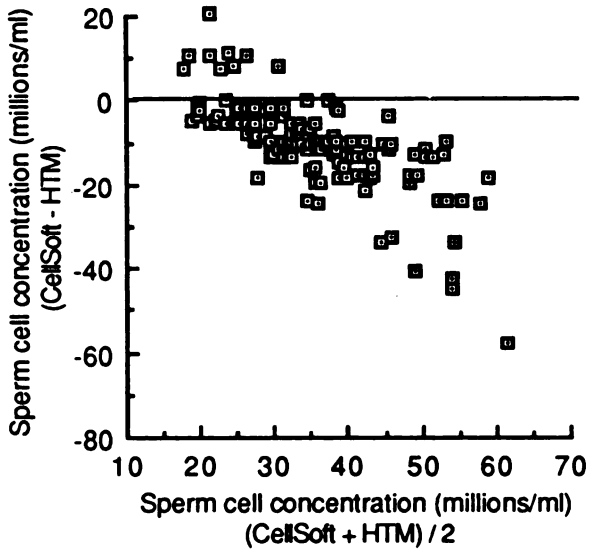


Fig 5.—Plot of the differences between the CellSoft Semen Analyzer and the HTM-2000 Motility Analyzer determinations of sperm concentration ($\times 10^6$/ml) in videorecorded samples of extended stallion semen against the average determinations obtained with the instruments (n = 144).

responding to different ejaculates of extended stallion semen were analyzed with the CellSoft Automated Semen Analyzer and the HTM-2000 Motility Analyzer. Exact scenes within segments were not analyzed by both instruments since we desired to compare results for ejaculates as if each instrument was used independently. Set-up values were similar between instruments for the perceived depth of sperm suspension, pixel to micrometer conversion, framing rate, and the number of frames analyzed; these have been reported to influence results (Mack et al, 1988; Knuth et al, 1987; Blach et al, 1989, Vantman et al, 1988; Budworth et al, 1988).

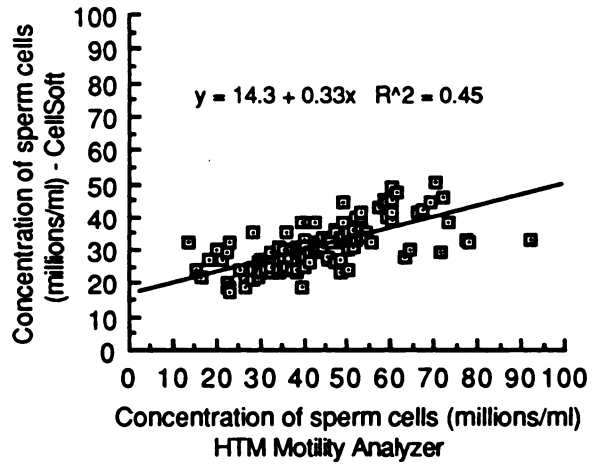


Fig 4.—Scatterplot of the concentration of sperm cells ($\times 10^6$/ml) in videorecorded samples of extended stallion semen determined with the CellSoft Semen Analyzer and the HTM-2000 Motility Analyzer (n = 144).

If the methods used to determine sperm motion characteristics were similar between instruments, the mean of the difference in determinations between instruments should equal zero. In addition, if the determinations from both instruments are plotted, a line of correspondence with a slope of one and a high coefficient of determination ($R^2$) should be obtained. Some scatter of the data would be expected due to machine error and from the analysis of different scenes of the same ejaculate. However, with the repeated analysis of different scenes of the same ejaculate with the CellSoft instrument the coefficients of variation for determinations obtained were all less than 10% (Jasko et al, 1988).

For the determination of the percentage of motile cells, the mean of the differences between comparisons of subjective estimation and either computer-automated system was small. However, for the comparisons of the subjective estimation and the CellSoft system and of the two automated systems (Table 3) this difference was significantly ($P < 0.05$) different from zero, indicating that the methods were not equivalent. Based on the regres-

sion slope and coefficient of determination for each comparison, results obtained with the computer-automated systems were more similar than the comparisons of the subjective estimation with either automated system. But even between the automated systems, large differences in results were obtained; differences as great as 30 percentage units would be expected based on the standard deviation of the mean of the differences obtained in this study (Table 3). This is similar to the results of Mortimer et al (1988) when comparing CellSoft's determination of the percentage of motile cells to that of a manual method.

For the CellSoft system, the determination of the mean curvilinear velocity is equivalent to the velocity termed "track speed" of the HTM-2000 Motility Analyzer. When these two curvilinear velocities were compared, they appeared dissimilar (Table 3 and Fig 2). This dissimilarity may be due in part to the determination of the location of sperm head centroids, which is different in the two instruments. CellSoft determines centroid locations based on the area of the sperm head, whereas the HTM-2000 Motility Analyzer determines the centroid based on sperm head brightness.

The HTM-2000 Motility Analyzer linearity results were divided by 10 to make them equivalent to those of the CellSoft system (HTM-2000 values are expressed as a percentage and those of CellSoft as a number from 0 to 10). Although the mean of the differences in the determination of linearity between the instruments was significantly different from zero, the overall results were similar between instruments, judged by the slope of the regression line and the coefficient of determination obtained from the comparison of results (Table 3 and Fig 3).

The determinations of the concentration of sperm cells in the video recorded extended semen samples made by the instruments were dissimilar. The mean of the differences was significantly ($P < 0.05$) different from zero (Table 3), and the mean concentration determined from the HTM-2000 Motility Analyzer exceeded that of the CellSoft system. In addition, the regression slope was significantly different from one (Fig 4). Determined HTM-2000 concentrations were greatly in excess of the $20 \times 10^6$ cells/ml that the extended semen samples were thought to contain. After extension of semen, accurate concentrations were not obtained to verify the dilution. However, it appears that the CellSoft concentrations were closer to the expected values than the HTM-2000 Motility Analyzer determinations. Overestimation of sperm cell concentration in dilute semen samples by computer-automated semen analysis has been reported (Mortimer et al, 1988).

When these data were replotted, as done by Mortimer et al (1988), using the means of the concentration determinations and the differences of the concentration determinations between instruments (Fig 5), it became evident that, at higher mean concentrations, the values obtained with the HTM-2000 Motility Analyzer exceeded those of the CellSoft system. This may be a consequence of the HTM-2000 Motility Analyzer splitting more tracks (as evidenced by using the playback option) as the number of collisions increased with concentration. Pedigo et al (1989) optimized the HTM-2000 Motility Analyzer for the determination of sperm cell concentration in undiluted human semen samples and concluded that the use of lower framing rates gave more accurate values. In this study, we used a higher framing rate to optimize determination of sperm motion characteristics.

In contrast to the study of Mahony et al (1988) who compared the CellSoft system with the HTM-2000 Motility Analyzer, we found large differences in the determinations of sperm motion characteristics even when an attempt was made to standardize instruments. The instruments were more similar in the determination of the percentage of motile cells than were comparisons between subjective estimates and either instrument, however, values obtained may still differ by as much as 30 percentage units between instruments. The instruments varied with in the determinations of mean sperm curvilinear velocity and sperm concentration, but mean sperm linearity was similar between instruments. We conclude that the determinations of sperm motion characteristics by subjective estimation, CellSoft Automated Semen Analyzer, and HTM-2000 Motility Analyzer are often dissimilar for many determinations, making direct comparisons of results difficult.

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**11th North American Testis Workshop**

**1991 Montreal, Canada—April 24–27**

The 11th North American Testis Workshop entitled "The Male Germ Cell: Spermatogonium to Fertilization" will be held at the Holiday Inn Crowne Plaza, Montreal, Quebec, Canada, April 24–27, 1991. The Workshop will consist of an inaugural session on the evening of April 24th, followed by two and one half days of oral presentations by invited lecturers and poster presentations. The major topics to be covered include: genetic and hormonal control of spermatogenesis, specific gene expression during spermatogenesis, structure and function of specific organelles, and epididymal and post-epididymal changes in spermatozoa.

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