

[Int J Androl](#). 1998 Feb;21(1):41-6.

Performance of the sperm quality analyser in predicting the outcome of assisted reproduction

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The present study was undertaken to assess the relationship between the results of conventional semen analysis and the sperm motility index (SMI) as measured by the sperm quality analyser (SQA), and to evaluate these in relation to the fertilization and/or pregnancy outcome of assisted reproduction. SMI determinations and conventional semen analyses were performed on 223 samples from subfertile men in two laboratories in Leuven (n = 136) and Antwerp (n = 87), and on spermatozoa prepared on a Percoll gradient (n = 136) used for treatment of male factor infertility in 57 cycles of intrauterine insemination (IUI), 44 attempts at in vitro fertilization (IVF) and 31 attempts at intracytoplasmic sperm injection (ICSI). SMI values for native semen correlated significantly with sperm concentration, motility and morphology. Multiple regression analysis revealed sperm concentration after preparation, and the concentration of motile spermatozoa with normal morphology and SMI (before preparation) to be the independent determinants for SMI after preparation. SMI values were significantly higher after, than before, preparation ($p < 0.0001$). In regular IVF (n = 44) the percentage of fertilized oocytes correlated significantly ($p < 0.05$) with sperm motility (A + B%, $r = 0.33$), with the percentage of spermatozoa with normal morphology ($r = 0.46$) before preparation, with the values of SMI both before and after preparation ($r = 0.54$, $r = 0.48$), with sperm concentration ($r = 0.34$) and with the motile sperm concentration ($r = 0.29$) after preparation. For the occurrence of pregnancy (all treatment methods), comparison of areas under ROC curves (AURC) indicated motile sperm concentration after preparation, as well as SMI both before and after preparation, to have the highest AURC, with no significant difference between these values as far as predictive power was concerned. These results indicate that the SQA allows for rapid evaluation of sperm characteristics and of the effectiveness of sperm preparation techniques. However, it is not superior to conventional semen analysis in predicting the outcome of assisted reproduction.

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Evaluation of Sperm Quality Analyzer

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In order to assess the usefulness of the Sperm Quality Analyzer (SQA, United Medical Systems Inc., Santa Ana, CA) in evaluating fertilizing ability of men, sperm motility index (SMI) values, which were determined using the SQA, were compared with the results of manual and computer-assisted semen analyses and various sperm function tests, including hypoosmotic swelling test, Penetrak test, acrobeads test, and zona-free hamster ovum human sperm penetration test (ZSPT).

The SMI value demonstrated statistically significant correlation with sperm concentration, sperm motility, motile sperm concentration, and linearity, Penetrak value and the percentage of sperm penetration in ZSPT also related to the SMI values.

These results indicate that the SQA is an easy and useful method for routine semen evaluation, and also has a possibility to be used as a substitute for complicated sperm function tests, such as Penetrak test and ZSPT.

Key words: semen analysis, sperm quality analyzer, sperm motility index.

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Evaluation of sperm fertilizing ability using the Sperm Quality Analyzer

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The Sperm Quality Analyzer is an inexpensive device which provides a quantitative estimation of sperm motility. To evaluate the fertilizing ability of human spermatozoa using a Sperm Quality Analyzer, correlations amongst the sperm motility index, the sperm penetration index (as assessed using the sperm penetration assay; SPA), and the fertilization rate in the treatment of standard IVF-ET were analysed retrospectively. The sperm motility index demonstrated a significant correlation with sperm concentration ($p < 0.001$), sperm motility ($p < 0.001$) and the motile sperm concentration ($p < 0.001$) in a total of 104 fresh semen samples from 81 men donating samples for IVF-ET. The sperm motility index also showed a significant correlation ($p < 0.001$) with the sperm penetration index in 60 patients, assessed using the SPA, before they were treated by standard IVF-ET. The correlation between the sperm motility index and the IVF-ET fertilization rate was higher than that between the sperm penetration index and the fertilization rate. The sperm motility index was classified into three categories: 'poor' (sperm motility index < 80), 'medium' (sperm motility index 81-160) and 'good' (sperm motility index > 160). The relationships between the IVF-ET fertilization rate and each category of the sperm motility index values were also evaluated. For the three categories in the sperm motility index, the fertilization rates (76.0%) of 60 samples judged as 'good' were significantly higher than those (44.2%) of 15 samples judged as 'medium' ($p < 0.001$) and those (34.7%) of 13 samples judged as 'poor' ($p < 0.001$). These results indicate that the Sperm Quality Analyzer provides a reliable estimation of the fertilizing ability of human spermatozoa.

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Assessment of the Sperm Quality Analyzer

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OBJECTIVE: To assess the relationship between the results of the Sperm Quality Analyzer (United Medical Systems Inc., Santa Ana, CA), which measures motile sperm concentration by light scattering, conventional manual semen analysis characteristics, and computer-assisted sperm motility analyses. **DESIGN:** Sperm Quality Analyzer measurements and manual and computer-assisted semen analyses were performed on 150 (50, 62, and 38) samples in three laboratories and the results were compared. **SETTING:** The study was performed in the Andrology Laboratory of Prince Henry's Institute of Medical Research, Monash Medical Centre, and Andrology Laboratory and Reproductive Biology Unit at the Royal Women's Hospital, Melbourne, Victoria, Australia. **PATIENTS:** Patients presented to the laboratories for routine fertility evaluation in the male and were selected at random to reflect the range of normal and abnormal samples seen in the laboratories. **INTERVENTIONS:** None. **MAIN OUTCOME MEASURES:** Sperm count, motility (percent motility, motility index, velocity, and amplitude of lateral head displacement [ALH]), morphology, and normal

acrosomes were evaluated by manual and computer-assisted semen analysis and sperm quality analyzer motility index. RESULTS: Spearman nonparametric univariate analysis showed strong correlations between sperm motility index and manual sperm concentration, motility, abnormal morphology, and normal acrosomes by *Pisum sativum* agglutinin; and computer-assisted sperm motility analysis sperm concentration, motile concentration, and percent static. Curvilinear velocity, straight-line velocity (VSL), and linearity also were related significantly to sperm motility index values. By multiple regression analysis, the significant covariates of the sperm motility index were motile sperm concentration, abnormal morphology, ALH, and straight-line velocity and these accounted for 85.5% of the variance of the sperm motility index. CONCLUSIONS: The Sperm Quality Analyzer is easy to use. The good correlation between the sperm motility index, motile sperm concentration, and, in addition, a number of other semen parameters supports the use of the Sperm Quality Analyzer for screening patients and in situations that warrant a rapid verification of semen quality, such as in the IVF or artificial insemination clinic. Further investigation of the Sperm Quality Analyzer in the management of male infertility is warranted.

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SQA LINE OF SPERM QUALITY ANALYZERS: VETERINARY APPLICATION

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The Optimum Semen Dilution for the Sperm Quality Index that is Most Predictive of Broiler Breeder Fertility

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Abstract: The sperm quality index (SQI) can accurately predict overall semen quality and fertility when broiler breeder semen is diluted, at most, 10-fold prior to analysis. The objective of the present study was to determine if a lower semen dilution rate yields an SQI that is an even better predictor of semen quality and fertility when hens are inseminated with a constant volume of semen. Individual ejaculates from 28 males were analyzed for sperm concentration, viability, and the SQI prior to insemination into 15 hens/male. Semen was diluted 2-, 4-, 8-, 10-, and 25-fold prior to analysis for the SQI. The SQI from 25-, 10-, and 8-fold dilutions produced the strongest correlations with total sperm concentration ($r = 0.85, 0.82, \text{ and } 0.80$, respectively). Correlation coefficients for live sperm concentration were highest for the SQI from 25-, 10-, 8-, and 4-fold dilutions ($r = 0.86, 0.86, 0.83, \text{ and } 0.73$, respectively). Correlation coefficients were similar for the SQI with fertility from the 4-, 8-, and 10-fold dilutions ($r = 0.75, 0.72, \text{ and } 0.72$, respectively). It appears that an 8- to 10-fold SQI dilution is the most consistent at predicting fertility and semen quality.

Key words: sperm quality index, broiler breeder, fertility, semen dilution.

Use of a sperm quality analyser on semen of turkey breeders to monitor storage time effects and age-related changes during a reproductive cycle

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1. A relatively new instrument known as a Sperm Quality Analyzer (SQA) offers a rapid assessment of sperm quality and quantity by providing a sperm quality index (SQI). The SQA measures the intensity of sperm activity and motile concentration by determining the number and amplitude of sperm movements per second in a capillary tube as detected through light beam interference. 2. The objectives of the current study were to determine if the SQA could accurately reflect changes in semen quality that occur with prolonged storage of semen and to determine the variation and change in SQI values among individual breeding male turkeys during their semen production cycle. 3. The effect of storage time on SQI values was evaluated by diluting semen with extender and placing the semen on an oscillating shaker at 4 degrees C for 8 h. The SQI values and sperm viability, expressed as % dead sperm, were recorded hourly. The SQI readings declined linearly with increased storage time while % dead sperm increased linearly with increased semen storage. 4. Semen from 220 individual males was analysed monthly for 9 months. Semen diluted 50-fold with saline had lower SQI values during pre- and post-peak phases of production (months 1, 7, 8, and 9 as compared with months 2 to 6 of semen production). The highest SQI values occurred during months 2 to 6. The largest variation in SQI values occurred during months 1 (CV = 26%) and 9 (CV = 31%) with a CV that averaged 16% for the remaining months. 5. Correlation analysis of SQI values for each bird averaged over 9 months with individual male SQIs for each month showed monthly correlation coefficients that ranged from 0.22 to 0.63. 6. These results indicate that the SQA accurately assessed the decline in sperm quality that occurs with prolonged storage of turkey semen and reflected age-related changes in semen quality and quantity that occurred during a semen production cycle of turkey breeders. In addition, the semen quality rank of some turkey breeders in a population changed with age.

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Utilisation of a sperm quality analyser to evaluate sperm quantity and quality of turkey breeders

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1. A relatively new instrument known as a Sperm Quality Analyzer (SQA) offers a rapid assessment of sperm quality and quantity by providing a sperm quality index (SQI). The SQA measures a combination of the intensity of sperm activity and motile concentration by determining the number and amplitude of sperm movements per second in a capillary tube as detected through light beam interference. 2. Because the SQA has not been tested for its potential use in turkeys, the objective was to determine if the SQA could accurately respond to changes in turkey sperm concentration, viability, and motility in semen collected from turkey breeders. 3. The effect of varying concentrations of sperm on SQI values was evaluated by diluting replicate pools of semen from 4 different aged turkey breeder flocks with saline. Results from all 4 flocks showed that semen dilutions greater than 20-fold resulted in a linear decline in SQI values. 4. Additional in vitro analysis evaluated the effects of

turkey sperm viability on the SQI under conditions of constant sperm concentration. Incubated, live sperm was mixed in various proportions with thawed, dead sperm to determine changes in viability. Increased proportions of dead sperm caused a decline in the SQI. 5. To assess sperm motility, turkey semen was incubated under either aerobic (motile) or anaerobic (immotile) conditions. Varied amounts of immotile and motile sperm samples were mixed. A linear increase in the SQI was observed as per cent motile sperm increased. 6. These results indicate that the SQA can respond to differences in turkey sperm concentration, viability, and motility using in vitro analyses.

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The effects of heat stress and sperm quality classification on broiler breeder male fertility and semen ion concentrations

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ABSTRACT

1. The present study was undertaken to determine the effects of heat exposure on fertility, semen quality, and semen ion concentrations of broiler breeders classified on sperm quality index (SQI) before heat stress.
2. Cobb males (108) were individually caged in 6 temperature-controlled rooms. Each room contained an equal number of males from each of the 4 SQI population quartiles as follows: best (B), good (G), fair (F), and poor (P). Three rooms were heated to 35°C, and the other three rooms were maintained at a constant 23°C as controls. For each SQI group in each room, 15 Leghorn hens were artificially inseminated (5×10^7 sperm/hen) once a week for 8 weeks for fertility observations.
3. Body weight, sperm concentration, SQI, and fertility of P males were lower than in the other three SQI groups. Body temperature of the top three SQI groups was increased by heat exposure, but body temperature was not altered by heat stress in the P group. Fertility, sperm viability, and SQI of the top three SQI groups, but not the P group, was decreased by heat stress. Seminal plasma K^+ of P males was lower than that of B males. However, seminal plasma Ca^{2+} concentration of P males was higher than that of B males.
4. In conclusion, high ambient temperatures had more impact on semen quality and fertility of males in the top 75% of the SQI population than in males in the bottom 25% of the population. In addition, calcium ions (Ca^{2+}) appear to play a major role in heat stress infertility.

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Selection of Young Broiler Breeders for Semen Quality Improves Hatchability in an Industry Field Trial

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SUMMARY

Previous laboratory research has shown that the sperm quality index (SQI) is predictive of broiler breeder fertility. The SQI is a tool to estimate overall semen quality by monitoring the number of times that sperm movement causes defections within a light path. An industry field trial was undertaken to determine if life of flock hatchability could be improved by selecting young males for house placement based on the SQI. The SQI was used to select males at 26 wk of age. Males with an SQI in approximately the top 80% of the population were moved into two hen houses, whereas the lower

20% of the SQI population was culled. Two control houses received males selected solely on physical appearance. Life of flock hatchability was improved by 1.1% in the SQI-selected houses over that of males selected for house placement based on physical characteristics alone. The males selected for the SQI numerically outperformed the control males in 64% of the hatches with the greatest difference in hatch occurring during postpeak production. This increase in hatch resulted in 21,000 more chicks being produced in the two houses containing Sol-selected males. In conclusion, the SQI is a useful tool for accurately identifying the reproductive ability of broiler breeder roosters throughout a complete laying cycle.

Key words: broiler breeder, fertility, hatchability, semen, sperm quality index

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Elevated Body Temperature Directly Contributes to Heat Stress Infertility of Broiler Breeder Males

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ABSTRACT

Alterations in the male reproductive tract, sperm, or both may be responsible for heat stress infertility of broiler breeder males. The present study was conducted to determine the direct effects of hyperthermia during heat stress on sperm viability, the sperm quality index (SQI), and seminal plasma ion concentrations by incubation of semen *in vitro* at and above normal body temperature. Thirty-seven Cobb males were divided into the upper (best group = B) and lower (poor group = P) 50% of the population according to their SQI. Semen characteristics and seminal plasma ion concentrations (Ca^{++} , Na^+ , K^+ , and Cl^-) for B and P males were evaluated at two temperature treatments (41.5 and 42.5 C) and four incubation times (0, 30, 60, and 90 min). The results revealed that sperm viability and the SQI were decreased by increasing incubation temperature and duration of exposure. Seminal plasma ion concentrations were not affected by semen incubation temperature; however, plasma Ca^{++} concentration in the P-SQI group was higher than that of the B-SQI group. Seminal plasma K^+ concentration increased in both SQI groups over time. In conclusion, it is apparent that changes in semen characteristics due to elevated body temperature alone contribute to heat stress infertility of broiler breeders.

Key Words: sperm quality index, semen, fertility, broiler breeder, heat stress.

[PhD work, PURDUE UNIVERSITY, 2001](#)

Evaluation of a sperm quality analyzer and the effect of dietary antioxidants on semen traits of breeder birds

[Neuman, Stacey Lee](#)

A relatively new instrument known as a Sperm Quality Analyzer (SQA) offers a rapid assessment of sperm quality and quantity by providing a sperm quality index (SQI). The SQA measures the intensity of sperm activity and motile concentration by determining the number and amplitude of sperm movements per second in a capillary tube as detected through light beam interference. Though tested in humans and chickens, its application for assessing semen quality in turkey breeders had not been previously evaluated. Our *in vitro* results showed that the SQI generated by the SQA was indicative of turkey sperm concentration, viability, and motility. The SQA detected the decline in sperm quality that occurred with prolonged storage of semen as well as age-related changes during a semen production cycle of a flock of turkey breeders. The effect of antioxidants, ascorbic acid (AA) and L-carnitine, on semen traits of breeder birds was evaluated. Dietary treatments of 0, 75, and 150 mg/kg AA were fed

to turkey breeders during the first 4 months of their reproductive cycle and then were doubled to 150 and 300 mg/kg during months 5 to 9. Semen traits were unaffected by dietary AA; however, multinucleated giant cells, indicative of degeneration, were quantitatively detected in the testes of control birds, but were absent from AA-supplemented birds, suggesting that the antioxidant properties of AA may have delayed the formation of these degenerative cells. Feeding 500 mg/kg of dietary carnitine to young (32 to 37 wk of age) and aging (58 to 62 wk of age) White Leghorn roosters for 5 weeks not only improved sperm concentration during the last half of supplementation, but also reduced sperm lipid peroxidation. Testicular tissue was preserved as indicated by a reduction in multinucleated giant cells in the carnitine-fed birds. These results suggest that dietary carnitine has antioxidant properties that may preserve sperm membranes in roosters, thereby extending the life span of sperm.

[Poultry Science](#) 1998; 77:888-893

Use of a Sperm Analyzer for Evaluating Broiler Breeder Males.

1. Effects of Altering Sperm Quality and Quantity on the Sperm Motility Index

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ABSTRACT

A new instrument for assessing mammalian semen attributes, the Sperm Quality Analyzer[®], was evaluated as a potential tool for determining rooster sperm quality. The Sperm Quality Analyzer[®] measures the "activity" of sperm in a semen sample as the sperm motility index (SMI). The SMI is defined as the number and amplitude of deflections in a light path per second as a result of sperm movement within a capillary tube. In the present study, effects of sperm concentration, viability, and motility on the SMI were evaluated. Peterson broiler breeder males (n = 40) were used as semen donors. In the initial experiment, semen was diluted from 2- to 25-fold and SMI readings were obtained. The SMI was very low in neat semen samples but increased when semen was diluted up to threefold. However, at dilutions greater than fivefold, the SMI decreased. Apparently, sperm concentration in undiluted semen is so great that sperm are unable to move freely within the capillary tube. Maximum SMI values were obtained at sperm concentrations of approximately 1 billion sperm per milliliter. When thawed, dead sperm were mixed with incubated, live sperm, the SMI decreased with decreasing sperm viability even though sperm concentration was constant. Obviously, fewer sperm move across the light beam as sperm mortality increases. When motile, aerobically incubated sperm were mixed at different rates with immotile, anaerobically incubated sperm samples, the SMI increased with increasing concentrations of motile sperm, whereas total sperm concentration was static. In addition, the SMI was strongly correlated with motility scores obtained by microscopic analysis. The Sperm Quality Analyzer[®] provides an estimate of the overall quality of sperm from broiler breeder males by reflecting sperm concentration, viability, and motility in a single value, the SMI.

Key Words: sperm motility, sperm viability, sperm concentration, fertility, broiler breeder

[Reprod Domest Anim.](#) 2005 Jun;40(3):237-44.

Validation and usefulness of the Sperm Quality Analyzer (SQA II-C) for bull semen analysis

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In this study, an upgrade version of the Sperm Quality Analyzer (SQA), the SQA-IIC was tested for the assessment of bull semen quality. In Expt 1, the device showed good repeatability of measurements within and between capillaries, as evidenced by the low coefficients of variation (CVs; < 13%) at concentrations between 35 and 705 x 10(6) spermatozoa/ml. In Expt 2, 10 semen concentrations (1-1000 x 10(6)/ml) were stored in HEPES TALP for 48 h at room temperature. A time-dependent decrease in sperm motility index (SMI) values was noticed. SMI values increased linearly with increasing sperm concentrations, but remained constant around 500, corresponding to a concentration of approximately 50 x 10(6)/ml. For sperm concentrations below 50 x 10(6)/ml, SMI values were highly correlated with concentration ($p < 0.05$) and with semen parameters, expressing the overall semen quality ($p < 0.05$; Expt 3). In Expt 4, a correlation of only 0.44 ($p < 0.05$) between SMI values of frozen-thawed semen samples of 35 bulls and the corrected 56-day non-return rate (56dNRRc) was found. Prediction of the 56dNRRc based on the SMI value of a semen sample was inaccurate. The present study indicates that the SQA-IIC is suitable for a rapid screening of bull semen diluted to a concentration of approximately 50 x 10(6)/ml. Furthermore, the device seems inappropriate for fertility prediction.

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Motility assessment of porcine spermatozoa: a comparison of methods

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Although widely used in practice, visual motility assessment of boar spermatozoa is a very subjective method. The aim of this study was to compare the visual motility assessment of boar spermatozoa with two objective, automated systems, namely the Sperm Quality Analyzer (SQA-IIC) and the Hamilton-Thorne computer-based semen analyzer (HTR). In addition, concentrations as determined by the Burkner counting chamber and HTR were compared. Motility of 30 semen samples from 30 different boars (22 Pietrain, seven Landrace boars and one Large White) was examined during three consecutive days, subjectively by two independent persons (visual motility assessment) and objectively with both automated systems. The use of the SQA-IIC and HTR for assessing boar sperm motility was evaluated and the repeatability of the measurements was estimated. The Sperm Motility Index (SMI), determined by SQA-IIC, and the percentage motile spermatozoa determined by the HTR showed a good correlation ($r=0.71$; $p < 0.05$). The visual examination performed by Person 2 showed a good correlation with the SMI ($r=0.81$) and with the percentage of motile spermatozoa measured by the HTR ($r=0.66$) ($p < 0.05$). There was a very poor correlation and a limited agreement between the visual assessments of both persons emphasizing the subjectivity of visual motility assessment. Nevertheless, motility scores of each person during the three consecutive days were highly correlated ($r=0.67$ and 0.72 , $p < 0.05$). The limits of agreement plots showed poor agreement between both persons and the HTR. The repeatability of measurements for most parameters evaluated by the HTR and by the SQA-IIC was good with coefficients of variation below 10%. In addition, for fertile Pietrain

boars (n=22), reference values for the different HTR-parameters are presented showing a high curvilinear velocity (157.3 +/- 19.5 microm/s) and a low straightness and linearity of the movement of the spermatozoa (62.7 +/- 8.7 and 35.5 +/- 7.6%, respectively). Concentration as determined by the Burkner counting chamber (56.0 +/- 16.8 x 10(6)/ml) was significantly higher compared with HTR measurement (37.6 +/- 7.7 x 10(6)/ml). The high number of counted cells and the low variation render the HTR concentration measurement more reliable. It can be concluded that visual motility assessment is highly subjective and should therefore be replaced by automated systems that allow for a more objective and detailed motility assessment of boar spermatozoa. In addition, based on the present results, highly repeatable results were obtained by the SQA-IIC and especially by the HTR.

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Motility characteristics of boar spermatozoa after addition of prostaglandin F2alpha

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Addition of prostaglandin F2alpha (PGF2alpha) to extended boar semen has been shown to slightly increase reproductive parameters in sows such as the conception rate and the total number of piglets born alive. The mechanisms by which PGF2alpha affect these parameters have not yet been elucidated, but it is possible that the sperm transport after insemination is increased. This study investigated whether the sperm motility from 20 Pietrain boars improved when PGF2alpha (Dinolytic; 5 mg PGF2alpha/ml) was added to diluted semen. Different amounts of PGF2alpha (0, 0.5, 1 and 2 ml/100 ml) were tested and the motility was evaluated immediately after addition of PGF2alpha, after 30 min, 2 h, and 24 h. Two computer-assisted semen analysis (CASA) systems, namely the Sperm Quality Analyzer (SQA-IIC) and the Hamilton Thorne (HTR Ceros 12.1) were used to assess the motility parameters. With the SQA-IIC, sperm motility index values of the treated groups were only slightly higher (P>0.05) compared to the negative control group. The different motility parameters measured with the HTR Ceros 12.1 were similar between the treatment groups, except for beat cross frequency, which was higher in the control group (1.5-5%; P<0.001). This study documented that the addition of 2.5, 5 or 10 mg PGF2alpha to 100 ml diluted boar sperm does not increase any sperm motility parameter. Further research is necessary to elucidate mechanisms by which PGF2alpha in diluted semen may improve the reproductive performance in swine farms.

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Validation of the sperm quality analyzer and the hypo-osmotic swelling test for frozen-thawed ram and minke whale (*Balaenoptera bonarensis*) spermatozoa

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The object of the present study was to investigate the validation of the sperm quality analyzer (SQA) and the hypo-osmotic swelling (HOS) test with standard sperm analysis methods in frozen-thawed

ram and minke whale spermatozoa. In rams, highly significant correlations were observed in the percentage of motile spermatozoa ($P < 0.01$) and sperm concentration ($P < 0.01$) between the standard and SQA methods. But, the percentage of morphologically normal spermatozoa did not significantly correlate between the standard and SQA methods. The percentages of swollen spermatozoa at 15 minutes by the HOS test were significantly correlated with the motility by the standard ($P < 0.05$) and by the SQA ($P < 0.05$) methods. For minke whale spermatozoa, the SVI (sperm viability index) values by the standard method were significantly ($P < 0.001$) correlated with the sperm motility index (SMI) values by SQA. The percentage of motile spermatozoa was also significantly correlated ($P < 0.01$) with the motility measured by SQA. Using different hypo-osmotic solutions and incubation times, the HOS test with 25, 100 and 150 mOsM did not show significant variations. Motility observed by the standard method and the percentage of swollen spermatozoa were significantly correlated ($P < 0.05$). These results indicate that the SQA and HOS test can be utilized to assess the post-thawing motility of ram and minke whale spermatozoa, and that the SQA and HOS test values are significantly correlated in ram spermatozoa. However, sperm concentration and morphologically normal spermatozoa are not assessed accurately by SQA in minke whales.

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Using the Sperm Quality Analyzer (SQA Ilc) to evaluate dog ejaculates

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ABSTRACT: 38 fresh ejaculates were examined, of which 23 were cryopreserved in three diluents differing in concentration of glycerol and examined after thawing. For each ejaculate we established the concentration, motility, speed of sperm, percentage of live sperm and the morphological image of the ejaculate using routine laboratory diagnosis and SQA parameters at the initial value and after a 120-minute survival test. The values of the parameters found through routine diagnosis and using the SQA device were statistically confronted. From the results it emerges that the device functions on the basis of the turbulence effect of the semen, and therefore on the intensity and character of the moving mass of sperm. From a research perspective, the SQA device is less usable, since the values it provides are not absolutely comparable with the values found through routine laboratory diagnosis. For the practitioner, however, the device may be useful, since the value of the sperm motility index (SMI) indicates the quality of the semen through the close connection of two parameters, concentration and motility. For a more precise assessment of the ejaculate, however, it is necessary to also analyse the remaining parameters of routine laboratory sperm analysis. To be useful in clinical practice for the evaluation of semen, it would be necessary to establish SMI limit values for ejaculates of varying quality.

Keywords: dog; ejaculate; sperm analysis; Sperm Quality Analyzer (SQA)

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Use of the Sperm Quality Analyzer (SQA II-C) for the assessment of dog sperm quality

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In the present study, an automated system for sperm analysis, the Sperm Quality Analyzer (SQA II-C), was tested as a potential tool for the assessment of dog sperm quality. In the first experiment the device displayed a good repeatability of measurements for semen of medium and high quality, as evidenced by a low coefficient of variance (CV; 0.08), whereas a high CV (0.46) was obtained for one dog with semen of inferior quality. In the second experiment, seven different sperm concentrations (25-300 x 10⁶/ml), obtained by dilutions in HEPES-TALP medium were stored for 48 h at room temperature. A concentration dependent increase in sperm motility index (SMI) was shown, reaching a plateau at 150 x 10⁶ spermatozoa/ml. For all sperm concentrations, the SMI value decreased significantly after 24 h, indicating the importance of sperm motility for SMI values. For sperm concentrations lower than 150x10⁶/ml, highly significant correlations [$r=0.80$; $p<0.05$] were established between SMI values on one hand and sperm concentration, and semen parameters expressing the overall semen sample quality on the other hand (experiment 3) while non-significant or low correlations were found between SMI values and other individual sperm parameters. In experiment 4, significantly high correlations ($r=0.97$) were found between mean SMI values and post-thaw motility and progressive motility assessed subjectively. In conclusion, our study indicates that both motility and concentration largely influence SMI values and that the SQA II-C saturates at 150 x 10⁶ fresh spermatozoa/ml. In our opinion, the SQA II-C may be a useful and objective device to assess the post-thaw motility of dog sperm.

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Validation of the sperm quality analyzer (SQA) for dog sperm analysis

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In the present study, a simple and inexpensive unit (the Sperm Quality Analyzer-SQA), was evaluated for dog sperm analysis. Our objective was to propose a cheap, accurate and convenient device to be used in veterinary practices involved with dog fertility assessment and artificial insemination. The device was tested by analyzing repeatability and accuracy at different sperm concentrations and motility characteristics. The Sperm Motility Index (SMI), a numeric index provided by the SQA, was compared with the results obtained using a computer-aided sperm analyzer (Hamilton Thorn IVOS 10). The correlation between SMI and some sperm parameters as well as predictive values of the SMI were established. The dog sperm data provided by the SQA were consistent and repeatable (coefficient of variability below 10% for all concentrations tested). The SMI was significantly dependant on motile sperm concentration and a positive significant correlation was established for the different motile sperm concentrations from a concentration of 25 x 10⁶ up to over 200 x 10⁶ cells/mL. Zero motility did not affect SMI because non-motile cells, regardless of their concentration, do not cause any fluctuations in the optical density (OD). Over the tested 200 x 10⁶ cells/mL value, a correlation still could be observed but it was not statistically significant, possibly because of a saturation of the system. In dog semen, the correlation is better between SMI values and the number

of motile spermatozoa than with the overall motile concentration. Based on this observation, a predictive value was given to the SMI allowing for a sorting of dog ejaculates in 3 sperm categories (SMI <100, 100<SMI<250, SMI>250) each characterized by a range of sperm number and motility. If a positive correlation between the SMI categories and fertility has been demonstrated in humans, such a correlation needs to be established in dogs.

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SQA LINE OF SPERM QUALITY ANALYZERS: TOXICOLOGICAL APPLICATION

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Measuring mouse sperm parameters using a particle counter and sperm quality analyzer: A simple and inexpensive method

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This study examined a method for analyzing the count, motility, and morphology of mouse epididymal sperm, optimizing the diluent, incubation time, sample concentration, and temperature, using a particle counter (CDA-500) to count and size sperm and a sperm quality analyzer (SQA-IIC) to measure sperm motility, quantified as the sperm motility index (SMI). The optimal conditions consisted of a 30-min incubation in D-MEM (Dulbecco's modified Eagle's medium; considering cost and availability) at 37 degrees C, with 5×10^6 cells/mL (-1) in the original solution. Furthermore, the influence of formalin fixation, and the correlation between the automated counter and a manual method were investigated. The sample fixation had no marked effect on the sperm count or morphology assessment. A linear correlation was observed between the manual and automated methods ($y=0.920x+0.276$; $r(2)=0.571$; $p<0.001$; range: $(3-6) \times 10^6$). The suitability of the proposed method was confirmed using spermatozoa prepared from mice treated with the reproductive toxin diethylstilbestrol (DES). Using sperm from the cauda epididymidis on one side per mouse, we confirmed that measurement of these sperm parameters using the two devices was simple, rapid, inexpensive, and reproducible.

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Comparative Investigation of Several Sperm Analysis Methods for Evaluation of Spermatotoxicity of Industrial Chemical: 2-Bromopropane as an Example

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Abstract: Reproductive toxicity of 2-bromopropane (2BP), a substitute for ozone layer-depleting chloro-fluorocarbon, was found among the workers in an electronics factory in Korea in 1995. Furthermore the importance of testicular toxicity has been realized since the problem of endocrine disruptors arose all over the world, but manual methods must rely on subjective assessment. Recently, computer-assisted sperm analysis (CASA) was proposed but this system requires vast investment. We then investigated the applicability of the MTT method with a microplate and sperm quality analyzer (SQA) as simple, rapid, and economic instrumental methods for the examination of sperm quality in rats, comparing it with the manual microscopic method and CASA. Epididymal fluid derived from male F344/N Slc (Fischer) rats intraperitoneally injected with 2BP in the dose range of 125–1,000 mg/kg/d twice a week (total 8 times) were examined by these methods as a model experiment. Sperm count measured by the manual method and CASA in the epididymal fluid, absorbance by the MTT method and sperm motility index value by the SQA method were significantly lower in the 2BP 1,000 mg/kg administered group than in the control group. This result suggests that the MTT method can detect oligospermia. With the microplate and microplate reader, the efficiency of detection becomes much better. Sperm analyses by the MTT method with the microplate reader and the SQA method are available for reproductive toxicity study in rats.

Key words: 2-Bromopropane, Tetrazolium salt, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), SQA (sperm quality analyzer), CASA (computer-assisted sperm analysis), Manual microscopic method, Reproductive toxicity, Rat.

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Comparative investigation of several sperm analysis methods for evaluation of spermatotoxicity of industrial chemical: 2-bromopropane as an example

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Reproductive toxicity of 2-bromopropane (2BP), a substitute for ozone layer-depleting chloro-fluorocarbon, was found among the workers in an electronics factory in Korea in 1995. Furthermore the importance of testicular toxicity has been realized since the problem of endocrine disruptors arose all over the world, but manual methods must rely on subjective assessment. Recently, computer-assisted sperm analysis (CASA) was proposed but this system requires vast investment. We then investigated the applicability of the MTT method with a microplate and sperm quality analyzer (SQA) as simple, rapid, and economic instrumental methods for the examination of sperm quality in rats, comparing it with the manual microscopic method and CASA. Epididymal fluid derived from male F344/N Slc (Fischer) rats intraperitoneally injected with 2BP in the dose range of 125-1,000 mg/kg/d twice a week (total 8 times) were examined by these methods as a model experiment. Sperm count

measured by the manual method and CASA in the epididymal fluid, absorbance by the MTT method and sperm motility index value by the SQA method were significantly lower in the 2BP 1,000 mg/kg administered group than in the control group. This result suggests that the MTT method can detect oligospermia. With the microplate and microplate reader, the efficiency of detection becomes much better. Sperm analyses by the MTT method with the microplate reader and the SQA method are available for reproductive toxicity study in rats.

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Comparison of sperm motility test methods (except computer-assisted sperm analysis) in rats under the condition of alpha-chlorohydrin treatment--collaborative investigation

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A comparison among rat sperm motility test methods including percent of motile sperm (% Motile), scoring method (Scoring), Ishii's method, Progressive Motility Test (PMT) and Sperm Quality Analyzer (SQA), was conducted using data gathered from eleven laboratories. As a unified study design, mature male rats were orally treated daily for approximately 1 week with alpha-chlorohydrin (ACH), which is known to affect the sperm motility at the epididymis, at dose levels of 2.5, 5 and 10 mg/kg, and then subjected to more than two test methods for sperm motility in each laboratory. Scoring (4 or 5 grades), Ishii's method, PMT and SQA showed high sensitivity for the detection of the effects of ACH, which were not considered to be inferior to a computer-assisted sperm analyzer (CASA). Longer incubation time before testing was considered to contribute to detecting the effects of ACH. In particular, we realized that Scoring was a favorable method even if the demerit of poor objectivity was allowed for. Percent Motile showed lower sensitivity than other test methods. The differences in sensitivity between % Motile and other methods were considered to be based on whether the defects of progressive motion could be detected. Although % Motile cannot clearly judge whether immotile sperm are dead or alive, the value is a great help for the interpretation of the result from other methods. Based on the characters for detectability, objectivity and efficiency, the most suitable method of sperm motility should be selected according to the purpose of the toxicity study.

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Simple Methods for Objective Assessment of Sperm Viability and Motility with MTT Assay and Sperm Quality Analyzer (SQA) in Rats

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To confirm the applicability of MMT assay and sperm quality analyzer (SQA) to the examination of sperm viability and motility in rats, epididymal spermatozoa derived from male rats (Sic:SD) treated with nitrobenzene at a dose of 60 mg/kg/day for 16 days were examined by these methods.

Epididymal fluid was suspended in Dulbecco's modified Eagle's medium containing 25 mM fructose in ratio of 1:125. Sperm concentrations in the epididymal fluid were 2.48 ± 0.55 ($\times 10^9/\mu\text{l}$, mean \pm SD, $n = 8$) in the control and 2.20 ± 0.54 in the nitrobenzene group, and did not significantly differ between the groups. Both absorbance unit of the MTT assay (1.497 ± 0.406 vs. 1.084 ± 0.350) and SMI (sperm motility index) value of the SQA (204.0 ± 15.3 vs. 182.5 ± 21.6) were significantly decreased in nitrobenzene group. The results suggest that the examinations of sperm viability and motility with MTT assay and SQA are available for the reproductive toxicology in rats.
Key words: sperm viability, sperm motility, MTT, SQA, rat.

Application to halogenized propanes of Simple and Rapid Sperm Toxicity Tests by Tetrazolium Salt Methods in Rats

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[Introduction] Spermatotoxicity of Dibromochloropropane (DBCP) or 2-bromopropane (2BP) was evidenced by incidents of workplace exposure in foreign countries. This finding suggested the importance of testicular toxicity testing in the industrial toxicology. It has also been recognized since the issue of endocrine disrupters arose all over the world. Manual (microscopic) observation is, however, still used widely in sperm quality testing, and thus development of more reliable and objective methods is needed. We examined the applicability of six tetrazolium (MTT/WST-1/-3/-8/MTS/XTT) assays to the assessment of sperm quality as a simple, rapid and objective instrumental method, comparing these assays with other instrumental methods, such as the uses of Sperm Quality Analyzer (SQA) and Computer-Assisted Sperm Analyzer (CASA; HTM-IVOS).

[Methods] The epididymal fluid derived from male F344 rats treated subcutaneously with DBCP, 2BP, 1-bromopropane (1BP) or 1,2-dichloropropane (DCP) twice a week, totally 8 times, was examined by all these assays.

[Results] Significant changes in absorbance levels by MTT and WST-3 assays were observed in the high dose of DBCP, 1BP and 2BP treated groups as well as in the Sperm Count by CASA, while no significant difference was evident only in WST-1 assay. Furthermore, significant decreases in the sperm motility indices obtained by SQA and CASA were observed in the similar DBCP and 1BP groups, while no such decrease was observed in all the 2BP treated groups. As concerned with DCP treated groups, no significant difference was observed in all the assays except WST-8 assay.

[Discussion] These results indicate that most tetrazolium (MTT, WST-3, MTS, XTT) assays can detect sperm toxicity caused by introduced chemical agents to the comparable extent as other methods such as CASA or SQA method. While the formazan formed by MTT is insoluble in water, WST-3 generates water-soluble one therefore the step of dissolving formazan prior to spectrometry is unnecessary in WST-3 assay. The MTT and WST-3 assays proved to be more sensitive than other tetrazolium salt assays. MTS, XTT and WST-3 have advantage of forming water-soluble formazan making procedure simpler. Accordingly, WST-3 is thought to be the best salt for the tetrazolium method of sperm analysis.

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