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# Thermoresistance sperm tests are not predictive of potential fertility for cryopreserved bull semen

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ABSTRACT

Different studies demonstrate positive correlations between seminal variables determined in the laboratory and subsequent fertility after artificial insemination. It is clear, however, that there is still a deficiency in predicting *in vivo* fertility results of semen samples. The present study intended to verify the efficiency of rapid and slow thermoresistance tests in predicting fertility of frozen semen of bulls. Sperm from 64 ejaculates of 39 Nelore bulls (*Bos indicus*), aged 2–10 years, were cryopreserved in 0.5 mL straws. Thawed straws containing $30 \times 10^6$ sperm were analyzed for seminal variables in the laboratory and used to inseminate 4920 cows to evaluate fertility in the field. The ejaculates were frozen in a Tris-based extender and samples were evaluated for total motility after rapid (46 °C/30 min) and slow (38 °C/5 h) thermoresistance tests by conventional and computerized (CASA) methods. Sperm samples were grouped according to their ability to retain motility after thermoresistance testing: group 0 (0% motility), group 1 (1–20% total motility), group 2 (21–40% total motility) and group 3 (>40% total motility). Correlation and association between these groups and fertility diagnosed by rectal palpation at 90 days were verified. Chi-square test demonstrated no association between motility groups and fertility ($P > 0.25$) and both rapid and slow thermoresistance tests had a lesser correlation to fertility ($r = 0.11$ and 0.14, respectively). These results demonstrated that these tests are not reliable in predicting *in vivo* behavior of bull frozen semen and are not effective to estimate fertility.



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## 1. Introduction

Despite the number of studies reporting positive correlation between seminal characteristics evaluated in the laboratory and fertility, it is clear that there is still a deficiency in predicting the real fertility rate of semen samples (Dimitropoulos, 1967; Arruda et al., 1992). Besides this difficulty, there is still the tendency to simplify the biological nature of subfertility simply blaming the male or the inseminator (Saacke et al., 2000).

Tests of sperm exhaustion at different temperatures have been developed to evaluate the ability of sperm to survive and maintain fertility through long periods at a determinate temperature (Dimitropoulos, 1967; Barnabe et al., 1980; Arruda et al., 1992). Incubation for 2.5 and 4.5 h affected the percentage of motile sperm at 15, 20 and 30 °C (Pursel et al., 1972). Other authors incubated frozen-thawed semen of bulls at 37 °C for 0, 3 and 6 h periods and verified a marked decrease on total motility and acrosome integrity (Coulter and Foote, 1974).

Dimitropoulos (1967) described a slow thermoresistance test in which sperm were incubated at 38 °C for a period of 5 h, after which the percentage of progressively motile sperm was evaluated and positively correlated to fertility using 60–90 days post-insemination non-return rates. This test has been used for several years and all the samples of thawed semen that had less than 20% motile sperm after this test have been systematically eliminated (Jondet and Rabadeux, 1976).

Barnabe et al. (1980) used semen samples frozen in ampules to evaluate thermoresistance test and verified that the average progressive motility was 46.2% immediately post-thawing, decreasing to 19.8% after the rapid thermoresistance test and to 6.1% after the slow thermoresistance test. These results suggest that systematic elimination of all samples with less than 20% motile sperm after slow thermoresistance test was not applicable to those samples, as they have been previously used commercially and there were no complaints concerning fertility rates.

Nagy et al. (2004) submitted bull sperm to incubation at 37 °C for a period of 4 h to evaluate alterations in plasma and acrosomal membranes and observed that the population of live sperm with intact acrosome decreased slightly, dead sperm with intact acrosome decreased from 37.4% to 17.4% while dead sperm with damaged acrosome increased from 18% to 35%. In this experiment, an increase in acrosomal exocytosis was verified by PE-PNA (peanut agglutinin fluorescent conjugate) staining.

## 2. Materials and methods

Semen from 64 ejaculates of 39 Nelore bulls (*Bos indicus*), aged 2–10 years, from Jacarezinho Genetics Artificial Insemination Centre (16880-000, Valparaíso/SP, Brazil) were cryopreserved and used for laboratory analysis and artificial insemination. Fresh semen samples were evaluated for mass motility, subjective total motility, vigor, concentration and sperm morphology and were then diluted, refrigerated (0.5 °C/min) during 4 h and frozen in Tris-based extender at −140 °C for 15 min.

Each semen sample was thawed in water bath at 37 °C for 30 s. The content of straws were transferred to 5 cm closed assay tubes and evaluated for motility after slow and rapid thermoresistance tests (38 °C/5 h and 46 °C/30 min, respectively) by conventional and computerized (HTM IVOS 12) techniques.

Total motility (TM) results were separated in groups: G0 (0% TM); G1 (1–20% TM); G2 (21–40% TM); G3 (>40% TM), for both slow (STT) and rapid (RTT) thermoresistance tests. To verify fertility of each group, 4920 first inseminations were performed in Nelore cows of different ages and fertility was verified by rectal palpation after 90 days.

For statistical analysis, InStat 3 (Graph Pad Software Inc., USA) was used. Associations among seminal variables and fertility were examined using the Chi-Square test, calculating $\chi^2$ and $P$ statistics. Data were presented in tables containing the number of pregnant cows, the number of inseminations and pregnancy rates in each class of considered variable. Correlation between total motility after thermoresistance tests and fertility was verified by Spearman test (Curi, 1998). For all statistical analysis, significance was established at $P < 0.05$.