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Assessment of boar semen parameters

[1]Laura Parlapan, [2]Inmaculada Parrilla, [2]Tatiana Tarantini, [1]Emoke Pall, [1]Mihai Cenariu, [1]Iulia Maria Balaci, [1]Simona Ciupe, [1]Ioan Ştefan Groza

[1]University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania; [2]University of Murcia, Faculty of Veterinary Medicine, Murcia, Spain. Corresponding author: I. Ş. Groza, isgroza@yahoo.com

Abstract. Normal morphology, high motility and viability are sperm characteristics that ensure a good fertilizing capacity, being considered crucial for sexing technology. The purpose of this study was to evaluate semen parameters and to estimate its suitability for subsequent sperm sexing. We analyzed five ejaculates (concentration, morphology, motility and viability) from one boar using different computerized analysis systems. The results showed that all 5 sperm ejaculates were suitable for use in the sexing processes and the methods of analysis used were fast and appropriate for evaluating semen characteristics.
Key Words: boar semen, ISAS, flow cytometry.

Introduction. Mankind has always been interested in the sex preselection of animals (Garner & Seidel 2008). Semen quality is considered a crucial parameter of sperm sexing technology. Various methods have been reported to separate X- and Y-bearing spermatozoa (Espinosa-Cervantes & Córdova-Izquierdo 2012) based on: gradient swim-down (Ericsson & Ericsson 1999), surface antigenic differences (Hendriksen 1999), volumetric differences (van Munster 2002), centrifugal countercurrent distribution (Ollero et al 2000), differing mass and motility, surface changes, differing protein content or immunological properties (Blecher et al 1999), but none of these methods has been able to produce satisfying results with repeatable success (Seidel & Garner 2002). The most accurate method is based on the relative DNA differences between X and Y chromosome bearing populations, using flow cytometric sperm sorting (Johnson 2000; Jain et al 2011).

During the last decades appreciable progress in semen assessment techniques has been achieved. Integrated Semen Analysis System (ISAS) (Mortimer 2000) and flow cytometry (Gillan et al 2005) provide fast, simple, accurate and quantitative assessment of semen quality (Arruda et al 2012).

The aim of our study was to estimate the concentration, motility and viability of fresh semen from one boar, in order to evaluate its suitability for subsequent sperm sexing.

Materials and methods. State-of-the-art techniques (described below) were utilized to assess the following boar semen parameters: concentration, sperm morphology, total and progressive motility and viability.

Semen collection

The sperm-rich fraction of ejaculates was collected using the gloved-hand method and after filtration it was diluted (1:1) using a commercial semen extender (BTS, Beltsville Thawing Solution). Once collected, the samples were immediately analyzed for semen concentration and morphological abnormalities.

Semen concentration

Concentration of the ejaculates was assessed using a NucleoCounter system (NucleoCounter® SP-100, Denmark) which is an integrated fluorescence microscope designed to detect light signals emitted by sperm nuclei previously stained with

propidium iodide (PI). PI is located inside the disposable SP1-Cassette and when semen is loaded in the cassettes, the PI lyses the cell and the cellular DNA is stained. The recorded fluorescent signal is analyzed and correlated to the total number of cells/mL, which is displayed on the NucleoCounter screen.

Morphological analysis

The evaluation of sperm morphology was performed using phase contrast microscopy (Nikon Phase Contrast 0.90 Dry, Japan), which allows the analysis of unstained and unfixed cells, highlighting both primary and secondary sperm anomalies. Semen samples were fixed with 0.3% formaldehyde-citrate solution and a total of 200 cells/sample were evaluated in order to determine the percentage of normal sperm and of sperm anomalies (sperm with abnormal tails, abnormal heads and proximal and distal cytoplasmic droplets).

Computer-assisted analysis of sperm movement

The different motility characteristics of the spermatozoa were evaluated objectively using a computer-assisted analysis system (ISAS®, Spain). Prior to the ISAS analysis, the sperm samples were suspended in BTS to a concentration of 25–30 $x10^6$ cells/mL, and warmed at 38°C for 3 minutes. A 7 µL droplet of each sample was placed in a Makler counting chamber and analyzed using ISAS. A minimum of 300 spermatozoa of each sample were analyzed and different motility parameters were determined. This study only presents the percentage of motile sperm and progressive moving sperm.

Triple fluorescent staining for flow cytometry

Sperm viability and acrosome integrity were assessed by using flow cytometry (FACSCanto™ II, United States) and a triple-fluorescence procedure. The semen samples were labeled with a combination of 3 fluorochromes: Hoechst 33342 (H42, 0.05 mg/mL), PI (0.5 mg/mL) and fluoresceine isothiocyanate-conjugated peanut agglutinin (FITC-PNA, 200 mg/mL), and incubated in the dark for 10 minutes at 38°C.

Results and discussions. The 5-ejaculate mean values for the analyzed semen parameters, assessing the quality of fresh semen, are presented in Table 1.

Table 1

Quality parameters of fresh semen

Semen parameters	Mean ± SEM
Sperm morphology (%)*	97.3 ± 0.56
Concentration ($x10^6$/mL)	262.68 ± 11.42
Total motility (%)	95.75 ± 0.12
Progressive motility (%)	58.2 ± 3.75
Viability (%)**	91.08 ± 0.5

* The value is the percentage of sperm cells with normal morphology.

** The value is the percentage of sperm with intact plasma and intact acrosomal membranes.

Abnormal morphology of sperm cells directly affects their fertilizing ability and is an indicator of semen suitability for the sexing process. After analyzing 200 sermatozoa per ejaculate, using phase contrast microscopy, both primary and secondary morphological anomalies were revealed. The most common abnormalities encountered were: sperm with detached heads, bent middle piece, coiled tails, proximal and distal cytoplasmic droplets (Figure 1).

Semen concentration is an important parameter reflecting the testicular function. Its average value was 262.68 ± 11.42 $x10^6$/mL, considered a normal value for boar semen.

The total motility is considered the sum of progressive and non-progressive motility. According to the results obtained regarding the total motility, the mean value was 95.75 ± 0.12%. These values were within the allowed limit of >80% for fresh sperm (Vázquez et al 1997). The semen progressive motility is one of the important parameter that is correlated with the fertilization rate. The average value for the progressive motility was 58.2 ± 3.75%.

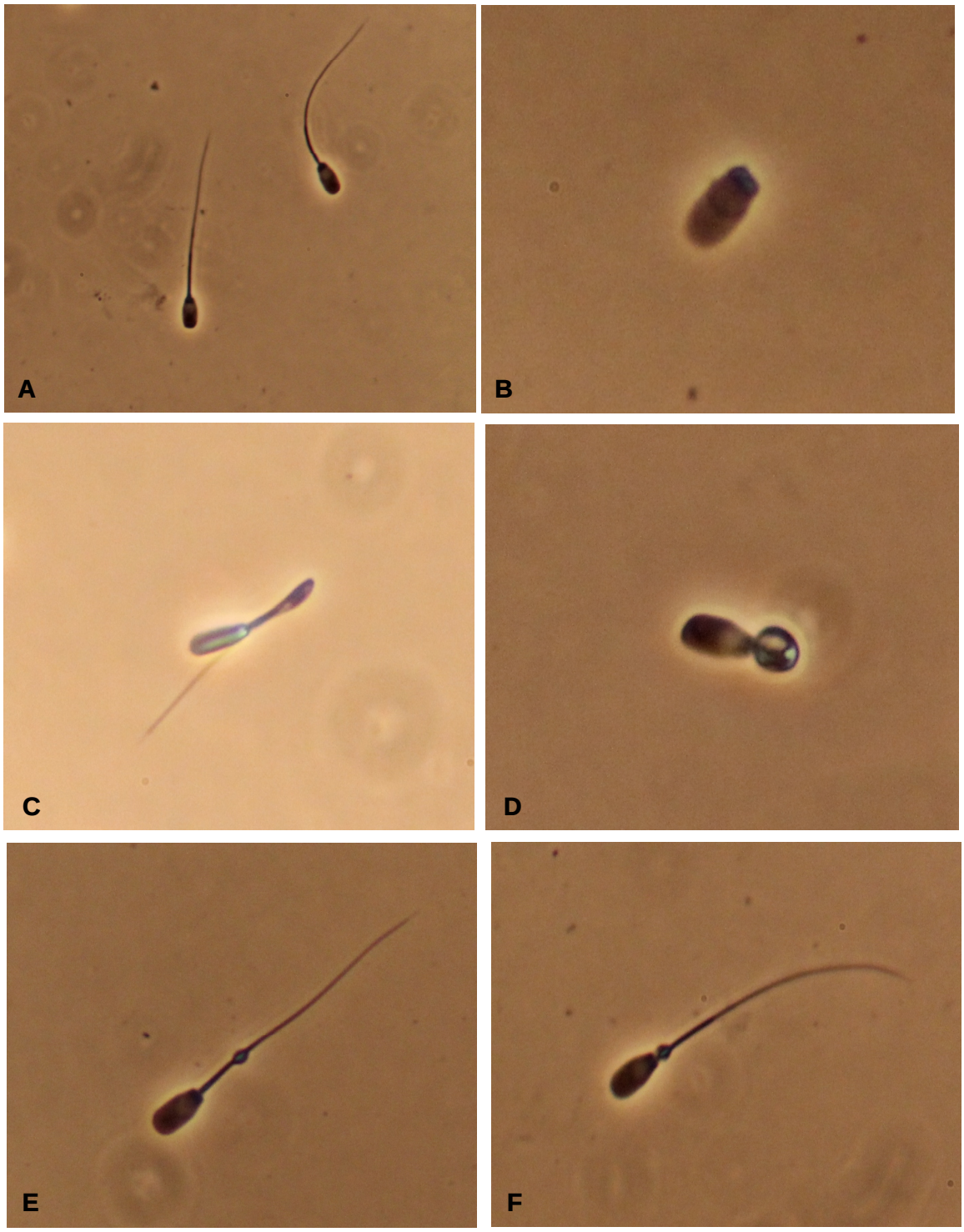

Figure 1. Phase contrast microscopy captures of normal and abnormal sperm morphology (40x): **A:** Normal morphology of boar spermatozoa;
B: Detached heads;
C: Bent middle piece;
D: Coiled tails;
E: Distal cytoplasmic droplet;
F: Proximal cytoplasmic droplet.

The viability of sperm cells assessed using flow cytometric and the triple-fluorescence techniques (that also evaluates plasma and acrosomal membrane integrity) was divided into four categories:

- live sperm with intact acrosome (H42+/PI-/FITC-PNA-) – 91.08%;
- sperm with intact plasma membrane and acrosome lesions (H42+/PI-/FITC-PNA+);
- damaged sperm plasma membrane and intact acrosome (H42+/PI+/FITC-PNA-);
- dead sperm with damaged plasma membrane and acrosome lesions (H42+/PI+/FITC-PNA+).

Conclusions. The methods of analysis used were fast and appropriate for evaluating sperm characteristics. All 5 sperm ejaculates were considered suitable for subsequent sexing procedures.

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Authors:
Laura Parlapan, University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania, e-mail: parlapan_laura@yahoo.com
Inmaculada Parrilla, University of Murcia, Faculty of Veterinary Medicine, Murcia, Spain, e-mail: parrila@um.com
Tatiana Tarantini, University of Murcia, Faculty of Veterinary Medicine, Murcia, Spain, e-mail: tatiana_vet@yahoo.com
Emoke Pall, University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania, e-mail: pallemoke@gmail.com

Mihai Cenariu, University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania, e-mail: mihai.cenariu@usamvcluj.ro
Iulia Maria Balaci, University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania, e-mail: balaci_i@yahoo.com
Simona Ciupe, University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania, e-mail: simona_ciupe@yahoo.com
Ioan Ştefan Groza, University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania, e-mail: isgroza@yahoo.com

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