each other, implying that either could be reliably used as an assessment of frozen thawed bull semen motility. The significant associations ($P < 0.001$) which were obtained between CASA motilities (TMOT and PMOT) and CONC do, however, reinforced the use of recommended sperm concentrations for CASA assessments as well as routine replay to ensure all sperm are appropriately tagged. Finally, the similarities between consecutive CASA readings for TMOT, PMOT and CONC for individual samples confirmed the validity of using rapid consecutive CASA measures to routinely check initial readings. This approach, however, is recommended in conjunction with other QC measures, such as image replay, to provide assurance that all sperm are being appropriately designated.

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Prevalence of uncompensable sperm defects in imported bull frozen semen categorized as unsound for AI in Costa Rica

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The quality of a frozen semen sample is of outmost importance in an AI breeding system. The standard testing at AI centers, must include sanitary and seminal characters -including sperm morphology- before freezing in order to minimize the chance of breakdowns in the conception rate. Frozen bull semen straws (n = 922), imported into Costa Rica mainly from Europe, North and South America, were submitted for quality testing to the andrology lab at UNA-Costa Rica from 1998-2021 (1 straw per batch). The same operator performed the evaluation after thawing the straws in water at 38°C for 30 s. Motility was determined by observation under phase contrast microscopy (200x) using prewarmed (38°C) slides and cover glasses. Sperm number per straw (NTS) was calculated after diluting the sample (1:100) with Hancock solution (HS) in a Neubauer hemocytometer. Sperm head abnormalities (size and shape) were determined in a smear stained with carbol fuchsin (200 cells/slide-1000x). Other defects were assessed in a wet smear fixed with HS (phase contrast, 200 cells/slide-1000x). Semen quality was categorized as sound for AI when $\geq 7.5 \times 10^6$ viable spermatozoa (VS) and $\leq 15\%$ uncompensable sperm defects (USD).

Motility (%), USD (%) and NTS ($x10^6$) for straws were 57 ± 23.3 (0-95), 14.1 ± 18 (0-100) and 27 ± 17.7 (1-154), respectively, with 25.7% (n = 237/922) of straws ranked as unsound for AI due to unacceptable levels (>15%) of uncompensable sperm defects (38 ± 23.3; range 16-100). The most common USD were: knobbed acrosomes, 2.0 ± 4.1 (0-33); narrow at the base heads, 13.0 ± 13.4 (0-62); tapered heads, 1.0 ± 1.7 (0-13); diadem, 11.0 ± 20.3 (0-100); nuclear crater, 1.0 ± 3.1 (0-33); simple nuclear pouch below the apical acrosome ridge, 3.0 ± 9.2 (0-63); undeveloped heads, 3.0 ± 5.3 (0-33); abnormal midpiece, 0.4 ± 1.8 (0-26); and proximal cytoplasmic droplets, 3.0 ± 6.8 (0-50).

These results underlined the need to improve quality controls, specially in the screening of sperm abnormalities in the native ejaculate before freezing, giving the negative impacts on the conception rate caused by an undesirable prevalence of these sperm defects. Finally, there is a need in the country to set clear threshold quality parameters, as a prerequisite to determine the suitability of imported frozen bull semen.

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Chemotaxis of capacitated ram spermatozoa to ewe follicular fluid

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Sperm orientation mechanisms, as chemotaxis, are essential for the sperm to reach the oocyte and then fertilize it. The follicular fluid is the biochemical environment where the oocyte develops until its ovulation, and its composition changes throughout the estrous cycle. The follicular fluid is released together with the oocyte at the moment of ovulation, and it has been proposed as a chemoattractant for spermatozoa. This chemotactic ability has been demonstrated in some mammals, but to date, no studies have been done in the ovine species. Also, it has been reported that only capacitated and hyperactivated spermatozoa can respond to chemotactic stimuli. Therefore, the aim of this work was to study the chemotactic response of ovine spermatozoa to ewe follicular fluid, obtained at various stages of the estrous cycle, and to evaluate the influence of the capacitation status in the chemotaxis mechanism. In order to perform the experiments, ovine follicular fluids were obtained in the early and late follicular and luteal phases, and two concentrations, 2 and 10%, were tested. Semen samples were collected from nine *Rasa Aragonesa* rams, and spermatozoa were selected by the swim-up/dextran method. *In vitro* capacitation was induced by incubation without (cap-TALP) or with cAMP-elevating agents (cap-Cocktail). After sperm quality evaluation (motility, viability, and capacitation state), the chemotactic assays were carried out with the aim of the commercial device (IBIDI® µ-Slide Chemotaxis chamber, Ibidi GmbH). Swim-up, cap-TALP or cap-Cocktail samples were loaded in the chambers in the presence of follicular fluid. Individual trajectories were analyzed by the OpenCASA software *(Alquezar-Baeta* et al., *PLoS Comput Biol, 15(1), 2019)*, which calculates a chemotaxis index. Differences were analysed by χ^2 test, except for the chemotaxis index (ANOVA) using GraphPad PRISM® (Version 8.0.1). Follicular fluid at 2 and 10% concentrations, obtained in luteal and early follicular