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Effect of different extenders on sperm motion characteristics, viability and acrosome integrity during liquid storage of dromedary camel semen

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The objective of this study was to compare the effect of different extenders on dromedary camel sperm motion characteristics, viability and acrosome integrity during liquid storage at 4 °C. Semen was collected by AV at weekly intervals for five weeks from five dromedary bulls, using an oestrous female as a mount animal. Ejaculates ($n = 25$) were diluted 1:1 using split-sample technique, in five different extenders [Optixcell (IMV Technologies, France), EquiPlus (Minitube, Germany), egg yolk based Green buffer (IMV Technologies), Biladyl (Minitube) and Triladyl (Minitube) extenders] and incubated in a water bath (37 °C) for liquefaction. Immediately after complete liquefaction, semen samples were further diluted to 100×10^6 spermatozoa/ml with the same diluent as used earlier and stored at 4 °C in a cold handling cabinet (IMV, France). Motion characteristics of spermatozoa were evaluated using CASA (CEROS, Version12, Hamilton Thorne Biosciences, USA) pre-adjusted for camel sperm analysis. Three microliters of semen (50×10^6 spermatozoa/ml) was placed in a 20 μm standard count analysis chamber (Leja, Nieuw-Vennep, The Netherlands). Five randomly selected microscopic fields were scanned five times each and approximately 500 sperm counted. The total motility (TM%) progressive motility (PM%), path velocity (VAP, μm/s), progressive velocity (VSL, μm/s) and track speed (VCL, μm/s), lateral head amplitude (ALH, μm), beat cross frequency (BCF, Hz), straightness (STR%) and linearity (LIN%) of spermatozoa were analyzed. Sperm viability and acrosome integrity were also evaluated (FITC-PNA/PI; Kershaw-Young et al., *Anim Reprod Sci*, 138:3–4, 2013). Semen samples were analysed at 0, 24 and 48 h after storage. Statistical analysis was performed using mixed models regression in GENSTAT (version 17, VSN Int.). The TM at 0, 24 and 48 h was lower ($P < 0.05$) for EquiPlus samples when compared to samples diluted in other extenders, whereas PM for EquiPlus when compared to other extenders was higher at 0 h, similar at 24 h and lower at 48 h. At 48 h, TM and PM for Optixcell and Triladyl treatments were higher when compared to Biladyl samples, and similar when Green buffer or Biladyl were used for dilution. There was no difference in the TM between 0 and 24 h for any extender, with the exception of EquiPlus. PM declined with the period of storage for Optixcell, Green buffer and EquiPlus, but PM at 0 and 24 h was similar for Triladyl. The percentage of viable, acrosome intact spermatozoa did not differ between extenders or over time of storage, with the exception of EquiPlus which declined over storage period. In conclusion, Optixcell, Green buffer and Triladyl are recommended for the liquid storage of semen at 4 °C for up to 48 h in dromedary camels.

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Deferred bulls: Are cycles per conception and pregnancy rate determined by the type of spermiogramme deviation under natural mating?

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Deferred bulls undergo clinical deviations that could be transitory or have an uncertain potential fertility output. Data in Costa Rica showed no significant differences in conception rate (CR) between sound vs. deferred sires (DS), although it was 5% lower in the latter group (Navarro *et al.*, *Rep. Dom. Animals*, 43, Suppl:3, 2008). Decreased potential fertility in (DS) could be concealed by using multiple sired breeding and/or when the mating season length allows many estrous cycles/cow. When impaired semen quality justified the deferred classification, the fertility output could be influenced by the type of spermiogramme abnormality. We aimed to determine whether the rate cycles/conception, as well as CR achieved by DS diverge according to the type of spermiogramme deviation under natural mating in tropical cattle farms.

Twelve breeding DS (*B. indicus* $n = 8$ and *B. indicus* × *B. taurus* $n = 4$) were ranked as theratozoospermic (T) (≥15 and ≤30% total uncompensable morphological abnormalities) ($n = 6$; 3.0 ± 1.0 years-old), or oligozoospermic (O) (<3000×10^6 sperm output) ($n = 6$; 3.3 ± 1.1 years-old) after an examination performed just prior beginning the breeding season. Sires were allocated in 5 farms situated in the North area of Costa Rica. Simultaneously, 221 healthy cycling cows (2.0–10.0 years-old), were randomly distributed in 12 single-sired groups, at a cow/bull ratio of 19.7 ± 11.1 and 17.2 ± 10.4 for (T) and (O) respectively. Bulls were retested at the end of the breeding period (5 months length). Cows were diagnosed by ultrasound every 3 weeks, determining retrospectively the cycles/conception rate.

Prevalence of non-compensable abnormalities (%) and sperm output ($\times 10^6$) in (T) and (O) was respectively 19.3 ± 2.7 (17–24); 4229.4 ± 945.6 (3000–5000) and 3.0 ± 5.1 (0–13); 1290.2 ± 872 (202.5–2541). Overall CR (%) for (T) and (O) was respectively 86.4 ± 11.2 (66.7–97.3) and 63.1 ± 36.6 (7.4–100) ($p = 0.0001$). Besides, CR at first service was higher ($p < 0.05$) for (T) versus (O), being respectively 53.4 ± 22.8 (26.7–83.8) and 36.9 ± 23.9

(3.7–64.5). Likewise, the cycles/conception rate achieved by theratozoospermic and oligozoospermic bulls was 2.1 (213/102) and 3.1 (203/65) respectively ($p < 0.05$). The parameters studied diverged according to the type of spermiogramme deviation under natural mating in tropical farms. Although, CR at first service and cycles/conception index are likely impaired in deferred compared to sound for breeding bulls.

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Thermographic scrotal pattern in sound for breeding zebu bulls extensively managed in tropical Costa Rica

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Infrared thermography (IR) is relatively a novel procedure in animal andrology for studying bull's scrotal thermal pattern. The accuracy of this technique lies onto camera's performance and operator skills, and although the high cost of the equipment is still a constraint for its broad use, it could assist on diagnosis and prognosis the sire's potential fertility, since bulls with a disrupted testicular thermoregulation are linked with impaired semen quality. This report aims to outline the thermal scrotal pattern in sound for breeding Brahman sires, in order to reference these values when using IR for assisting during bull's breeding soundness examination.

IR files were recorded from the scrotum's posterior surface on thirty-seven Brahman sires (3.5 ± 1.4 years-old) ranked as sound for breeding. The bulls were breeding naturally in beef farms managed under extensive rearing in tropical Costa Rica. IR images were studied individually for left (L) and right (R) side of the scrotum through the software LumaSpec Offline Analyzer® using the tool "temperature point" and "region" on the following areas of interest: Top-Botton Neck Gradient (SNG), Top-Botton Testicular gradient (STG), Average Testicular Temperature (AST) and Average Cauda Epididymis Temperature (AET).

Environmental temperature showed a significant and positive correlation with rectal bull's temperature ($r = 0.26$; $p < 0.01$), (AST) ($r = 0.71$ to 0.72; $p < 0.0001$) and (AET) ($r = 0.74$ to 0.78; $p < 0.0001$). (AST) for (L) and (R) was highly and positively correlated with (AET) (coefficient ranging from 0.86 to 0.91; $p < 0.0001$). Mean temperatures (C°) on left and right side of the scrotum for (SNG), (STG), (AST) and (AET) were respectively: 1.05 ± 0.41 and 1.13 ± 0.43; 1.01 ± 0.52 and 0.96 ± 0.52; 32.12 ± 1.00 and 31.99 ± 1.04; 31.04 ± 1.08 and 31.00 ± 1.19. Minimal dissimilarities on average temperature were observed between (L) and (R) only for (SNG) and (AST) (0.08 ± 0.33 $p < 0.05$, and 0.13 ± 0.27 $p < 0.0001$ respectively). These slight differences are detectable only by IR software analysis using highly accurate cameras. Besides, they are indicative of a symmetrical temperature pattern in different regions of the scrotum from Brahman bulls ranked as sound for breeding.

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Flagella parameters used as descriptors of fish spermatozoa motility

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In many fish species, spermatozoa are immotile in the male reproductive tract but their flagella are activated when delivered into the surrounding medium, where various physico-chemical interactions regulating or influence their motility. Our aim is to describe quantitatively how such interactions affect flagella waves in their form and efficiency.

Optical microscopy (40 or 100×) with high-speed video or stroboscopic illumination with up to 5000 images/sec can be used to observe and record flagella movement of fish sperm. Various physical agents can be used to influence such movement: [1] ionic or sugar solutions to control osmolality combined, e.g. DMSO; [2] the interface of air/water or the egg surface; [3] methylcellulose to adjust viscosity; [4] temperature controlled microscope; and [5] gas effect using a gentle stream of CO_2. Carp, trout, sturgeon, and/or turbot spermatozoa can be studied.

The main signal activating fish sperm motility is osmotic pressure. Osmotic shock leads to serious damage to membranes and shortens motility. Biochemical steps of activation are well understood but the latter occur during cell dispersion in the swimming medium. Using osmotic agents (DMSO), we could delay for several second the appearance of the first waves at the head-tail junction and video-record this step.

Spermatozoa reaching surface vicinity remain swimming but "trapped" very close to it. Detailed waves properties are dependent on surface suppleness. Such accumulation of swimming spermatozoa is clearly of biological importance when approaching the egg surface as we demonstrated complementarily by simulation approach.

Viscosity is important for sperm swimming in the follicular or ovarian fluid. Addition of polymers, e.g. methyl-cellulose, drastically changes wave properties.

Temperature is important, especially for species of fish that reproduce at low temperature, such as trout (4–8 °C), or below 4 °C, for example burbot. Thermodynamic aspects of mechano-chemical properties of flagella are affected differentially by temperature.

In flatfishe, for example turbot, CO_2 can block transiently flagella wave propagation. But such an effect is fully reversible. This allows observing initiation sequence of first bending waves.