Title: DYNAMICS OF SPERM DNA FRAGMENTATION IN MAMMALIAN SPECIES AS ASSESSED BY THE SCD METHODOLOGY

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Objective: To determine the differential dynamic increase of sperm cells with fragmented DNA in semen samples from different mammalian species after exposure to a Temperature Excursion Episode (TEE) and 37°C temperature recovering, to simulate insemination.
**Design:** Prospective study in 10 different species. Sperm samples chilled and/or frozen ordinarily, as used for artificial insemination, were incubated at 37°C and sperm DNA fragmentation was assessed at different times (from T0 to T48h).

**Materials and Methods:** Species: Human, boar, bull, ram, goat, stallion, donkey, dog, rabbit and mouse. A total of 856 individuals were included in the analysis. Sperm DNA Fragmentation (SDF) was assessed using the Sperm Chromatin Dispersion test (SCD-test; commercial modification Sperm-Halomax®) which was adapted for application in each species by ChromaCell SL.

**Results:** The SDF index commonly found among individuals which were selected for reproductive characteristics was relatively low (5 to 10%), while in those species selected for other characteristics (human, stallion, donkey) exhibited a wider range of SDF. In all species when spermatozoa experience a severe (frozen) or mild (cooled) TEE, SDF is induced and causes the subsequent decline of sperm quality. In all analyzed species, SDF index increases when the biological sperm temperature is resumed. However, the period of time for SDF triggering varies from one species to another and could be detected just at the onset of the biological temperature recovery (human, ram, goat, stallion, rabbit, donkey) or could be delayed for a period of 24-48 h of incubation (bull) or even several days (some boars). In most species, SDF in frozen-thawed samples was detected at the onset of sperm incubation at 37°C, but an increase of SDF was not observed if the fragmentation was immediately assessed after thawing. Interestingly, the general pattern of SDF timing was not totally strict within the same species and differences were observed when different individuals were compared.

**Conclusions:**

1) Alterations of the sperm chromatin by handling for artificial insemination triggers SDF in mammalian species. 2) The dynamics of SDF is species-specific. 3) Within the same species differences were observed in the timing and intensity of SDF when different individuals were compared. 4) The level of SDF tends to be lower in animals which were selected for reproductive characteristics.

**Support:** ChromaCell SL provided the prototype kits for donkey, rabbit and stallion.

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