

The hypo-osmotic swelling test for fresh yak semen

V S CHOUHAN¹, G KRISHNAN², K P RAMESHA³, M A KATAKTALWARE⁴,
P CHAKRAVARTY⁵ and G KANDEEPAN⁶

National Research Centre on Yak, Dirang, West Kameng District, Arunachal Pradesh 790 101 India

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Commonly employed tests for evaluating sperm quality are the zona-free hamster egg penetration assay (Brackets *et al.* 1982, Ramesha and Goswami 1993) and the hypo-osmotic swelling test (HOST) (Jeyendran *et al.* 1984). HOST test is used in various species of livestock including cattle (Correa and Zavos 1994, Revell and Mrode 1994). As no report is available on HOST for yak semen, the present study was conducted to determine the best hypo-osmotic solution (HS) for testing membrane integrity in fresh yak semen.

Ejaculates (36) were collected from 6 genetically superior yak males (6 from each yak bull) maintained at Nyukmadung farm (2750 msl) under the NRC on Yak, Dirang, Arunachal Pradesh. All the ejaculates were evaluated for physio-morphological characteristics using standard procedures. Hypo-osmotic solutions of 50, 100, 150, 200, 250 and 300 mOsm/l were made according to Jeyendran *et al.* (1984) using sodium citrate and fructose. After macroscopic evaluation,

0.1 ml of semen was immediately added to 1.0 ml of each solution and incubated for one hour at 37°C. A total of 200 spermatozoa were counted in at 7 different fields and spermatozoa were classified as non-coiled, coiled, and strongly coiled as described by Revell and Mrode (1994) under 40× magnification using green filter under Image analyzer. The data were analysed using Duncan's multiple range test following two-way analysis of variance using SPSS (version 10).

The effect of different concentrations of HOS on coiling of fresh yak semen is presented in Table 1. The highest percentage of strong as well as total coiling of spermatozoa occurred in 150 mOsm/litre. For total coiling, 150 mOsm/l HS was superior to all other HS tried. For strong coiling, 150 mOsm/l solution was similar to 100 mOsm/l and significantly superior from all other concentrations attempted. Similar results were reported by Revell and Mrode (1994)

Table 1. Effect of different concentrations of hypo-osmotic solutions (mOsm/l) on the coiling of fresh yak spermatozoa

Concentration (mOsm/l)/ parameter (%)	50	100	150	200	250	300
Strong tail coiled	6.84±0.55 ^b	8.04±0.74 ^a	8.77±0.47 ^a	6.52±0.46 ^b	4.2±0.36 ^c	3.2±0.28 ^c
Total tail coiled	54.18±1.18 ^c	60.61±1.06 ^b	74.12±0.86 ^a	58.96±0.99 ^b	53.99±1.14 ^c	47.46±0.93 ^d

Means with different superscript differ significantly (P<0.05).

Table 2. Average percentage of coiling in fresh yak spermatozoa for different yak bulls exposed to hypo-osmotic solution

Animal no./parameter (%)	161	177	158	204	200	246
Total tail coiled	57.94±1.6 ^{bc}	56.26±1.4 ^{bc}	57.53±1.90 ^{bc}	63.08±1.5 ^a	55.66±1.90 ^c	58.83±1.65 ^b
Strong tail coiled	4.48±0.37 ^d	4.86±0.46 ^{cd}	7.63±0.61 ^a	6.93±0.74 ^a	5.94±0.52 ^{bc}	6.88±0.54 ^{ab}

Means with different superscript differ significantly (P<0.05).

Present address: ¹Scientist (Animal Physiology) (e mail: vikrant.chouhan@gmail.com); ²Scientist (Animal Physiology); ³Senior Scientist (AGB); ⁴Scientist (LPM), SRS of NDRI, Bangaluru. ⁵Principal Scientist (Animal Physiology); ⁶Scientist (LPT).

and Correa *et al.* (1997) in cattle. Among the 6 bulls, bull no. 204 showed higher percentage of coiling as well as strong coiling. Bull to bull variation was observed in average percentage of total tail coiling and also in strong tail coiling (Table 2). Similar results were reported earlier in cattle by

Prasad *et al.* (1999). On exposure to HOS, biochemical-active sperm cells with intact membranes absorb water and swell, increasing in volume to establish equilibrium between the fluid compartment within spermatozoa and extra cellular medium. It culminates into a spherical expansion of the cell membrane covering the tail, thus forcing the flagellum to coil inside the membrane. Tail coiling begins at the distal end of the tail and proceeds towards the mid piece and head as the osmotic pressure of suspension media is lowered (Jeyendran *et al.* 1984). It is important to differentiate loops and bends produced by exposing normal spermatozoa to a hypotonic solution (coiling) from cytoplasmic droplets in principal piece. Coiling means that spermatozoa are viable, while cytoplasmic droplets indicate pathological condition. Combining data of both strong and total coiling, 150 mOsm/litre solutions yielded best result. It was reported that Hypo osmolarity at 150 mOsm/litre is strongly correlated to mass movement, progressive motility, live sperm, total intact acrosome, sperm concentration (Prasad *et al.* 1999) and fertility (Jeyendran *et al.* 1984).

SUMMARY

A study was conducted to determine suitable hypo-osmotic solution for testing membrane integrity in fresh yak semen. The study revealed that 150 mOsm/litre solution would be suitable for use in HOST for evaluation of fresh yak semen.

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