USE OF CRYOPRESERVED SPERMATOZOA FOR CAPRINE IN VITRO FERTILIZATION (IVF)

A.A.Simplicio[1,2], B.G.Brackett[1], and L.Keskintepe[1]

[1]Department of Physiology & Pharmacology, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602; [2]Brazilian Agricultural Research Agency National Goat Research Center, PO Box D-10, Sobral, Ceara, Brazil.

Feasibility of using frozen-thawed semen in caprine IVF outside the breeding season was investigated. Two Nubian and 1 Nubian-Boer bucks were electroejaculated. Sperm were washed 2x (Proc.Rec.Adv. in Goat Prod. 2:1089-1094), frozen in skim milk (Brazilian Soc.Anim.Reprod. 171-177), or in egg-yolk (World Rev.Anim. Prod. 8:80). Oocytes with 2-4 layers of cumulus cells from 3-6 mm follicles washed in Tyrode's with pyruvate and PVA, were incubated in 1 ml of Hepes-TCM-199 + 10 μg oFSH and 10 μg bLH (NHPP, NIDDK, NICHD, USDA)/ml + 20% FBS (MM) at 38.5°C. After 4.5 h oocytes were further incubated (23 h) in 75 μl of MM under paraffin oil and 5% O_2, 5% CO_2, 90% N_2. Frozen sperm were thawed in a water bath (37°C, 15 sec), and selected by swim-up. IVF was in mDM (Theriogenology 37:1049-1060), supplemented as below, for 24 h. Embryo culture was in 50 μl of c-SOF+NEA (Biol. Reprod. 55:333-339) for 9 d. Data analysis was by ANOVA and Bonferroni t-test. Percentages of oocytes exposed to heparin-capacitated (HC) sperm that reached cleavage (C), morula (M), blastocyst (B), and expanded B (EB) were 82.8, 57.1, 35.7, 30.0 %, respectively; without heparin treatment of sperm data for C, M, B, EB were 44.3, 31.4, 18.6, 8.6 %, respectively. Further work employed HC sperm. Use of cryopreserved sperm with BSA for IVF yielded no C. Although extenders containing 8 to 20 % egg-yolk enabled good motility after crypreservation in vitro fertilizing ability was lost in our conditions. By contrast, commercial semen processed in season with egg-yolk was effective for IVF (see table).

Table. Caprine IVF with Cryopreserved Spermatozoa

Freezing extender	Suppl.of IVF medium	No. of oocytes inseminated	Development stages / Inseminated oocytes (%) Cleaved 48 h	Morula 120 h	Blastocyst (B) 168 h	Expanded B 216 h
Skim Milk	LS[1]	40	33 (82.5)[a]	20 (50.0)[d]	12 (30.0)[h]	9 (22.5)[l]
Skim Milk	FCS	37	4 (10.8)[b]	1 (2.7)[e]	0[i]	0[m]
Egg Yolk C[2]	LS	41	24 (58.5)[c]	11 (26.8)[f]	7 (17.1)[j]	4 (9.7)[n]
Egg Yolk C	FCS	38	6 (15.8)[b]	4 (10.5)[g]	3 (7.9)[k]	0[m]

[1]Lamb serum. [2]Sperm processed in season in egg yolk commercially. [a]Different superscripts in the same column denote significant differences ($P<0.05$).

Highest proportions of blastocysts resulted after sperm cryopreservation in skim milk extender, heparin capacitation, and insemination in media containing lamb serum.