

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

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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<sub>2</sub>, 5% CO<sub>2</sub>, 90% N<sub>2</sub>. 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 cryopreservation 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 <sup>1</sup>	40	33 (82.5) <sup>a</sup>	20 (50.0) <sup>d</sup>	12 (30.0) <sup>h</sup>	9 (22.5) <sup>†</sup>
Skim Milk	FCS	37	4 (10.8) <sup>b</sup>	1 (2.7) <sup>e</sup>	0 <sup>i</sup>	0 <sup>m</sup>
Egg Yolk C <sup>2</sup>	LS	41	24 (58.5) <sup>c</sup>	11 (26.8) <sup>f</sup>	7 (17.1) <sup>j</sup>	4 (9.7) <sup>n</sup>
Egg Yolk C	FCS	38	6 (15.8) <sup>b</sup>	4 (10.5) <sup>g</sup>	3 (7.9) <sup>k</sup>	0 <sup>m</sup>

<sup>1</sup>Lamb serum. <sup>2</sup>Sperm processed in season in egg yolk commercially. <sup>a</sup>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.