Table 1. Cleavage, embryo development rate, and cryotolerance of FSH of various origins

FSH	Oocytes, no.	Cleavage, no. (%)	Expanded blastocysts, no (%)	Post-thaw survival, no. (%)	Re-expansion, no. (%)	Hatching, no (%)
1× pFSH	452	256/452 (57)^{BC}	75/452 (17)^{AB}	66/75 (88)	53/75 (71)	36/75 (48)
1× rhFSH	407	220/407 (54)^{C}	54/407 (13)^{B}	47/54 (87)	33/54 (61)	25/54 (46)
1× pFSH + 1× rhFSH	488	329/488 (67)^{AB}	105/488 (22)^{AB}	98/105 (93)	79/105 (75)	64/105 (61)
2× pFSH	497	365/497 (73)^{A}	127/497 (26)^{A}	122/127 (96)	105/127 (83)	87/127 (69)
2× rhFSH	413	292/413 (71)^{A}	91/413 (22)^{A}	82/91 (90)	67/91 (74)	52/91 (57)

$^{A-C}$Values with different superscripts in the same column differ ($P < 0.05$).

30 Bull sperm kinetics after semen cryopreservation in extender containing propagermanium

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There is a negative association between a plentiful production of oxygen reactive species and spermatozoa kinetics parameters. Thus, antioxidants have been added to the freezing medium to improve sperm quality due to their protective effect against membrane lipid peroxidation. Propagermanium (GE132) is an organometallic compound that has never been used in freezing medium despite its known antioxidant effect as a free radical scavenger. This study aimed to investigate the effects of different concentrations of GE132 added in a commercial freezing medium (CFM) on frozen-thawed sperm motion. Nine ejaculates of 3 Nellore bulls (3 replicates), collected by an artificial vagina, were evaluated, pooled, and divided into groups D (semen was diluted and kept at 33°C for 30 min before cooling) and C (semen was cooled immediately after dilution). Both groups were submitted to the same experimental treatment, as follows: addition of 0, 500, and 1000 μg mL^{-1} of GE132 in a CFM resulting in subgroups D0, D500, D1000, C0, C500, and C1000. The sperm samples were diluted to a final concentration of 30×10^6 spermatozoa per straw (0.25 mL) and then cooled at 4°C for 5 h before freezing. Sperm samples were assessed using a computer assisted sperm analyser at 5 and 60 min post-thawing for total motility (TM; %), progressive motility (%), curvilinear velocity (μm s^{-1}), velocity straight line (μm s^{-1}), velocity average path (μm s^{-1}), amplitude of the lateral head displacement (ALH; μm), beat cross frequency (Hz), linearity (LIN; %), and straightness (STR; %). Data were analysed using the R software package version 3.4.4 (2018; https://www.r-project.org/). An ANOVA was applied to assess statistical differences, and Tukey's test was used to determine differences among subgroups. A significance level of $P < 0.05$ was adopted. No significant differences ($P > 0.05$) were observed among subgroups for all sperm parameters except for TM, in which C0 presented higher ($P < 0.05$) value (68.72 ± 3.36) than D0 (54.67 ± 5.59), D5 (57.10 ± 2.34), and C10 (54.20 ± 2.73), with similar results between D10 (59.5 ± 4.22) and C5 (59.52 ± 4.64). There were significant differences within subgroups when comparing 5 and 60 min post-thawing for TM, ALH, LIN, and STR. Total motility decreased 17.2 and 9.9% in C5 and C10, respectively. Similarly, values of ALH decreased 0.2, 0.4, and 0.2 μm in D0, D5, and C5, respectively. However, the increase in LIN was 11% in D10, whereas the values for STR increased in D10 (10%), C5 (6.3%), and C10 (7.3%). The addition of GE132 to the CFM did not enhance all the sperm parameters after cryopreservation except for a slight improvement in ALH, LIN, and STR over time and TM among groups. The lack of additive effect could be due to the presence of antioxidants in the CFM; therefore, further investigation with fluorescent probes using flow cytometry and free-antioxidants freezing medium could lead to a new approach for bull sperm freezing.

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31 Effect of fetal calf serum on production and cryotolerance of *in vitro* bovine embryos from ecuadorian creole heifers

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In the Ecuadorian Andes there is a Creole bovine biotype whose population is disappearing. *In vitro* embryo production and cryopreservation is an important biotechnology that allows the conservation of animals threatened with extinction. The objective of this study was to determine the *in vitro* production and cryopreservation of embryos from creole heifers raised in the highlands of Ecuador. Immature cumulus-oocyte complexes were retrieved by ovum pickup from 10 Creole heifers (OPU) and from abattoir ovaries (control). The experiment was completed within 8 replicates. Cumulus-oocyte complexes were cultured in a maturation medium (TCM-199 supplemented with 10% fetal bovine serum, 100 μg mL^{-1} of sodium pyruvate, 0.75 mg mL^{-1} of L-glutamine, 4 μg mL^{-1} of FSH-p, 100 μM cysteamine, and 250 μg mL^{-1} of gentamicin) following IVF (SOF medium supplemented with 10 μg mL^{-1} heparin) and *in vitro* culture (citrate SOF medium). After denudation (Day 1 after IVF), presumptive embryos from each oocyte source (OPU and control) were split into 2 groups: with (FCS+) and without (FCS−) FCS (2.5%), which was added on Day 5 after IVF. On Day 7, embryos were evaluated, and those with quality 1 were vitrified. After warming, embryo re-expansion at 2 h and embryo re-expansion and

hatching at 24 and 48 h were evaluated. Data were analysed by logistic regression in SAS software (SAS Institute Inc., Cary, NC, USA). Results of embryo rate at Day 7 and rates of vitrified, re-expanded, and hatched embryos are shown in Table 1. Regardless of the oocyte source, the addition of 2.5% FCS decreased embryo re-expansion at 2 h and reduced embryo hatching at 48 h in the OPU group. In conclusion, FCS did not improve embryo production and adversely affected the cryotolerance of embryos produced *in vitro* from Ecuadorian creole heifers.

Table 1. Production and cryotolerance of *in vitro* bovine embryos

Variable, % unless noted	Oocyte source			
	OPU		Abattoir	
	FCS+	FCS−	FCS+	FCS−
Oocytes (no.)	119	116	145	145
Embryos on d 7	39.5	29.3	33.8	29.6
Vitrified	21.0	19.0	20.7	16.5
Re-expanded 2 h	52.4[a]	85.7[b]	36.0[c]	64.7[d]
Re-expanded 24 h	66.7	92.9	84.0	82.3
Re-expanded 48 h	61.9	85.7	80.0	88.2
Hatched 24 h	19.0	42.8	32.0	35.3
Hatched 48 h	19.0[a]	78.6[b]	36.0	58.8

[a-d]Values with different letters in the same row for each oocyte source differ ($P < 0.05$).

32 Vitrification of *in vitro*-matured bovine oocytes in triacetate cellulose hollow fibres

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A prospective method of vitrification in triacetate cellulose hollow fibres (HF) introduced by Matsunari *et al.* (2012 J. Reprod. Dev. **58**, 599-608) allowed significant simplification and standardization of vitrification/warming procedures and was successfully used for group cryopreservation of various pre-implantation mammalian embryos. The goal of the current work was to evaluate the effectiveness of the HF vitrification method for cryopreservation of *in vitro*-matured bovine oocytes. The base medium for all the vitrification and rewarming solutions was calcium-free TBP-like protein-HEPES supplied with 20% of fetal bovine serum. Groups of 15 morphologically normal *in vitro*-matured bovine oocytes were equilibrated with 3% (vol/vol) ethylene glycol for 15 min, loaded into HF, and transferred into vitrification solution containing 30% ethylene glycol and either 0.5 M (Group 1) or 1.0 M (Group 2) sucrose. Hollow fibres were incubated for either 60 s (Group 1) or 30 s (Group 2) and immediately plunged into LN. Rewarming was conducted at 39°C. Oocytes within HF were placed in decreasing concentrations of sucrose solutions to remove cryoprotectants. Then, oocytes were subjected to IVF. Non-vitrified denuded oocytes were used as a control. Survival rates were evaluated at 21 h post-rewarming. Part of the presumptive zygotes were fixed and stained with acetolacmoid for fertilization rate. The remaining zygotes were cultured for 10 days. Developmental rates were evaluated at 44 h and 7 and 10 days post-IVF. All results are presented as mean percentage ± standard deviation. Data were analysed using Mann-Whitney U test. Significance was set at $P < 0.05$. Survival rate was significantly lower in Group 1 (79.0 ± 8.0%) and Group 2 (75.0 ± 5.0%) compared with the control group (97.0 ± 4.0%). Fertilization rate in Group 1 differed significantly from the control (80.5 ± 18.3% *v.* 95.5 ± 9.1%). Cleavage rates in Groups 1 and 2 did not differ significantly from the control (42.5 ± 15.7% *v.* 60.7 ± 11.1% *v.* 63.0 ± 15.8%, respectively). Blastocyst yields at 7 days post-IVF were 0.9 ± 2.3 (1/116) and 9.6 ± 5.4% (6/65) in Groups 1 and 2, respectively. The former was significantly lower than in the control group (17.0 ± 10.3%, 23/154). It should be noted that hatching in the control group started at 8 days post-IVF and was delayed in Groups 1 and 2 for at least 24 h. Day 10 blastocyst yields were 3.0 ± 3.3 ($P < 0.05$), 20.9 ± 13.8, and 30.4 ± 9.6% in Groups 1 and 2 and the control group, respectively. All obtained Day 10 blastocysts (3/116) in Group 1 hatched. Hatching rate in Group 2 was significantly lower than in the control group. Both Groups 1 and 2 showed relatively high survival and fertilization rates, but embryo development rates in both groups had a tendency to be lower than in the control. However, the obtained results indicate that the modifications of the protocol may increase the effectiveness of HF vitrification. The HF vitrification method remains a prospective option for simultaneous cryopreservation of a group of bovine oocytes.

33 Caffeine improves equine sperm motility after thawing

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The pattern of sperm transport and survival in the mare's reproductive tract is different between fresh and frozen-thawed semen. A probable reason for this difference is the biophysiological changes in sperm during cryopreservation of equine semen. These changes can impair motility of stallion sperm after thawing. The aim of the present work was to test the effect of different caffeine concentrations on stallion sperm motility after thawing. One ejaculate of 9 stallions was frozen with the INRA82 frozen extender, and after thawing, different caffeine concentrations were added to the semen