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(Article begins on next page)

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Effect of EGF on fertilizing potentials of cryopreserved beef bull semen

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AIM OF THE WORK

Evaluate the effects of EGF on the viability and integrity of frozen sperm from Piedmontese bulls

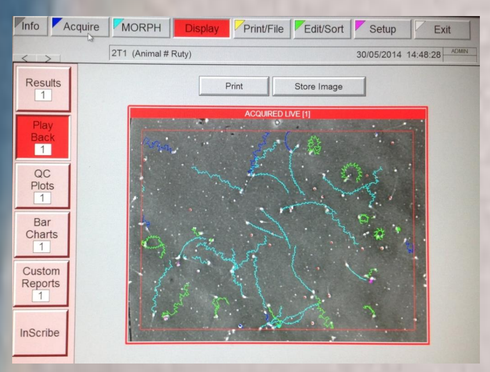
MATERIAL AND METHODS

- **Ejaculates of 4 Piedmontese breed bulls → POOL**
- **Weekly withdrawal for 8 consecutive weeks same bulls**
- **Dilution with Bullxcell**
- **Experimental groups addition of EGF at concentrations of 0, 50, 100, 200 and 400 ng / ml.**
- **Programmed freezing up to -150 ° C**
- **Storage in liquid nitrogen (-196 ° C)**

- **Motility and Velocity CASA - T0 T1 T2 T3 e T4 - Motility (total and progressive) Speed (curvilinear, average, linear)**
- **Vitality and acrosomal status → Trypan Blue & Giemsa**
- **Integrity of the plasma membrane → HOS test**
- **DNA integrity**
- **Apoptosis**
- **Mitochondrial activity**

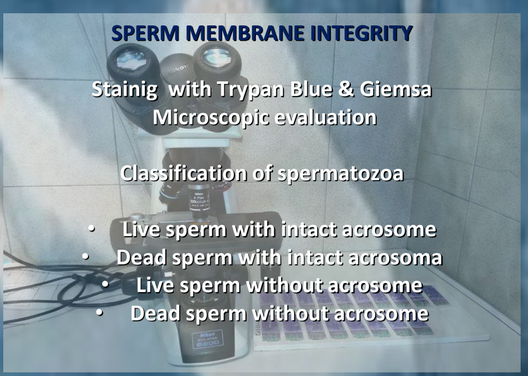
  } **Citofluorimetry**

- **Penetration of cervical mucus**
- **Antioxidant activity → SOD Colorimetric Kit**



MOTILITY	GROUP	POST THAWING	1 HOUR	2 HOURS	3 HOURS	4 HOURS
PROGRESSIVE MOTILITY (%)	Control	53.19 ± 0.66	43.56±0 .70[a]	26.63±1.30[a]	6.16±1.40	4.02±1.25
	EGF 50ng/mL	53.03± 0.85	44.56±1.00[a]	30.78±1.14[b]	9.28±1.26	3.84±0.99
	EGF 100 ng/mL	54.12 ± 0.57	45.58±0.64[a]	27.20±0.98[a]	8.38±1.29	4.09±1.16
	EGF 200 ng/mL	54.69±0.58	48.09± 0.71[b]	32.77±1.01[b]	9.89±1.45	3.66±0.96
	EGF 400 ng/mL	54.44±0.97	44.89±0 .60[a]	32.47±1.39[b]	8.06±1.00	4.75±1.34
	Sig.	NS	0.000	0.000	NS	NS

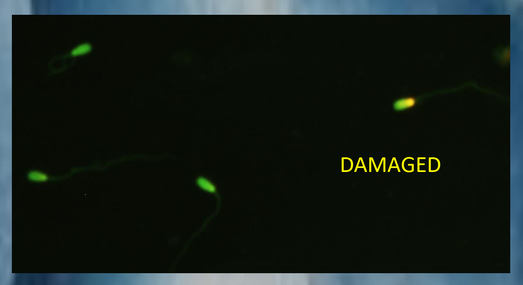
TABLE 1: Motility parameters



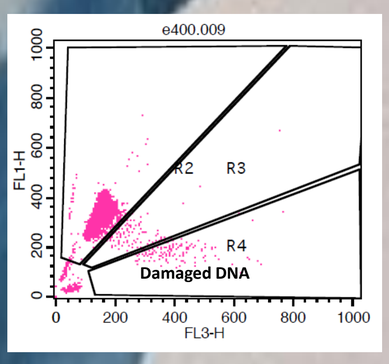
PLASMATIC MEMBRANE INTEGRITY
Hypo-osmotic swelling test (190 mOsm/kg)

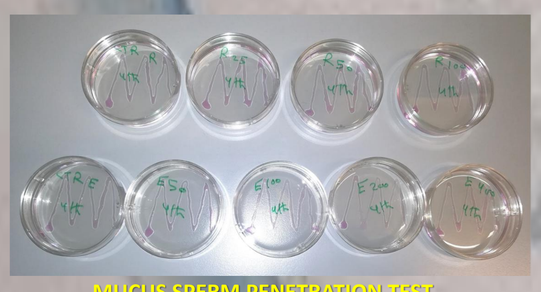
Experimental groups	Live sperm (%)	Acrosome Integrity (%)	Plasma membrane Integrity (%)	DNA Integrity (%)
Control	68.56 ±1.32[ab]	90.94 ± 1.75	60.94 ± 1.65	94.02±0.35
EGF 50 ng/mL	65.50 ± 1.10[a]	90.56 ± 1.71	61.94 ± 1.95	93.85±0.70
EGF 100 ng/mL	70.06 ± 1.34[b]	92.25 ± 1.52	64.63 ± 1.74	94.02±0.66
EGF 200 ng/mL	72.38 ± 1.50[b]	92.06 ± 1.78	63.00 ± 2.35	94.25±0.63
EGF 400 ng/mL	71.06 ± 1.50[b]	93.56 ± 1.36	62.88 ± 1.95	93.43±0.73

TABLE 2: Effect of EGF on cryopreserved Piedmontese bull semen livability, acrosome, plasma membrane and DNA integrities. Values are presented as mean± SEM. The different letters within the same column differed significantly at P < 0.05.

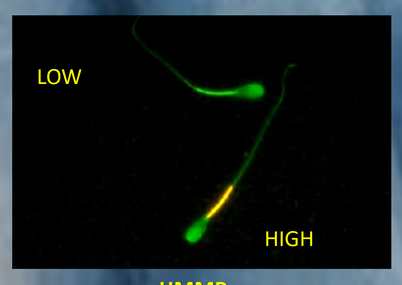


SPERM CHROMATIN STRUCTURE ASSAY
WITH ACRIDINE ORANGE





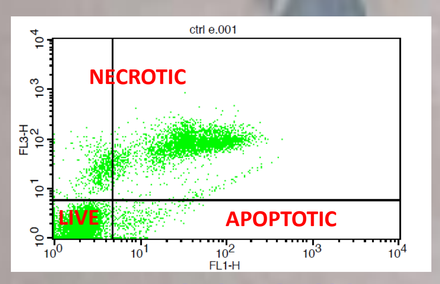
MUCUS SPERM PENETRATION TEST



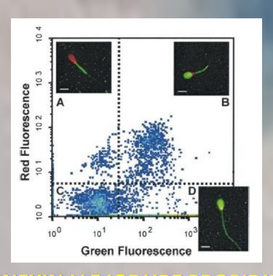
HMMP

Group	HMMP (%)	Mucus penetration distance (cm)	SOD activity (u/ml)
Control	12.44 ± 1.68	8.75 ± 1.93	1.68 ± 0.02
EGF 50 ng/mL	24.15 ± 12.44	10.00 ± 1.78	1.67± 0.04
EGF 100 ng/mL	30.67 ± 9.39	9.73 ± 1.98	1.72 ± 0.03
EGF 200 ng/mL	25.73 ± 10.35	10.38 ± 1.14	1.69 ± 0.03
EGF 400 ng/mL	22.61 ± 10.30	9.70 ± 0.83	1.64 ± 0.04

TABLE 3: Effect of EGF on mitochondrial activity, mucus penetration ability and SOD activity. Values are presented as mean ±SEM. HMMP: High Mitochondrial membrane SOD: Superoxide dismutase. The different letters within the same column differed significantly at P < 0.08.



EVALUATION OF APOPTOSIS



ANNEXIN-V E IODURE PROPIDIUM

Group	Normal Viable sperm (%)	Necrotic sperm (%)	Apoptic sperm (%)
Control	45.95 ±10.65	45.90 ± 5.61	8.15 ± 5.41a
EGF 50 ng/mL	50.41 ± 5.43	47.78 ± 5.28	1.80 ± 0.34ab
EGF 100 ng/mL	52.69 ± 3.64	46.29 ± 3.70	1.02 ± 0.23b
EGF 200 ng/mL	51.63 ± 3.64	47.36 ± 3.88	1.02 ± 0.31b
EGF 400 ng/mL	50.54 ± 2.85	48.48 ± 2.81	1.15 ± 0.23b

TABLE 4: Effect of EGF on sperm apoptosis and necrosis. Values are presented as mean ±SEM. Values with different superscripts differed significantly at (P <0.07)

CONCLUSIONS

The EGF significantly (p<0.05) improve the different velocity parameters after the different incubation periods mainly with the concentrations 100, 200 and 400 ng/ml. EGF significantly improved the sperm vitality (p<0.01) and decreased sperm apoptosis (p<0.05) with the concentrations 100, 200 and 400 ng/ml without affecting acrosome, plasma membrane and DNA integrities. In conclusions, incorporation of EGF especially at concentrations 100 and 200 ng/ml could improve the vitality parameters of cryopreserved bull semen.

Further studies would be needed to verify the positive effect of EGF on low fertility bulls.

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