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Title: Role of amino acids as additives on sperm motility, plasma membrane integrity and lipid peroxidation levels at pre-freeze and post-thawed ram semen

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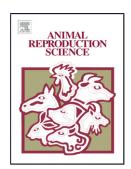
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ACCEPTED MANUSCRIPT

1	Role of amino acids as additives on sperm motility, plasma membrane integrity
2	and lipid peroxidation levels at pre-freeze and post-thawed ram semen
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7 8	Highlights
9	L-Glutamine, L-Proline improved ram sperm character at pre-freeze and frozen semen.
10 11	L-Glutamine, L-Proline reduced the lipid peroxidation level at pre-freeze and post-thawed semen.
12 13	L-Alanine has deleterious effect on the ram spermatozoa during cooling as well as freezing process.
14	
15	Abstract
16	The possibility of including amino acids for cryopreservation of ram semen to improve
17	the quality of frozen semen was explored in this study in sheep model. 24 samples were
18	collected in triplicate from 8 rams of 2-3 year old Bannur cross bred rams maintained at the
19	institute Experimental livestock unit. Semen was diluted in tris-egg yolk glycerol diluent and
20	made into 7 aliquots as follows: aliquot 1 served as control, "L"- alanine" was added at 100
21	and 135mM in the aliquot 2 & 3, "L"- glutamine" was added at 20 and 25mM in the aliquot 4
22	& 5 and "L"- proline" was added at 25 and 50mM in the aliquot 6 & 7, respectively. Diluted
23	semen was filled in 0.25 ml French straws and frozen in LN2. Inclusion of "L"- proline" and
24	"L"- glutamine" in the diluent increased the percent live sperm (P<0.001), total motility
25	(P<0.05) and maintained higher functional membrane and acrosomal integrity (P<0.001) by
26	decreasing lipid peroxidation (P<0.001) compared to the control group. In contrast, "L"-
27	alanine" decreased the percentage of total motility, fast progressive spermatozoa and

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