المعهد الوطني للبحث الزراعي
ⵓⵔⴰⵔⵉ ⵏⵜⵓⵙⵙⵏⵉ ⵏ ⵜⵓⵙⵙⵏⵉⵡⵉⵏ ⵏ ⵜⵓⵣⵔⵉⵄⵜ
Institut National de la Recherche Agronomique

Improvement Strategies in Ovine Artificial Insemination: The Moroccan case

by Bouchra El Amiri
INRA- Morocco

Outline

1-Introduction

2- Endegenous *vs* exogenous factors

3-Challenges and potential solutions

4-The case study : the Moroccan experience

5- Take-home messages

Introduction

- **Artificial insemination (AI) is a crucial tool in breeding programs for sheep**
- **Due to irregular and low fertility results, as well as difficulties in using enhancements like frozen-thawed sperm.**
- **It is not as widespread compared to other domestic species.**

.

Introduction: roles and impacts

Genetic Improvement

Widespread commercial AI in sheep would accelerate genetic improvement programs by enabling widespread use of superior sires and increasing selection intensity.

Breed Conservation

AI could play a vital role in conserving rare and endangered ovine breeds by disseminating genetic material and establishing gene banks.

Biotechnology Integration

Reliable intrauterine AI would enable integration of advanced reproductive biotechnologies, sperm sorting, and stem cell-derived gametes.

Economic Benefits

Improving the efficiency and accessibility of ovine AI could provide significant economic benefits to the sheep industry through increased production and genetic gain.

Sheep Artificial Insemination is a very complex equation

- **Semen handling and quality (extenders, additives,T°, osmolartity,…., type of collection,…)**
- **Oestrus synchronisation**
- **Type of AI**
- **Time**
- **Equipements,**
- **….**

Aims

The aim of this presentation is to explore in detail the most factors and challenges facing successful ovine AI in sheep and suggests strategies and best practices for its improvement.

This lecture will serve as a guide for understanding and optimizing the success of ovine AI.

Present what we did for AI in Moroccan sheep

I-Exploring Endogenous and Exogenous Factors for Successful Artificial Insemination in Sheep

veterinary sciences

MDPI

Review

Exploring Endogenous and Exogenous Factors for Successful Artificial Insemination in Sheep: A Global Overview

Bouchra El Amiri [1,2,*] and Abdellatif Rahim [1,3]

Vet. Sci. **2024**, *11*, 86

IA is not simple action

Endogenous factors

Genetic Factors

Heritability

Male fertility has low heritability (0.001-0.005).
Female fertility is higher (0.040-0.078).

Genomic Selection

Combining genomic selection with reproductive technologies like AI can boost genetic gain.

Breed Differences

Breed variations exist in reproductive traits affecting AI outcomes.

Age-Related Fertility

1

Young Ewes (<2 years)

Reduced fertility due to incomplete reproductive maturity.

2

Optimal Age (2-6 years)

Peak reproductive performance and highest conception rates.

3

Older Ewes (>6 years)

Declining fertility associated with age and parity.

Keep in mind the complex anatomy of ewe cervix while performing AI

Cervical Anatomy

Complexity

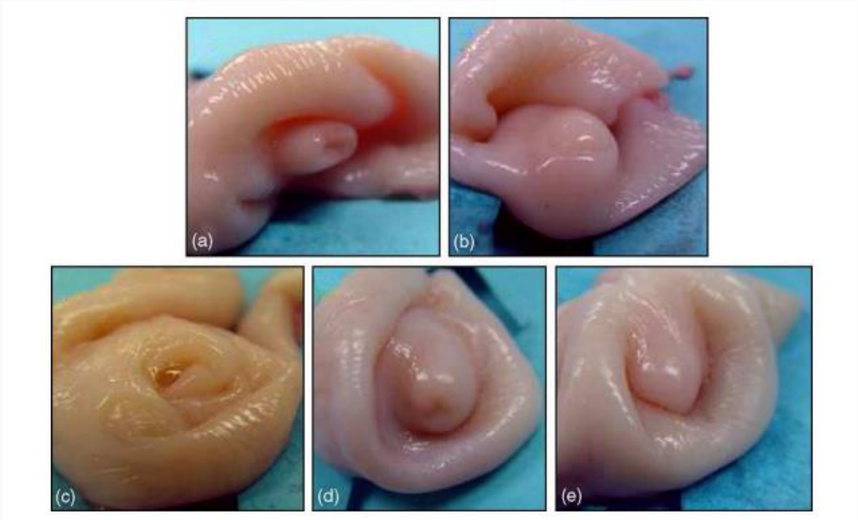
The ovine cervix has intricate folds, making transcervical AI challenging.

Breed Variations

Cervical anatomy varies across breeds, impacting AI success rates.

Age Effects

Older ewes tend to have less complex cervices, facilitating deeper AI gun penetration.



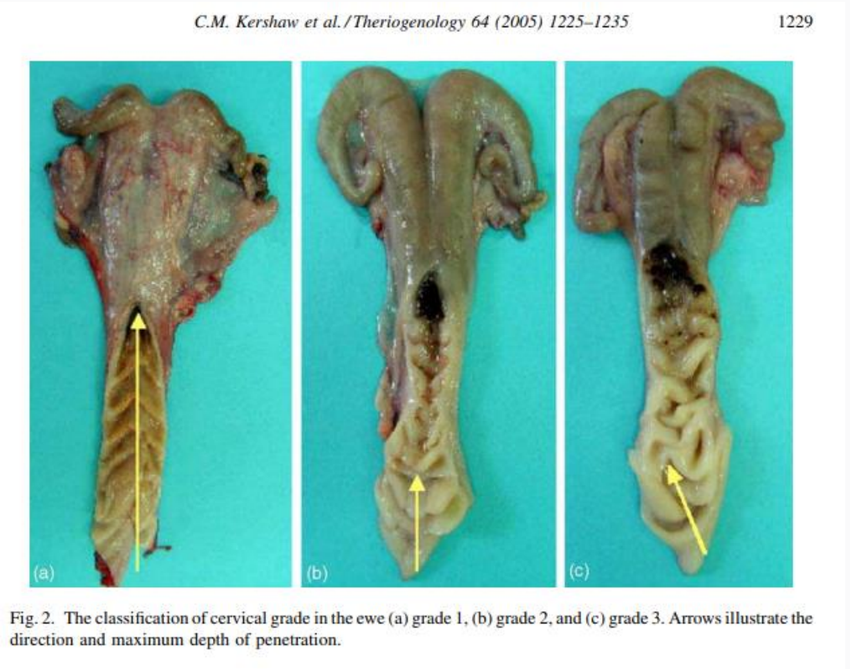


Fig. 2. The classification of cervical grade in the ewe (a) grade 1, (b) grade 2, and (c) grade 3. Arrows illustrate the direction and maximum depth of penetration.

Animal Reproduction Science 196 (2018) 43–47

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Morphometry and depth of inseminating catheter penetration in prolific and non- prolific ewes at different ages: A post mortem study

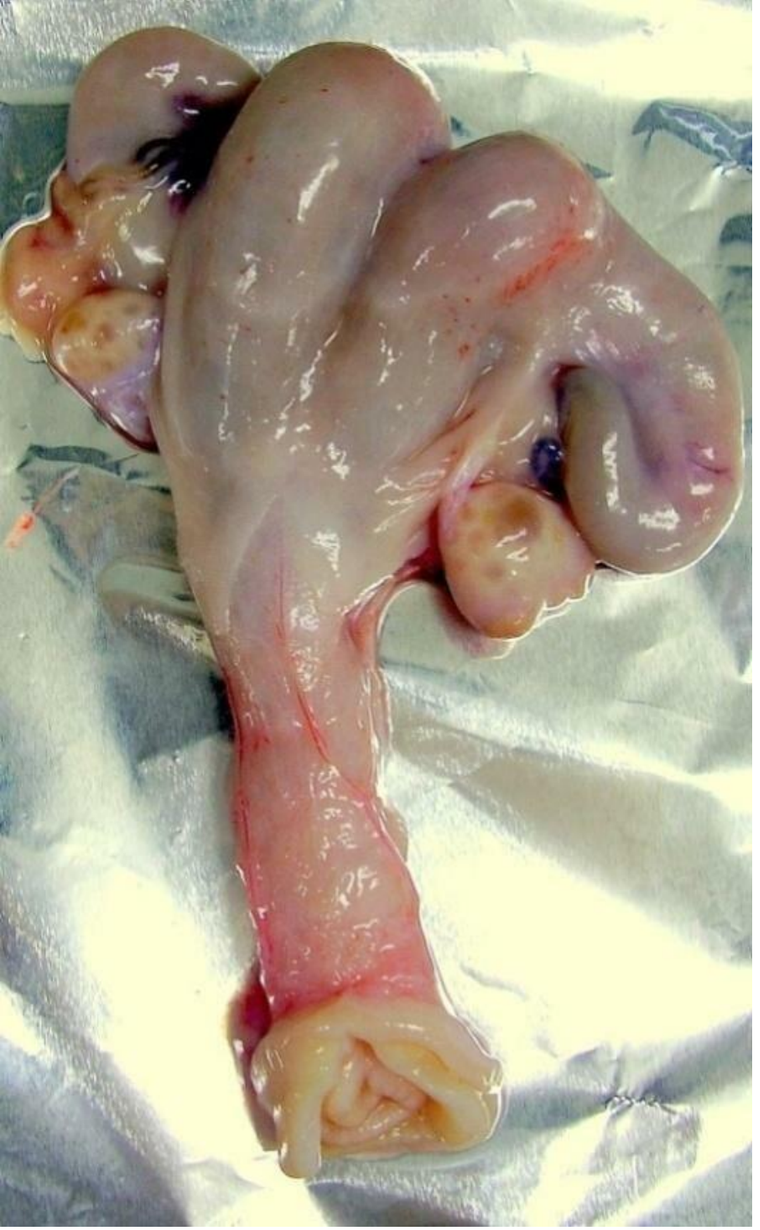
Kaoutar EL khalil[a,b], Larbi Allai[a], Alice Fatet[c], Anass Benmoula[a], Naima Hamidallah[b], Abdelmoughit Badi[a], Zineb Moussafir[a], Mustapha Ibnelbachyr[d], Bouchra El Amiri[a,*]

Effects of breed and age on the percentage of cervical grades in D'man (prolific sheep) *vs.* Boujaâd (non-prolific sheep).

Breed	Age	Number of samples	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)
D'man	2 T	16	25^{bAy}	56.25^{bAx}	18.75^{bBy}
	4 T	21	33.64^{aAy}	47.93^{bAx}	18.44^{bBz}
	6 T	12	13^{cBy}	72.22^{aAx}	14.78^{bBy}
	8 T	15	25^{bAy}	52.44^{bAx}	26.56^{aBy}
Boujaâd	2 T	38	14.57^{bBz}	51.41^{aAx}	34.01^{aAy}
	4 T	34	20.59^{aBy}	41.56^{bAx}	38.25^{aAx}
	6 T	49	23.24^{aAz}	44.63^{bBx}	32.13^{aAy}
	8 T	66	17.17^{abBz}	45.96^{bBx}	36.87^{aAy}

a, b, c. Different superscripts within columns indicate a statistically significant effect of age for each grade in each ewe breed ($P < 0.05$).
A, B, C. Different superscripts within columns indicate a statistically significant effect of breed for each grade in each age ($P < 0.05$).
x, y, z. Different superscripts within lines indicate the distribution of different cervix grades in each age for each breed ($P < 0.05$).

- ❑ The anatomical features of the cervical canal of Boujaâd ewes differ from those reported for D'man ewes.
- ❑ The cervices of Boujaâd ewes are more complicated than those of D'man ewes.
- ❑ In both D'man and Boujaâd breeds, the cervix becomes less complicated with progressing age (6–8 T).



This figure was uploaded by Laura Falchi

Table 1. Cervical length and number of rings in six ewe breeds (n=28–30 ewes per breed). Cervices were collected post mortem. Values are mean±SEM. [abc] Different superscripts differ significantly within each column ($P<0.05$).

Ewe Breed	Cervix Length (cm)	Number of Cervical Rings
Suffolk	7.52±0.169[a]	5.0±0.26[a]
Belclare	7.42±0.177[a]	4.9±0.18[a]
Fur	5.54±0.138[b]	5.6±0.15[b]
NWS	5.52±0.153[b]	5.4±0.16[b]
Ile de France	7.11±0.232[a]	4.7±0.15[a]
Romanov	6.02±0.173[b]	4.2±0.17[a]

NWS= Norwegian White Sheep.

L. Abril-Parreño et al., 2021 (Theriogenology)

Synchronisation and lambing compactness

- Choose the best mating time (maximize fertility)
- Adjust: nutritional requirements and provision of cheap natural feed
- Produce according the market requirements and the consumer demande

Estrus synchronisation

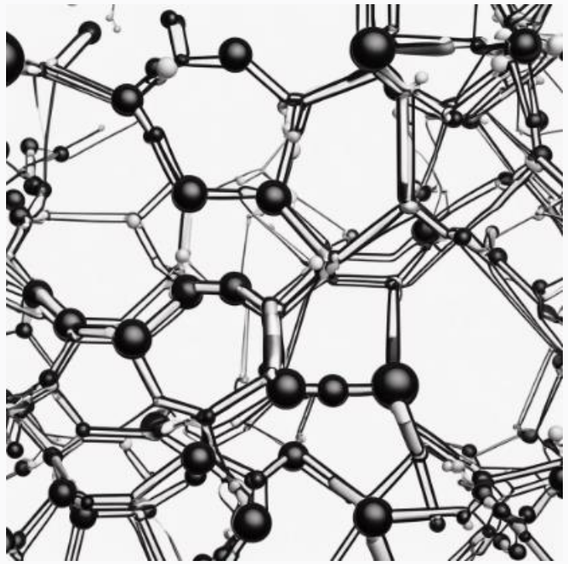
There have many benefits to estrus sychronisation

- Schedule lambing
- Concentrate lambing
  - Reduced mortality
  - Reduce labor
  - More uniform lamb
  - Reduced marketing cots
- Essential for artificial insemination

Hormonal Synchronization

Breeding Season

Prostaglandins are used for synchronization when ewes have a corpus luteum.



Non-Breeding Season

Progesterone-based protocols with eCG/PMSG are employed for anestrous synchronization.

Naturel methods

Photoperiod

Ram effect

Feeding

Hormonal methods

The most successful is based on the temporary suppression of the estrus with the help of progestins

Semen Handling

1

Collection

Careful handling during collection is crucial to maintain semen quality.

2

Dilution

Proper dilution protects sperm from pH changes and provides required nutrients.

3

Cooling

Controlled cooling preserves semen for transport and insemination.

4

Antioxidants

Antioxidants help counter oxidative stress during semen storage.

Artificial vagina

- Consider to be the best method of semen collection for all animals
- The device can be easily prepared and used

Disadvantages

- Need for female in estrus
- The male should be trained for collection
- Parts must be clean carefully to avoid contamination

Electroejaculator

- Do not need females

Disadvantages

- Harmful for animal
- Urine may be mixed with semen
- Animal may lie down due to paralysis of the leg
- Semen volume is larger and concentration is lower of that collected by AV

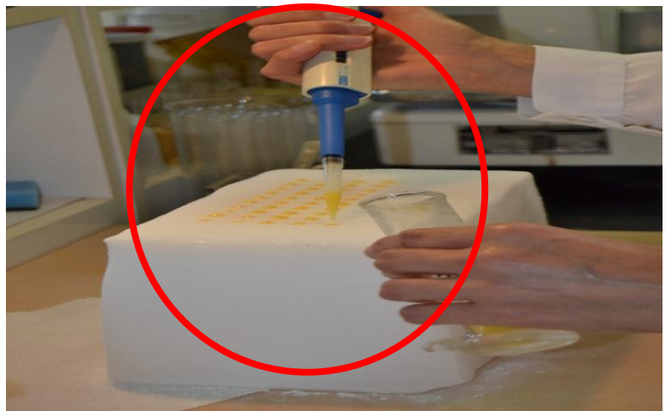
Preservation forms

Liquid/fresh or Chilled semen

- Collecte, examine, extend and coold to 5°C or 15°C

Frozen semen / 1 or two steps

- Cooling to 5°C
- Equilibration 3 to 4 h at 5°C in presence of cryoprotectant

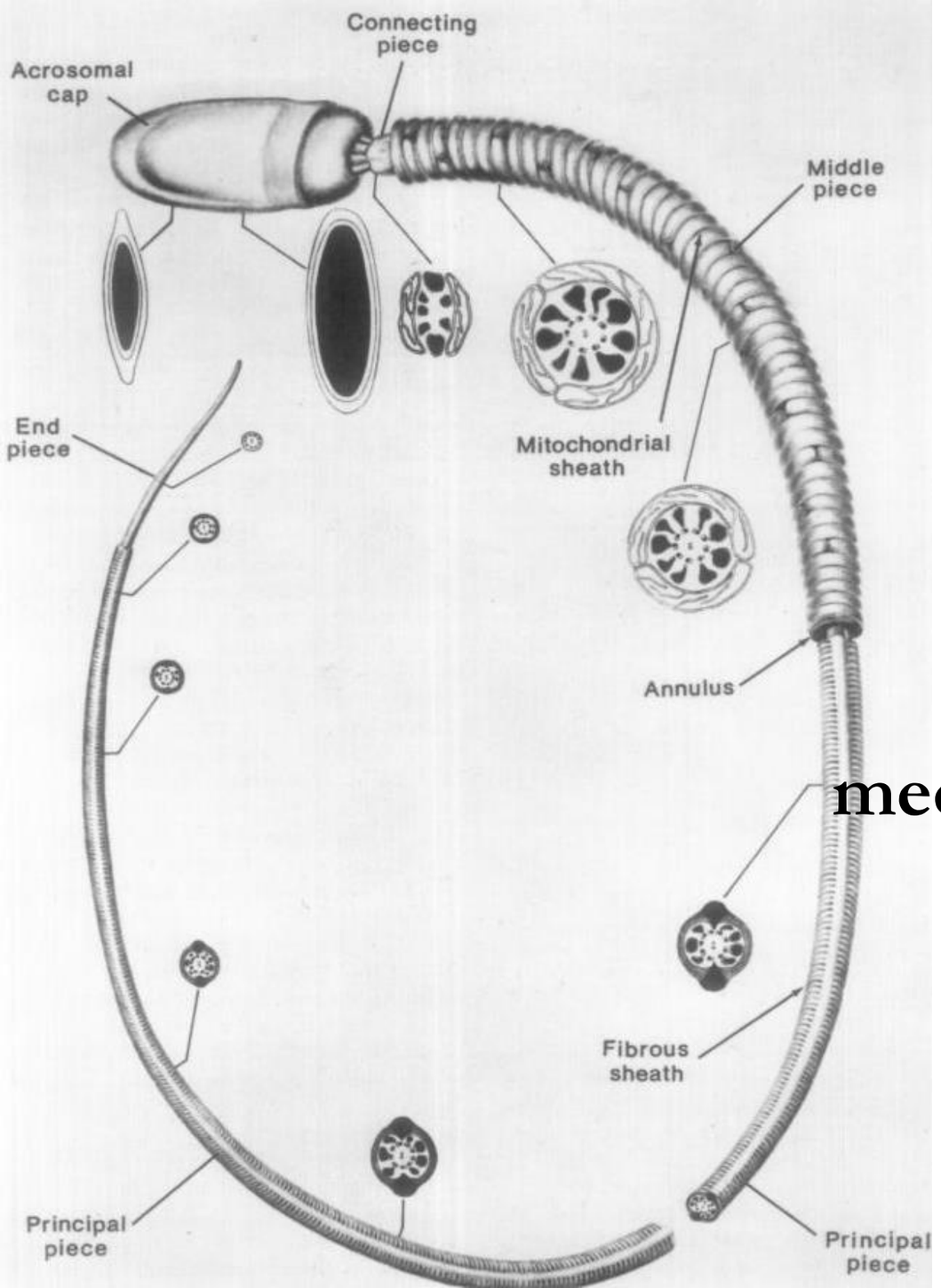


Extenders (7 components)

- Nutrients (Glucose, fructose)
- Cold shoch prevention (Milk, Skim Milk, egg yolk,…)
- Buffer (Citrate, Tris,…)
- Osmotic pressure (the buffer component)
- Inhibit bacterial growth (antibiotics)
- Increase volume
- Cryoprotectant (glycerol, DMSO, …) [cryoconservation]

Addition of cryoprotectant

- They are chemical which prevent cryo-damage
- These are sucrose, alcohols, glycols, some amino acid,DMSO (dimethylsulfoxide)
- Generally two cryoprotectant should be used together instead of single one as they are more effective



Semen

=

Spermatozoa

+

a fluid medium called seminal plasma (SP)

SP is a complex fluid portion and mediates the chemical function of the ejaculate

JUYENA & STELLETTA, 2015

Seminal plasma in ram

- It is an organic fluid that may contain spermatozoa

- Seminal plasma contains serval components that promote the survival of spermatozoa and provide a medium through which the spermatozoa can move or “swim”

Table . *Composition of seminal plasma in ruminants and camelids (values are mg/dL unless otherwise stated)*

Content	Bull[a]	Ram[b]	Goat[b]	Buffalo[c]	Old World Camelids[d]	New World Camelids[e]
Fructose	150–900	150–600	875	368–815	23.5	3–7
Glucose	300	0.9–1.6	4.8–8.8	13–52	29–42	4–8
Citric acid	340–1150	110–260	...	440–444	9.8	3.1–6.0
Total proteins, g/dL	3.8	2.30–2.50	0.77–1.48	...	1.6–2.6	3–4
Total lipids	29	254–396	...	150–175	87	51–115
Phospholipids	149.1	...	57	6.9–59.4	26–48	27–31
Cholesterol	312.16	...	...	117.83	15.3–25.9	0–8
Glutamic acid	1.0–8.0	4.5–5.2	...	4.28	...	...
Na	140–280	120–258	60–183	260–278	...	...
K	80–210	50–140	76–255	192–205	...	...
Ca	35–60	6–15	5–15	30	7.7–8.8	13–31
P	9	4.8–12.0	...	8–9	1.7–4.6	7–17
Cl	110–290	86	82–215	303–347	84–120	263–491
Mg	7–12	2–13	1–4	4.3–5.7	...	2.1–4.85
Zn	2.6–3.7	56–179	...	0.80–1.17	...	...
Testosterone, pg/mL	210–1310	25–375	...	970	...	...
Estrogen, pg/mL	20–166	...	...	43.67	...	...
Prostaglandins, ng/mL	5–10	500–20 000	...	...	...	...
ALP	246 BU/dL	14 895–40 818 mU/mL	...	315 BU/dL	...	50–3143 UI/L
AST	345–623 SFU/mL	190–256 mU/mL	...	166 units/mL	...	...
ALT	15.0–18.3 SFU/mL	39–148 mU/mL	...	34 units/mL	...	0–115 UI/L
LDH	1909 units/mL	968–1697 mU/mL	...	1621 BBU/mL	...	...

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate amino transferase; BBU, Berger-Broida units; BU, Bodansky units; LDH, lactate dehydrogenase; SFU, Sigma Frankel units; UI, international units.

[a] Pineda, 2003; Andrabi, 2009.
[b] Pineda, 2003; Gündoğun, 2006; Andrabi, 2009.
[c] Singh et al, 1969; Chauhan and Srivastava, 1973; Javed et al, 2000; Andrabi, 2009.
[d] El-Manna et al, 1986; Mosaferi et al, 2005.
[e] Garnica et al, 1993; Juyena, 2011.

JUYENA & STELLETTA, 2015

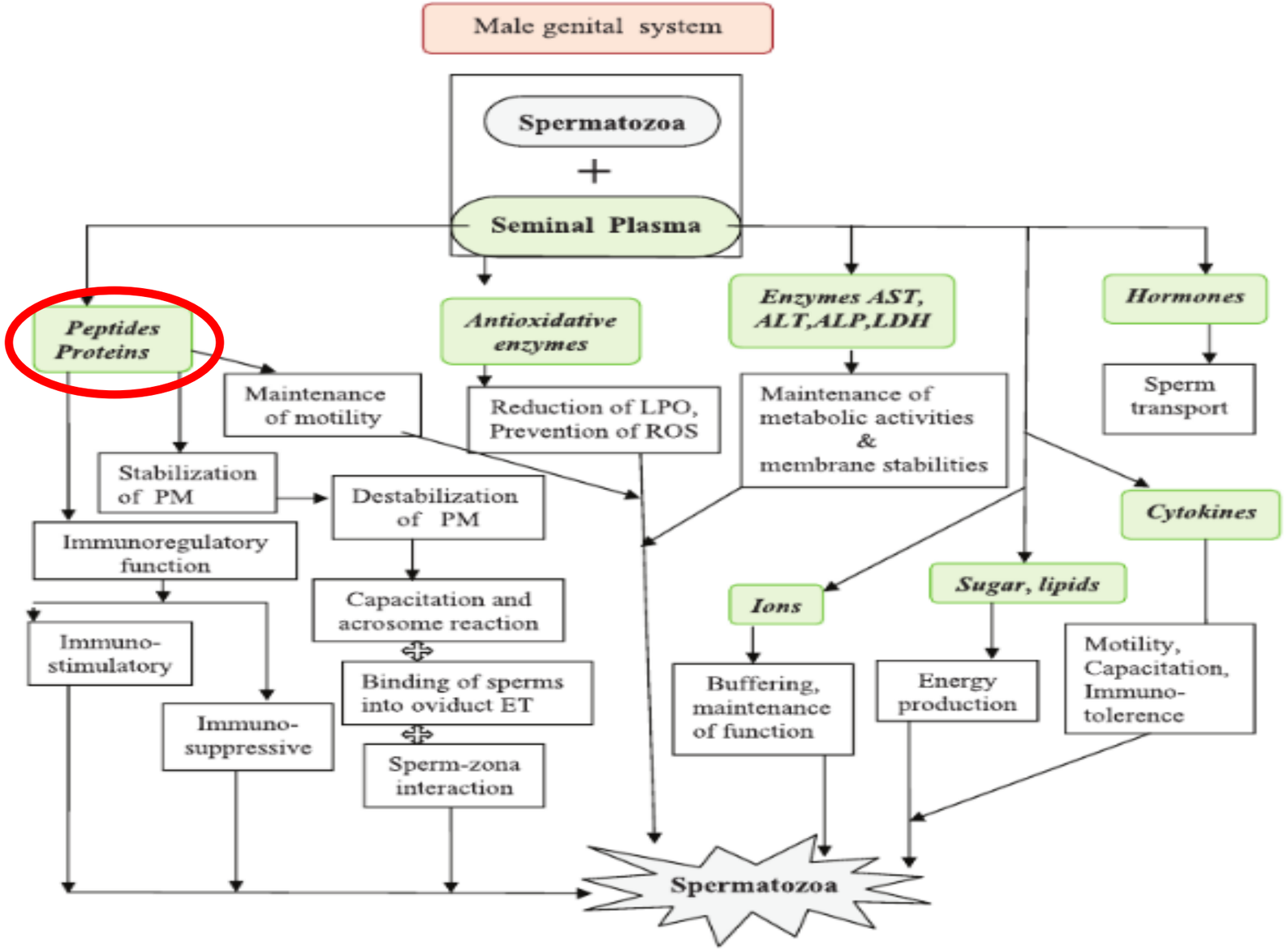


Figure . A model of seminal plasma (SP) structures and functions. The model focuses on the main components of SP and their functions on spermatozoa. PM indicates plasma membrane; AST, aspartate amino transferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; LPO, lipid peroxidation; ROS, reactive oxygen species; ET, epithelium.

JUYENA & STELLETTA, 2015

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Seminal plasma proteins and their relationship with sperm motility
Santa Ines rams

M.A.M. Rodrigues[a], C.E.A. Souza[a], J.A.M. Martins[a], J.P.A. Rego[a], J.T.A. Oliveira[b], G. Domont[c], F.C.S. Nogueira[c], A.A. Moura[a,*]

[a] Department of Animal Science, Federal University of Ceará, 60021-970 Fortaleza, Brazil
[b] Department of Biochemistry, Federal University of Ceará, 60021-970 Fortaleza, Brazil
[c] Institute of Chemistry, Federal University of Rio de Janeiro, 21941-909 Rio de Janeiro, Brazil

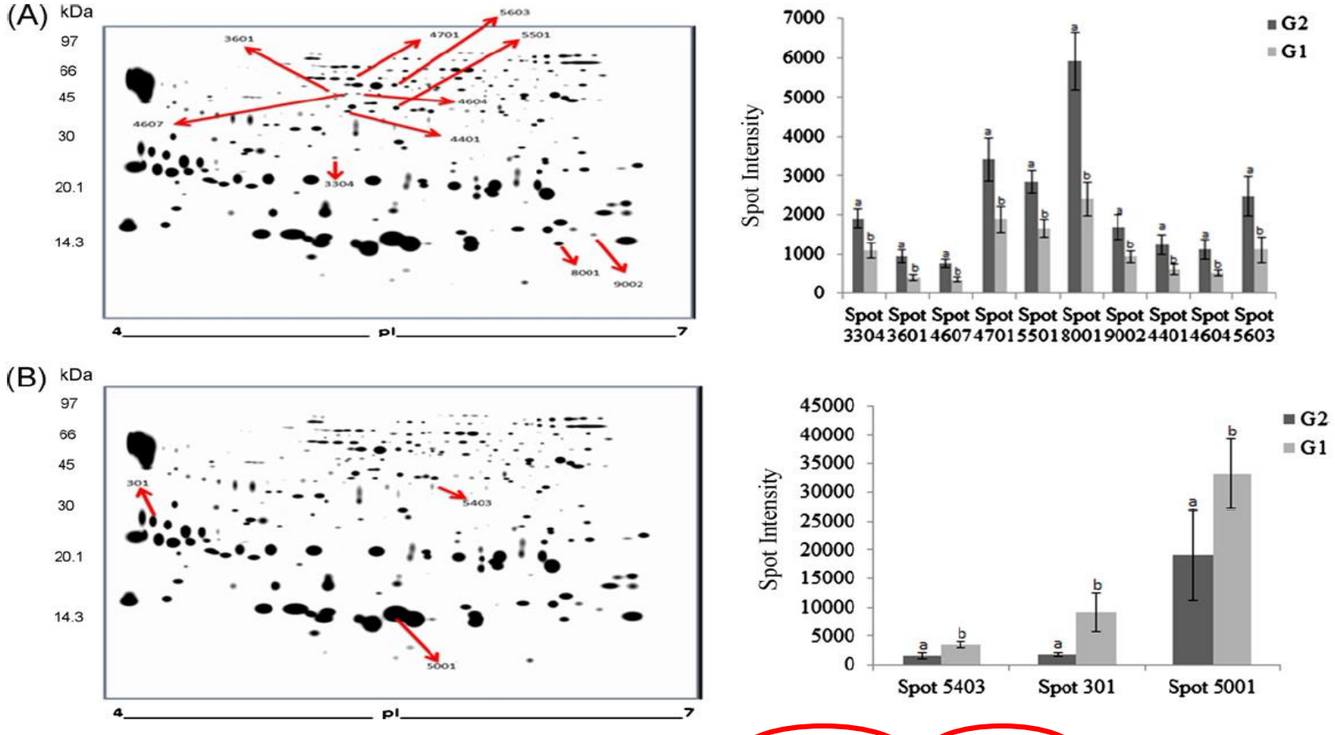
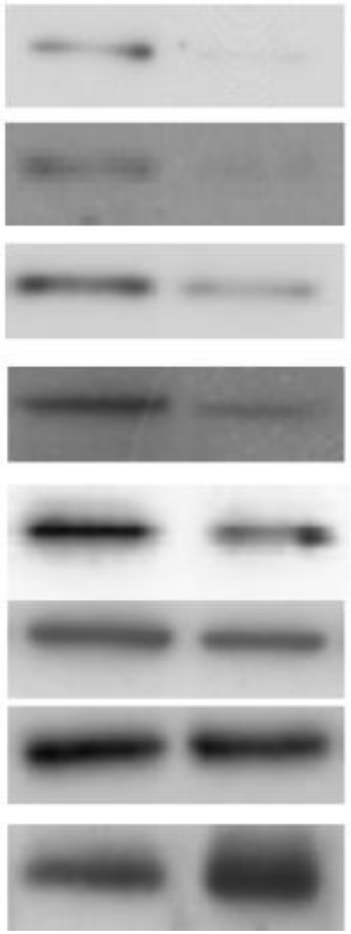


Fig. 2. Ram seminal plasma protein spots differentially expressed in groups of rams with higher (G2) and lower sperm motility (G1). (A and B) Master ge and respective spot intensities of the seminal plasma proteins from Santa Ines rams are differentially expressed in G2 and G1, respectively.

Somme proteins could be markers for male fertility

Seminal plasma and ability of semen to be conserved

Proteins	HPA / LPA
Gelsolin	4.9
TCP1 Zeta (CCT6A)	3.3
Hsp90	2.7
Alpha enolase	2.1
26 S Proteasome	1.9
Valosin Containing Protein	1.7
Glucose 6 Phosphate Isomerase	1.4
Zinc Alpha Glycoprotein	0.4



Ram seminal plasma proteome and its impact on liquid preservation of spermatozoa

C. Soleilhavoup, G. Tsikis, V. Labas, G. Harichaux, P.L. Kohnke, J.L. Dacheux, Y. Guerin, J.L. Gatti, S.P. de Graaf, X. Druart

(Journal of Proteomics, 109 (2014) 245-260)

Semen Quality

1 **Motility**

Sperm motility is a key factor for successful fertilization.

2 **Morphology**

Normal sperm morphology contributes to higher conception rates.

3 **Membrane Integrity**

Intact sperm membranes are essential for fertilizing ability.

4 **Capacitation Status**

Non-capacitated sperm correlate with improved *in vivo* fertility.

Ram Semen Preservation

Cooling

1

Cooling semen to 5°C or 15°C reduces sperm metabolism and extends fertile life, but cold shock can damage cells.

Gradual cooling,
extender composition,
and antioxidant additives like superoxide dismutase can help overcome these effects.

A medium-term refrigeration method allowing 3-5 days distribution would be ideal.

2

Freezing

Freezing methods exist but are restricted to intrauterine insemination due to low fertility with frozen-thawed semen used vaginally.

Cryopreservation damages membranes and reduces motility.

Improving cryoprotectants, extenders, freezing curves, and sperm selection could increase the viable sperm population recovered.

Assessment of Ram Sperm

1 Basic Techniques

Current semen assessment in AI centers uses basic techniques like visual evaluation, subjective motility estimation, and concentration measurement. While practical, these are highly subjective with low correlation to fertility.

2 Automated Analysis

Computer-assisted sperm analysis (CASA) provides objective motility assessment and can identify subpopulations with differing fertilizing ability. Flow cytometry techniques evaluate membrane and acrosome integrity, mitochondrial status, chromatin structure, and other functional parameters.

3 Fertility Biomarkers

Assays detecting uncompensable seminal defects like oxidative stress, DNA damage, and subtle membrane changes could identify subfertile males. In vitro fertility tests directly measuring ability to fertilize oocytes may also prove useful.

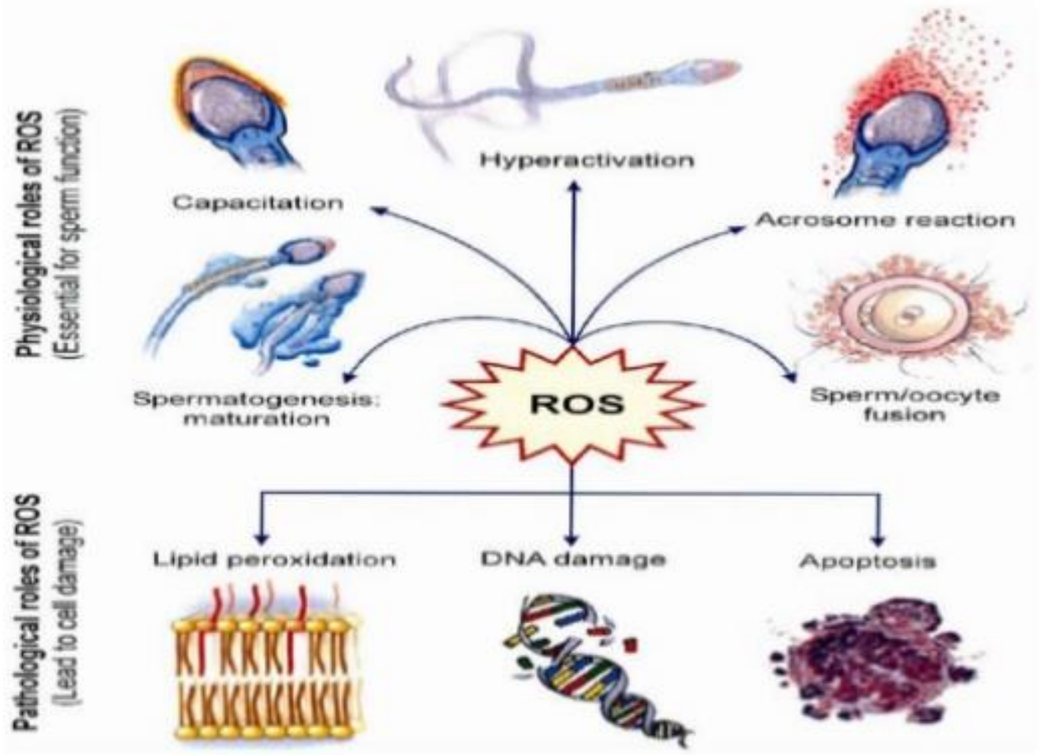
Extended semen and oxydative stress

Manipulated semen (*in vitro)*

- **Spermatozoa are susceptible to ROS (reactive oxygen species) attack**
- **The imbalance between the production of reactive oxygen species (ROS) and detoxification is known as oxidative stress**
- **An imbalance between ROS generation and scavenging activity is detrimental to sperm and associated with male infertility** (Sharma & Agarwal, 1996)

- **Reactive oxygen species are produced in more quantities during cooling**

- **The levels of antioxidant defenses are decreased in spematozoa after a cycle of freezing and thawing (Belodeau et al. 2000)**



(Aitken *et al.*, 2015)

Common source of ROS

- ROS produced intracellalarly, originating from spermatozoa or extracellarly, from environmental factors

- Most common sources of ROS
  Sperm cells themselves (immature.defective/damaged/dead sperm)
  Leucocytes and other cells

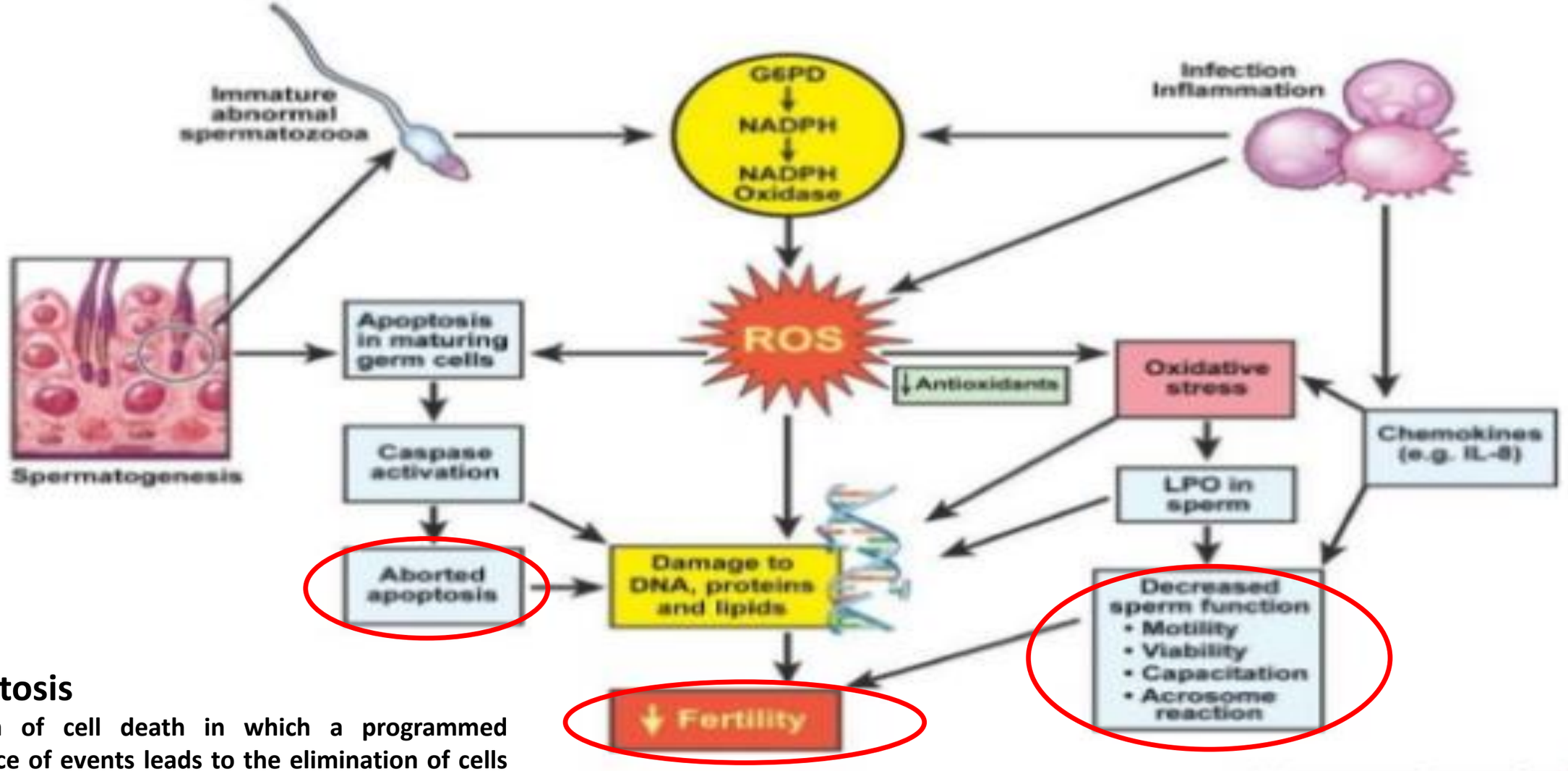
- Semen processing techniques and egg yolk diluted semen
  Dissolved oxygen in extender

Pathogenesis of ROS mediated sperm damage

- Pathogenesis of ROS mediated sperm damage

- Lipid pyroxidation
- Apoptosis and DNA damage
- Motility impairement
- Protein damage

Pathogenesis in general



(Agarwal *et al.*, 2014)

Apoptosis

A form of cell death in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area.

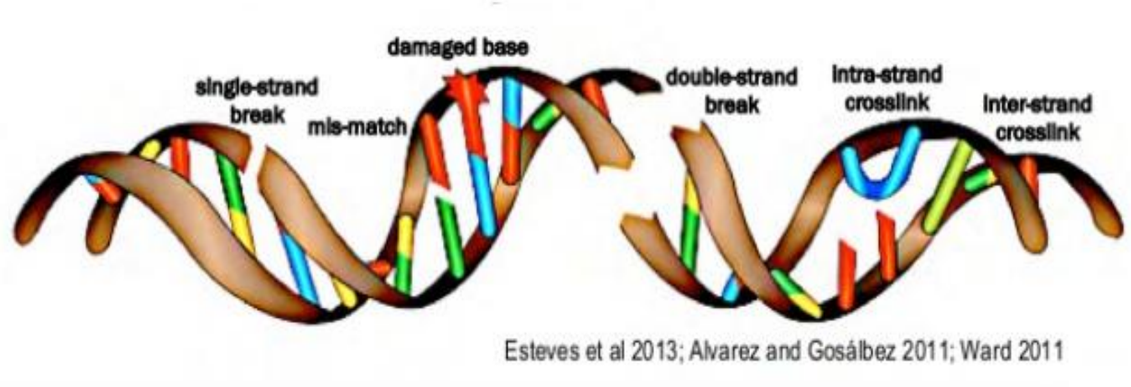
LPO level in fresh and frozen thawed buffalo spermatozoa

	Fresh stage	Frozen- thawed
LPO (nM MDA/10^9)	278.78 ± 18.2	364.67 ± 22.40
		(Kadirvel *et al.*, 2014)
	238.90 ±3.09	478.83 ±3.35
		(Balamurugan, 2015)

Spermatozoa are particularly vulnerable to lipid peroxidation because they contain high concentrations of unsaturated fatty acids (Jones et al, 1979)

What is DNA FRAGMENTATION?

- Separation or breaking of DNA strands into pieces
- Causes of DF: Testicular event or post testicular event
- The DFI tests are effective method for measuring % of sperms with fragmented DNA in the ejaculate

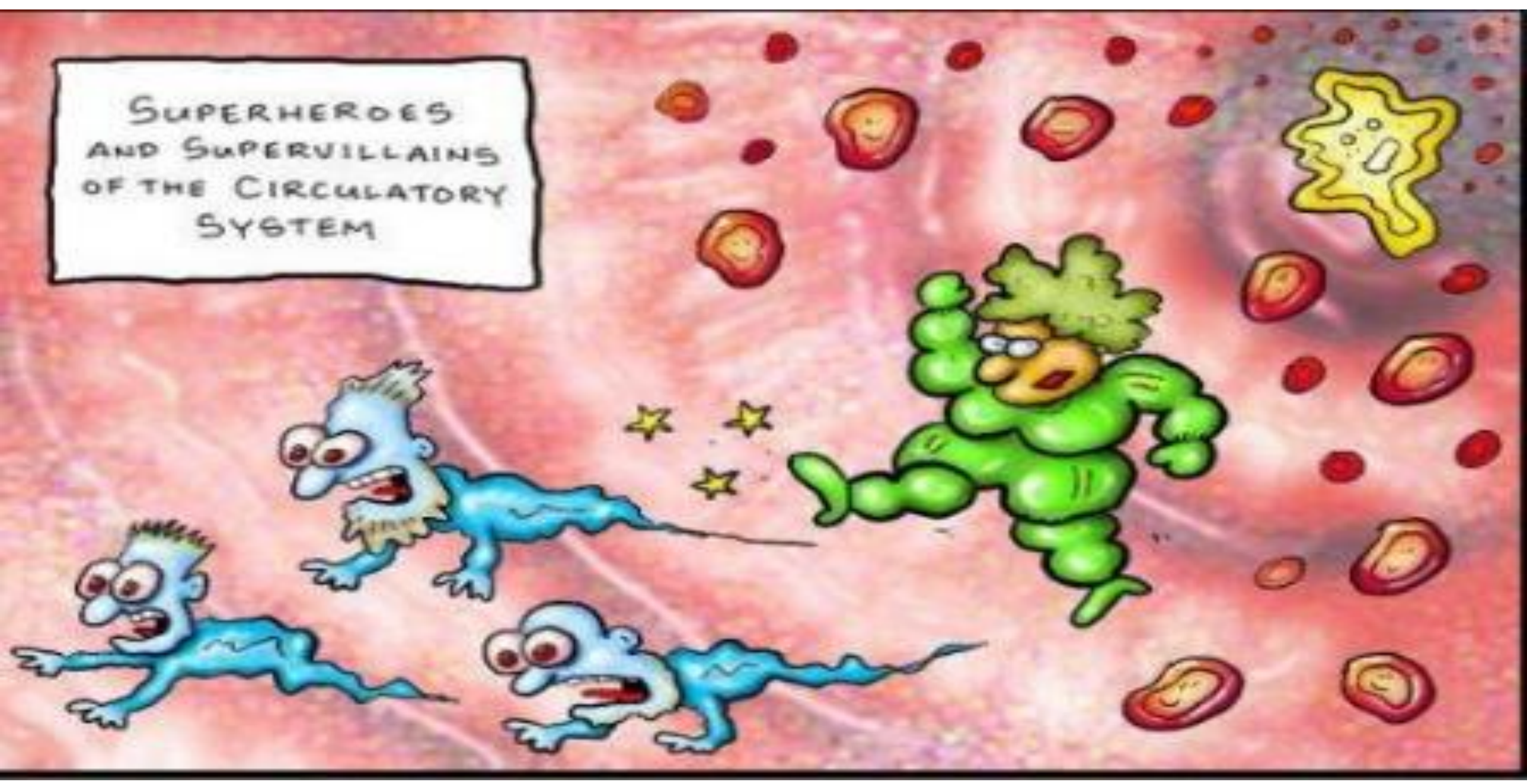


Esteves et al 2013; Alvarez and Gosálbez 2011; Ward 2011

- DNA fragmentation can be:

  Single strand DNA break
- Double strand DNA break

  Base deletion or modifications
- Inter and intra-strand cross linkage



Antioxidant kickout free radicals in semen

- To maintain normal cell function, the excess ROS is continuously inactivated by seminal plasma antioxidants

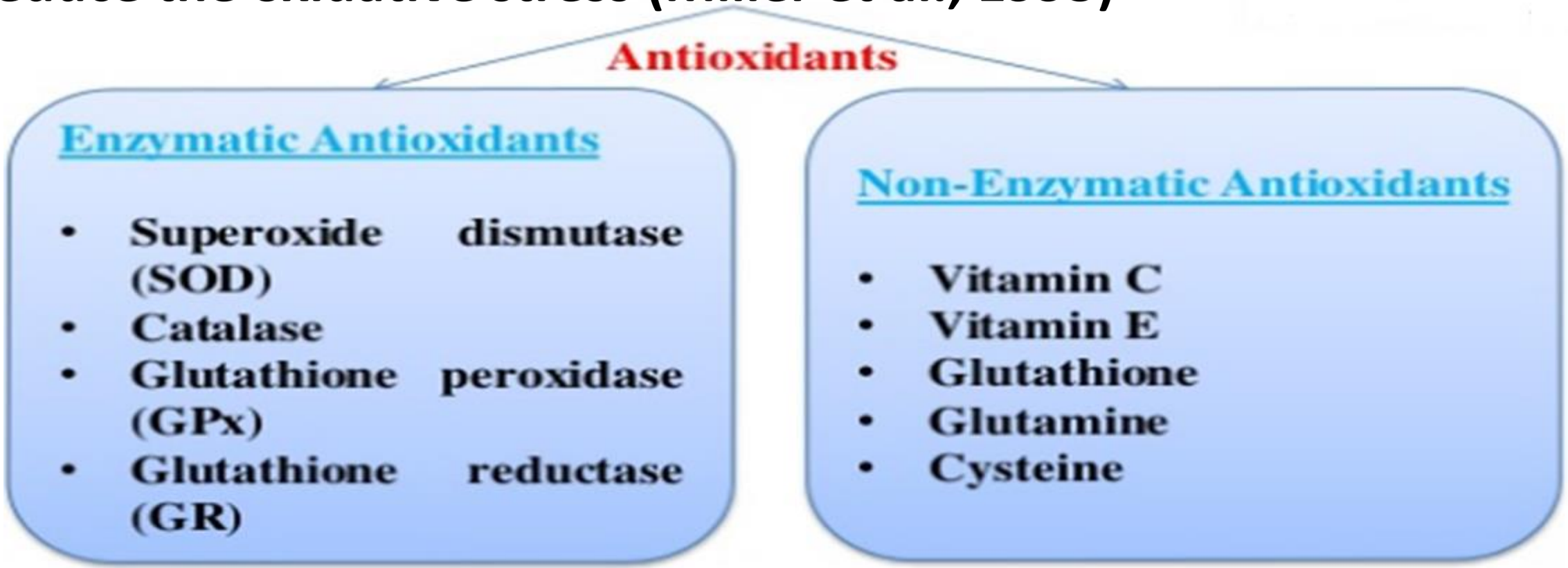
- The seminal plasma antioxidants block the formation of new ROS act as scavengers and remove ROS already generated

- Natural antioxidant enzyme systems include (catalase, Glutathione peroxidase, superoxide dismutase)

Antioxidants

Antioxidants are agents, which break the oxidative chain reaction

It reduce the oxidative stress (Miller et al., 1993)



Catalase

- Catalase @ 5 U/ml and Pyruvate @ 5 mM

Added to EYT-G

- Prevented loss of sperm ATP
- Prevent production of MDA
- Increased activity of superoxide dismutase

(Bilodeau *et al.*, 2002)

α-tocopherol

- ✓ A chain breaking antioxidant
- ✓ It improves metabolic activity and cellular integrity of frozen-thawed semen
- ✓ Breaks the lipidperoxidation chain reaction through its interaction with lipid peroxyl and alkoxyl radicals
- ✓ Improves post-thaw motility, viability along with reduced peroxidative damage.

(Becconi *et al.*, 1993)

Glutathione

- ✓ Maintains the intracellular redox status
- ✓ Prevents leakage of intracellular enzymes and damage of chromatin.
- ✓ Added @ 5mM to the extender
- ✓ Preserve the sulfhydryl groups of protein which play an important role in sperm motility and metabolism

(Linderman *et al.*, 1988)

Phosphodiesterase inhibitors

- Butylated hydroxy toluene (BHT)
  - Minimizes damage to the sperm cell membrane and sperm motility
  - Sustains sperm viability during freezing and thawing
  - Involved in the prevention of auto-oxidation reaction

(Prasjova *et al.*, 2004)

- Pentoxyfylline
  - Reduces superoxide anions responsible for DNA apoptosis
  - Increases intracellular cAMP
  - Boosts sperm motility
  - Decreases lipid peroxidation

(Zhang *et al.*, 2005)

Manganese

Table 3 **Manganese decreases the malondialdehyde production (mean ± SE) during cryopreservation of cattle bull spermatozoa**

Conc of Mn^{++} (μM)	Spermatozoa cooled at 4°C		Frozen-thawed spermatozoa	
	MDA produced μM/10^9 cells	Difference in MDA production (freshly diluted & cooled sperms)	MDA produced μM/10^9 cells	Difference in MDA production (cooled & frozen-thawed sperms)
Control	45.03 ± 3.3[a]	-	-	-
0	73.33 ± 1.6[b]	38.55	122.5 ± 6.9[e]	40.16
100	66.96 ± 2.0[c]	30.82	101.8 ± 1.4[f]	36.05
150	63.43 ± 4.2[c]	29.08	96.8 ± 7.7[g]	34.47
200	56.26 ± 1.6[d]	19.96	75.2 ± 2.2[h]	25.18

Superscripts (a, b, c, d, e, f, g and h) indicate the difference at 5% level of significance within the columns. This table depicts significant antioxidant effect of Mn^{++} in cooled as well as frozen thawed semen samples but to a maximum level on addition of 200 μM of Mn^{++}.

(Cheema *et al.*, 2009)

Ascorbic acid

- Biologically active reducing agent
- Reduces and neutralizes free radicals
- Improves carbohydrate metabolism and electron transport chain, thus the sperm motility
- Added @ 10 Mm in extender prevents lipid peroxidation and helps maintain structural integrity of plasma membrane
- Addition of ascorbic acid resulted in about 18% increase in the post-thaw motility, 11% increase in intact acrosome and prevented leakage of intracellular enzymes (AST, ALT and AKP)

(Srivastava and Kumar, 2014)

Addition of Oviductal protein

Effect of oviductal proteins on lipid peroxidation levels of pre-freeze and post-thaw spermatozoa

Protein[a]	Spermatozoa (nM/10^9)		Seminal plasma (μM/ml)	
	Pre-freeze	Post-thaw	Pre-freeze	Post-thaw
MDA production				
NLOP	505.75 ± 6.71 a	1067.50 ± 15.39 a	6.81 ± 0.04 a	11.82 ± 0.07 a
LOP	569.25 ± 5.71 b	1136.80 ± 25.74 ab	8.71 ± 0.04 b	12.65 ± 0.46 b
Control	675.75 ± 14.35 c	1162.50 ± 32.16 b	9.63 ± 0.19 c	14.00 ± 0.14 c

Data shown all means ($n = 2$) ± S.E. Mean with different letters in a column differ significantly ($P < 0.05$); NLOP: nonluteal oviductal proteins; LOP: luteal oviductal proteins.

[a] At the rate of 1 mg/ml of extended semen.

(Kumaresan *et al.*, 2006)

Addition of Seminal Plasma Heparin Binding Proteins

- HBPs protect sperm from lipid peroxidation during cryopreservation. (Kumar *et al.*, 2008)

Table 5. Effect of affinity purified SP-HBP on MDA production (μM/10^9 spermatozoa, mean ± SE) during pre-freeze and frozen-thaw phase of cryopreservation

Bull number	Pre-freeze		Frozen-thaw	
	Control[1]	Treated[1]	Control	Treated
1	73.5±51.3	51.6±35.0	112.4±38.0	94.9±37.8
2	46.8±19.4	24.9±12.8	83.3±19.4	60.3±15.1
3	22.1±10.3	50.2±33.4	115.2±34.1	98.9±31.6
4	72.9±20.8	32.8±5.4	130.3±47.5	65.6±16.8
5	97.0±24.5	62.3±22.6	132.8±49.6	85.4±31.5
6	51.6±20.7	45.8±20.9	159.7±69.9	132.7±51.1
Combination factor mean	66.7[ac]	44.6 [a]	122.3[bd]	89.6[cd]

SP-HBP, seminal plasma-heparin binding protein; MDA, malondialdehyde; SE, standard error.

[1] Control, without HBP; Treated, supplemented with SP-HBP.

Superscripts a, ac, bd, cd indicates the difference at 5% level of significance within the colums.

(Patel *et al.*, 2015)

Deoxygenation of Extender

- **Levels of enzymatic antioxidants (SOD, GPx, CAT) and TAC in seminal plasma and LPO and ROS in spermatozoa at post-thaw stage of buffalo semen (Mean ± SE, N=30)**

Groups	Dissolved O_2 (ppm)	CAT (U/mg of protein)	SOD (U/mg of protein)	GPx (mmol/min/ml)	TAC (mM)	LPO (nmol/10^8 spermatozoa)	ROS (Units of H_2O_2)
Group I (Control)	8.50±0.07 ^{A}	0.00051±0.030^C	.193±0.005^C	59.22±3.60^C	1.421±0.021^C	478.83±3.35^A	197.16±2.77^A
Group II (LN_2 Flushing)	3.71±0.02 ^{B}	0.0056±0.000^A	.257±0.002^A	69.27±3.38^A	1.667±0.015^A	314.50±6.93^C	123.50±1.461^C
Group III (Mechanical method)	5.34±0.02^C	0.0032±0.000^B	.215±0.006^B	64.23±3.32^B	1.532±0.014^B	364.10±5.77^B	146.66± 1.923^B

Means bearing different superscripts (A, B & C) differ significantly (p<0.001) in column

(Balamurugan, 2015)

Exogenous factors

Heat Stress

Body Temperature

Heat stress elevates body temperature, impacting reproductive functions.

Effects on Ram

It reduces libido, reaction times, and mounts during ejaculation in rams.

Effects on Ewe

It diminishes sexual behaviors, oocyte quality, and embryo production in ewes.

Journal of Thermal Biology 119 (2024) 103794

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Heat stress and ram semen production and preservation: Exploring impacts and effective strategies

Anass Ben Moula [a,*], Zineb Moussafir [d], Naima Hamidallah [c], Bouchra El Amiri [b,e]

Nutritional Stress

Delayed Puberty	Inadequate nutrition can delay the onset of puberty in ewes.
Irregular Cycles	Nutritional stress disrupts normal estrous cycles.
Reduced Conception	Poor body condition decreases conception rates and increases embryo mortality.
Semen Quality	Malnutrition negatively impacts ram semen quality and sexual behavior.

Insemination Techniques

Fresh/Cooled Semen

Non-surgical cervical or vaginal insemination is used with fresh/cooled semen.

Frozen-Thawed Semen

Laparoscopic insemination is preferred for frozen-thawed semen due to cervical complexity.

Timing

Timely insemination on synchronized estrus is critical for optimal results.

II-Challenges in Ovine AI

1

Seasonality

Sheep are seasonal breeders, so AI must account for variations in fertility associated with the breeding season versus artificially induced estrus. Ideally, AI should be possible year-round.

2

Sperm Transport

The complex cervical anatomy in ewes hinders effective sperm transport after vaginal/cervical insemination, reducing fertility especially with frozen-thawed semen which is more fragile.

3

Sperm Survival

Improving sperm survival during liquid cooling and cryopreservation is a key in extending the fertile lifespan and distribution range of insemination doses.

Potential Solutions

Extender Development

Optimizing semen extenders with better cryoprotectants, antioxidants, and membrane stabilizers could improve sperm survival during cooling and freezing.

Sperm Selection

Techniques to select sperm subpopulations with higher DNA integrity, motility, and fertilizing ability prior to cryopreservation may increase post-thaw viability.

Transcervical Catheters

Developing catheters that can gently traverse the ovine cervical rings to deposit semen in the uterus could enable widespread transcervical AI.

Fertility Diagnosis

Implementing advanced semen analysis techniques in AI centers to assess functional sperm parameters could allow better prediction of fertility outcomes.

Factors Influencing Cervical AI Success

1

Insemination Time

Studies have reported that the stimulation time of the speculum in the vagina can produce a release of oxytocin, altering uterine contractility and fertility.

Results suggest that when cervical insemination time is less than 10 seconds, fertility is improved.

2

Milk Production

The productive state of females at the time of insemination can influence fertility.

In the Assaf ewe, it has been observed that in advanced stages of lactation (>500 kg produced), the fertility of cervical insemination is higher, potentially due to a negative correlation between milk production and fertility.

3

Diluent and Storage Time

The diluent, storage time, and temperature, as well as the number of spermatozoa per dose, are important factors that influence fertility in cervical AI.

Extending the storage time to 72 hours at 5°C could be a potential strategy, but further studies are needed to design a valid medium-term preservation method.

Commercial Viability

Technique	Advantages	Disadvantages
Vaginal/Cervical AI	Simple, inexpensive	Low fertility with frozen semen
Laparoscopic AI	High fertility, low sperm numbers	Invasive, expensive, requires skill
Transcervical AI	Non-surgical, intrauterine	Unproven, risk of trauma

For widespread commercial adoption of ovine AI, a non-surgical transcervical method using frozen-thawed semen with acceptable fertility rates is needed.

This would combine the advantages of convenient vaginal insemination and laparoscopic intrauterine deposition while avoiding their drawbacks.

Improvements in semen preservation, cervical catheter design, and functional semen analysis are required to achieve this goal.

III-Artificial Insemination in Moroccan Sheep: Present and Perspectives

Despite the large number and importance of sheep in Morocco, artificial insemination (AI) in this species is not yet an effective tool for ovine breeding development and selection.

Early Trials and Field Studies

1987-1988: Initial Experiments

1

The first trials in Morocco started in 1987 and examined factors like breed differences, synchronization protocols, and PMSG doses on AI outcomes.
However, the overall fertility results were low, not exceeding 30%.

1990s: Field Trials and Exports

2

Two AI centers were established, and trials were conducted exporting frozen D'man semen to Egypt and Iraq. Fertility rates were modest, around 30-60% depending on the location and breed.

1992-1994: French Breed Program

3

A Moroccan-French program used laparoscopic AI to inseminate 2401 ewes with French breeds like Ile de France and Lacaune. Fertility rates ranged from 40-70% across the different breeds.

1995: Industrial Crossbreeding

4

A large field trial compared AI to natural mating for industrial crossbreeding of Sardi, Timahdit, and Boujaâd ewes with Ile de France, Merinos, and Lacaune rams. AI fertility was 42-90% depending on the breed combination.

Recent Research on sheep Moroccan Breeds

1 ## Seasonal Patterns

Studies found the testicular size and semen quality of Boujaâd rams peaked in May-July and were lowest in November-December, following a seasonal pattern.

2 ## Semen Preservation

Researchers assessed different extenders, storage temperatures, antioxidant supplements, and fresh vs frozen semen conservation methods for Boujaâd rams.

3 ## Reproductive Physiology

Investigations were made into the anatomy of the cervix in Boujaâd and D'man ewes, as well as characterizing the preovulatory LH surge patterns after synchronization.

4 ## Synchronization and AI Trials

Fertility after AI with fresh semen ranged from 45-55%, and was slightly higher using 300 IU vs 400 IU PMSG for synchronization in natural mating.

Animal Reproduction Science 192 (2018) 6–17

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Review article

Supplementation of ram semen extender to improve seminal quality and fertility rate

Larbi Allai[a,b], Anass Benmoula[a], Marciane da Silva Maia[c], Boubker Nasser[b], Bouchra El Amiri[a,*]

Table 1
Summary of noteworthy results on fertility of ram semen after insemination.

Supplement	Type of insemination	Conservation Type	Extender	Controls (%)	Improved fertility (%)	Reference
GSH	Cervical	Fresh	EquiPro® (Minitüb)	76	81	Kubovičová et al., 2010
(SOD) and (CAT)	Intra-uterine	Fresh	Tris-glucose-yolk diluents	16	41	Maxwell and Stojanov, 1996
(GSH)	Cervical	Fresh	Tris-citrate-fructose	7	37	Mata-Campuzano et al., 2014
TEMPOL 24 h of storage	Cervical	Fresh	Sodium citrate	19	67	Mara et al., 2005
TEMPOL 72 h of storage	Cervical	Fresh	Sodium citrate	0	52	Mara et al., 2005
Seminal plasma	Cervical	Fresh	Skim milk	5	86	Belibasak et al., 2000
Trehalose	Cervical	Frozen	Tris-fructose-egg yolk	18	47	Aisen et al., 2002
0.30 g fish oil	Intra-cervical	Frozen	Tris-citrate-fructose	18	47	Abdi-Benemar et al., 2015

EquiPro® (Minitüb) is an Equine Semen Extender that is composed of a blend of glucose, sucrose, non-fat dry milk, and antioxidants; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione or reduced glutathione; TEMPOL, (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl or 4-hydroxy TEMPO) is a stable nitroxyl antioxidant that has been used as a protectant in disorders that involve reactive oxygen species (ROS).

Effect of argan oil on liquid storage of ram semen in Tris or skim milk based extenders☆

Larbi Allai[a,b], Xavier Druart[c], Jesus Contell[d], Noureddine Louanjli[e], Anass Ben Moula[a], Abdelmoughit Badi[a,b], Abdelkhalid Essamadi[b], Boubker Nasser[b], Bouchra El Amiri[a,*]

[a] *INRA-Centre Régional de la Recherche Agronomique de Settat, BP589 Settat, Morocco*
[b] *Laboratoire de Biochimie et Neurosciences, Faculté des Sciences et Techniques, Université Hassan 1, BP 577, 26000 Settat, Morocco*
[c] *INRA, UMR 85 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France*
[d] *Recherche/Development Department, PROISER S.L. Catedrático Agustín Escardino, 9, Building 3, 1st Floor, 46980 Paterna, Valencia, Spain*
[e] *Biologie Médicale et Biologie de la Reproduction, LABOMAC, Casablanca, Maroc, France*

In conclusion,

Argan oil is able to be used safely in liquid storage of ram semen.

The use of some concentrations of argan oil may last storage period of ram semen related with extender and storage temperature.

Animal Reproduction Science 197 (2018) 40–47

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Does advanced age affect reproductive variables, semen composition, and liquid semen storage during different seasons in Boujaâd rams?☆

Abdelmoughit Badi[a,b], Anass Benmoula[a], Kaoutar El Khalil[a,b], Larbi Allai[a,b], Abdelkhalid Essamadi[b], Boubker Nasser[b], Bouchra El Amiri[a,*]

The results of the present study indicate that in Boujaâd Moroccan sheep, the SC, semen composition, semen
quality, and liquid storage (15 °C) were affected by age and seasonal variations.
Autumn and summer were shown to be the most favorable seasons for sperm production, particularly for the younger rams.
Advanced age negatively affects all studied variables during different seasons of the year.

Even though there are seasonal and age effects, Boujaâd ram semen can be stored at 15 °C for artificial insemination purposes throughout the year. The results of the present study also indicate there is a strong correlation
between semen composition (lipids, proteins and cholesterol) and sperm motility of stored ram semen in the Boujaâd breed.

Effect of extender and storage temperature on sperm motility parameters of liquid ram semen

A. BENMOULA[1,2], L. ALLAI[1], A. BADI[1], K. EL KHALIL[1], B. EL AMIRI[1]

(Reçu le 13/04/2017; Accepté le 10/10/2017)

Skim milk, Duragen®, and INRA96® gave the best motility results for INRA180 ram semen stored at 15°C.

However, for Boujaâd ram semen, Ovipro®, Triladyl®, Duragen®, and SM were the best extenders for maintaining motility parameters.

For storage at 5°C, SM, INRA96®, Duragen®, Andromed®, and Ovipro® are recommended for INRA180 ram semen.
While for Boujaâd ram semen, results were better in SM, TEY, Duragen®, Ovipro®, INRA96®, Triladyl®.

Animal Reproduction Science 180 (2017) 17–22

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Effect of season on scrotal circumference, semen characteristics, seminal plasma composition and spermatozoa motility during liquid storage in INRA180 rams☆

Anass Benmoula[a,b], Abdelmoughit Badi[a], Moussa El Fadili[c], Kaoutar EL Khalil[a], Larbi Allai[a], Abderaouf El Hilali[b], Bouchra El Amiri[a,*]

The present study suggest that rams do not show a reproductive seasonality (SC, sperm quality and total proteins of seminal plasma) which implies that this breed may have inherited the no seasonality character from its origin (D'Man breed).

Total lipid and cholesterol concentrations increased in winter and summer. The stored semen in SM at 15 ◦C from 0 h to 24 h throughout the twelve months showed a preference for winter and summer regarding the motility parameters.

Animal Reproduction Science 175 (2016) 1–9

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Protective effects of *Opuntia ficus-indica* extract on ram sperm quality, lipid peroxidation and DNA fragmentation during liquid storage

Larbi Allai[a,b], Xavier Druart[c], Mehmet Öztürk[d], Anass BenMoula[a], Boubker Nasser[b], Bouchra El Amiri[a,*]

- The ACTEX exacted from a natural plant has significant antioxidative effects on ram semen during liquid storage at 5 °C.
- The 1% concentration of ACTEX (1%) can improve sperm quality variables such as TM, PM, VIAB, HOST, ABN, oxidation and DNA fragmentation.
- However, greater concentrations of ACTEX cannot efficiently protect ram sperm preserved at 5 °C. In the future, more investigations are needed to identify the major chemicals compound present in ACTEX.

Theriogenology 215 (2024) 234–240

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Original Research Article

Effect of C-phycocyanin purified from *Spirulina platensis* on cooled ram semen quality and *in vivo* fertility

Abdellatif Rahim [a,b], Saad Salhi [a,b], Nora El Khelfaoui [a,c], Bouabid Badaoui [d,e], Abdelkhalid Essamadi [b], Bouchra El Amiri [a,e,*,1]

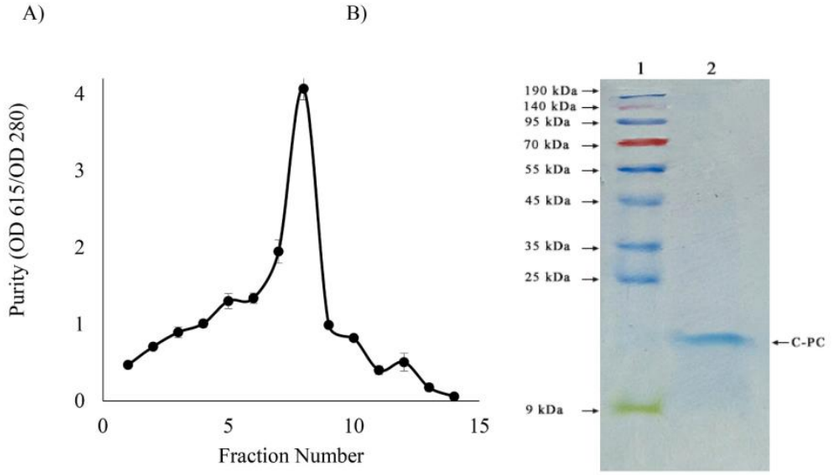


Fig. 1. Elution profile of purified C-PC on DEAE–Sepharose CL-6B ion exchange chromatography (A). SDS-PAGE of C-PC eluted from DEAE–Sepharose CL-6B column (B). Lane 1: Molecular weight marker; Lane 2: Fraction corresponding to purified C-PC.

2.4 µg/mL of purified C-PC improved the quality of ram semen cooled at 5 °C and increased its *in vivo* fertilization ability.

The C-PC purified from *Spirulina platensis* could be considered as a natural alternative to the most used additives for sperm preservation.

Animal Reproduction Science 196 (2018) 43–47

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Morphometry and depth of inseminating catheter penetration in prolific and non- prolific ewes at different ages: A post mortem study

Kaoutar EL khalil[a,b], Larbi Allai[a], Alice Fatet[c], Anass Benmoula[a], Naima Hamidallah[b], Abdelmoughit Badi[a], Zineb Moussafir[a], Mustapha Ibnelbachyr[d], Bouchra El Amiri[a,*]

The anatomical features of the cervical canal of Boujaâd ewes differ from those reported for D'man ewes.

The present study revealed that the cervices of Boujaâd ewes are more complicated than those of D'man ewes.

In both D'man and Boujaâd breeds, the cervix becomes less complicated with progressing age (6–8 T).

Journal of Thermal Biology 119 (2024) 103794

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Heat stress and ram semen production and preservation: Exploring impacts and effective strategies

Anass Ben Moula [a,*], Zineb Moussafir [d], Naima Hamidallah [c], Bouchra El Amiri [b,e]

Chapter 15
Effects of Heat Stress and Chemical Pollutants on Sheep Reproduction and Strategies to Mitigate Them

Abdellatif Rahim and Bouchra El Amiri

ANOC Conservation Programs

Hamra/Beni Guil Sheep

ANOC and the Algerian sheep breeding institute ITELV are collaborating on a FAO-funded to develop and preserve the Hamra/Beni Guil sheep breed. Selected Moroccan rams will have semen collected at the Ain Jemaa AI center, while Algerian ewes will be inseminated by laparoscopy.

New AI Center Plans

In addition to renewing the existing Ain Jemaa center, ANOC plans to create a new AI center in Morocco as part of their animal genetic resources conservation strategy.

Private Sector Involvement

1

New Private AI Center

For the first time, a private AI center has been founded in Morocco with ambitious programs planned, though currently it is focused more on goat projects.

2

Capacity Building

A major activity of the new private center is building technical capacity through training personnel in AI procedures and techniques.

3

Future Sheep Projects

Future sheep AI projects will target inseminating 2000 Boujaâd ewes, with plans to later expand to the Sardi breed as well.

Take-home messages

Sheep Artificial Insemination is a very complex equation.

Different factors can impact the success of artificial insemination.

Gatering sucessful pecies that are involved in the above mentonned complex equation can enhance the fertility rates

Acknowledgments

The authors express gratitude to Pr. Derqaoui Lahcen, Mr El Benani Mohamed from IAV-Hassan II, Dr. Manar Samira, Mr Choukri from ANOC, Mr Allai Larbi, Mr Lakrad Aberahim, and Mr. Meftah Mohamed for sharing valuable information and technical assistance. They also thank the financial support from PRAD02/2008 and PRAD 04/2012 projects.

Academic Partners

IAV Hassan II, INRA Nouzilly,….

Industry Partners

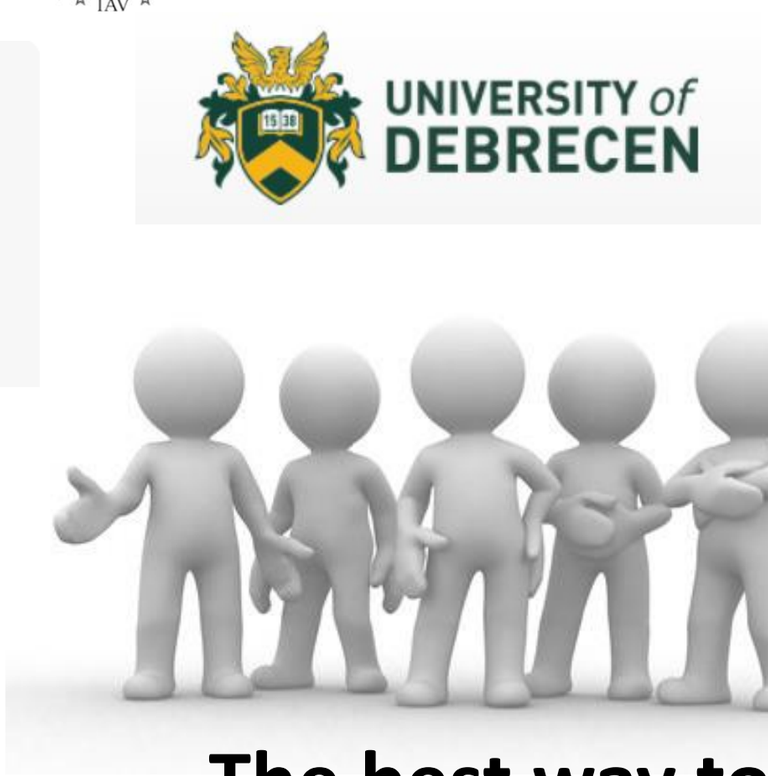
ANOC, Sheep Breeders

Funding Sources

PRAD02/2008, PRAD04/2012, ARES-Belgium


ELGA
ELGA LabWater
le spécialiste de l'eau de laboratoire
RIDERS ON THE STORM
Intel Concept

THANK YOU FOR YOUR ATTENTION



The best way to succeed the ART is a good connexion of people