*ADVANCES IN BIORESEARCH, Vol 1 [1] JUNE 2010: 144 - 148*

**Society of Education, India**
**http://www.soeagra.com**
**ISSN 0976-4585**

**RESEARCH ARTICLE**

# Physiological Aspects of Arabian Sand Gazelle Semen and its Cryopreservation

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**ABSTRACT**

*The distribution of Arabian Sand Gazelles (Gazella subgutturosa marica) has declined dramatically during recent decades, apparently due to excessive hunting and habitat degradation. The Saudi Wildlife Commission has a research centers for some endangered species, one of them is the sand gazelle. Also the activities includes captive breeding, habitat protection, and reintroduction. There are no studies of the semen characteristics and its cryopresercation and artificial insemination of the Arabian Sand Gazelle, so this study was focused to described semen characteristics and Cryopreservation by using Triladyl and Tris Diluents as extender for semen cryopreservation of the Arabian Sand Gazelle Males. Information on the Physiological Aspects of The Arabian Sand Gazelle semen and its cryopresercation is very limited in this respect and references on other species will be used where available.*

***Key words**: Sand Gazelle, (Gazella subgutturosa marica), Semen, Cryopreservation, Triladyl, Tris Diluent, Glycerol*

## INTRODUCTION

The Saudi Wildlife Commission in Saudi Arabia make huge efforts in keeping and breeding endangered species, with a view to preserve them from extinction and ideally to reintroduce them back in their natural habitats.

The distribution of sand gazelles (*Gazella subgutturosa marica)* has declined dramatically during recent decades, apparently due to excessive hunting and habitat degradation. Today, sand gazelles survive only in small numbers in a few isolated parts of their former range. The Saudi Wildlife Commission has a research centers for some endangered species, one of them is the sand gazelles. Also the activities includes captive breeding, habitat protection, and reintroduction [14].

Little is known about the reproduction of large mammals inhabiting arid environments, and less is known about the mammals of the Arabian Peninsula, especially the Arabian sand gazelles [1,2,3,4,5].

There are no studies of the semen characteristics, its cryopresercation, and artificial insemination of the Arabian Sand Gazelle except that published by [2,3,4]. That study was focused to described Semen characteristics and Cryopreservation by using Triladyl and Tris Diluents as extender for semen cryopreservation of the Arabian sand Gazelle Males.

The order Artiodactyla, the even-toed ungulates, is comprised of the major families *Bovidae* (antelope, cattle, bison, buffalo, sheep, and goats), *Cervidae* (deer), *Suidae* (pigs), *Tayassuidae* (peccaries), *Hippopotamidae* (hippopotamuses), *Giraffidae* (giraffe and okapi), *Camelidae*, and the smaller families of *Antilocapridae* (pronghorn antelope), and *Tragulidae* (chevrotains). According to the 2007 IUCN Red List, of 214 species of Artiodactyla, described, two are listed as extinct in the wild, 14 are critically endangered, 26 are endangered, and 35 are considered vulnerable [3,4]. For the purpose of this review, the term non-domestic bovids refers to members of the *Bovidae* family, but excludes those that can be considered domestic livestock such as cattle, buffalo, bison, sheep, and goats [19].

Semen storage technology was revolutionized approximately 50 years ago by the discovery that glycerol could act as a cryoprotectant. The discovery of glycerol as a cryoprotectant marked a quantum advance in semen cryopreservation, but subsequent research has only made relatively small improvements to the basic techniques established in the early 1950s.

Glycerol concentrations of less than 2% were necessary to achieve good fertility also after freezing of boar semen, although maximum sperm motility was seen at a concentration of 7% glycerol [8, 10, 15, 18].

In 1957, Canadians Barker and Gandier reported the first foaling following insemination with frozen epididymal spermatozoa. During the next decade a dozen more successful reports with ejaculated semen followed from Germany, Japan, Russia, and the U.S. Sugar-containing diluents were most commonly used. A study by Garde, J., *at al*., [12] on Sperm Cryopreservation in Three Species of Endangered Gazelles (*Gazella cuvieri, G. dama mhorr, and G. dorcas neglecta*) were used five diluents to compared cryopreservation for these three species by using Glycerol concentration between 6- 8 %.

This quantitative difference between species is an important determinant of the fertility of cryopreserved semen and means that development of successful freezing procedures necessarily involves more than the identification or application of novel cryoprotectants and additives [12].

Information on the Physiological Aspects of The Arabian Sand Gazelle semen and its cryopreservation is very limited in this respect and references on other species will be used where available .

## PRINCIPLES OF CRYOPRESERVATION

### Freezing rate

During the cryopreservation process, cells are exposed to cryoprotective agents such as glycerol, ethylene glycol or dimethylsulfoxide in an extender. The addition of 'cryoprotectant' agents exposes the cells to a hypertonic environment, this causes the cells to initially shrink, but they regain their normal volume as the cryoprotectant enters the cells. Cooling also changes the cell membrane. During freezing, cells again shrink as water flows out of the cell and ice forms in the extracellular space. Depending upon the cooling rate, ice may form inside the cell as well [22].

Formation of intracellular ice usually results in damage to the cell membrane, it loses its semi-permeable properties, resulting in death of the cell. During thawing, the cells undergo similar changes in volume as water moves back into the cell. The thaw rate and cell survival are dependent upon the cooling rate used. As stated by a researcher considering the difficulties in semen cryopreservation of other species compared to the bull, it causes one tomarvel at successful survival rather than to despair over the difficulties of its implementation to the preservation of a variety of other sperm cell types [9, 15, 17].

### Supercooling and seeding

The temperature of the semen will invariably cool below the freezing point before freezing occurs (super cooling). A high degree of super cooling is generally considered unwanted because it will cause a rapid upward and downward temperature fluctuation. Seeding at a minimum temperature below the freezing point will initiate ice crystal formation and avoid the temperature drop. Seeding may be initiated by vibration or touching of the straw with an instrument colder than the package as is done in embryo freezing. Seeding will otherwise be initiated in the straw where it touches the freezing rack. Most heat is conducted away from the straw through the metal rack if the rack is in contact with the liquid nitrogen and therefore colder than the vapor. Freezing racks offering multiple contact points have been used for maxi straws (2.55 ml) but there is no data indicating an advantage for such racks.

### Cryoprotectants

Life is a complex chemical process that happens in water. Without liquid water, there is no life, or at least no life process. Cryoprotectants are chemicals that protect living things from being injured by water freezing during exposure to cold [6].

Water expands when it freezes, but contrary to popular belief it is not expansion of water that causes injury. It is the purification of water during freezing that causes injury. Water freezes as a pure substance that excludes all else. It is this exclusion process that causes injury. Instead of remaining a solvent that allows the molecules of life to freely mix within it, water that freezes gathers itself up into crystals pushing everything else out. This is illustrated in Figure 1. (a, b).

Freezing causes damage by two distinct mechanisms. The first is mechanical damage as the shape of cells is distorted by ice crystals. The second is damage caused by chemical and osmotic effects of concentrated solutes in the residual unfrozen water between ice crystals. This is so-called “solution effects” injury [6, 7, 21, 23].

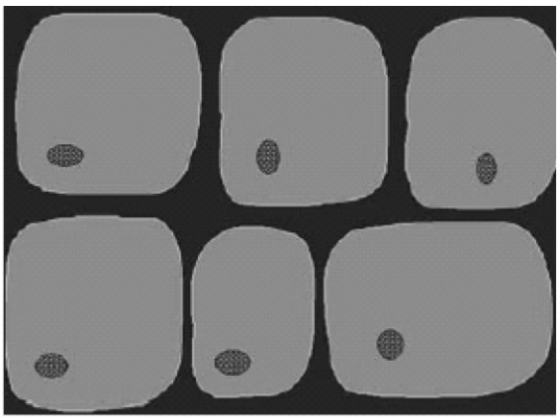

**Figure 1a. Cells before freezing**

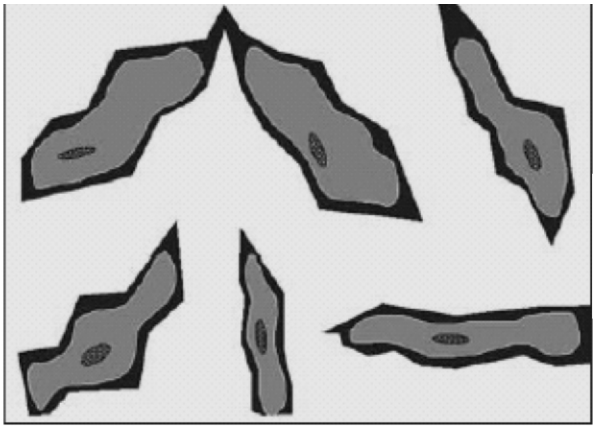

**Figure 1b. Cells after freezing.**

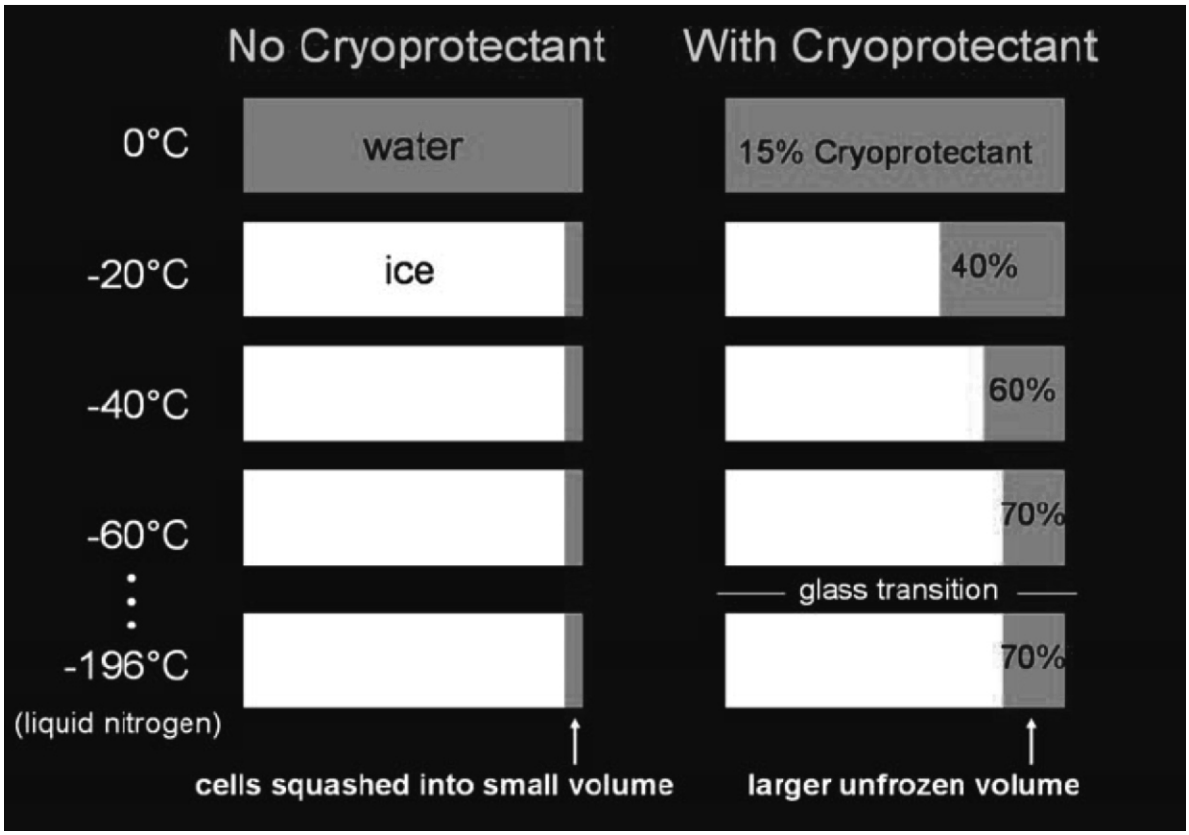


***Figure 2. Water when frozen without and with added cryoprotectant.***
Without cryoprotectant, almost the entire water volume freezes during cooling. Only salts and other dissolved molecules prevent water from freezing completely. With cryoprotectant, the percentage of cryoprotectant present in solution increases as ice grows. At any given temperature, ice growth stops when the cryoprotectant becomes concentrated enough to make the melting point equal to the surrounding temperature. Eventually the cryoprotectant reaches a concentration that cannot be frozen. No more ice can grow as the temperature is lowered, and there is more room for cells to survive between ice crystals. Below approximately -100ºC, the remaining unfrozen liquid pocket solidifies into a glass, permitting storage for practically unlimited periods of time. Cells survive freezing by existing inside the glassy solid between ice crystals. The larger the starting cryoprotectant concentration, the larger the unfrozen volume will be at the end of freezing.

On the other hand many of The Cryoprotectants used for semen cryopreservation of gazelles (like Gazella gazella

and Gazella dorcas) and one subspecies (Gazella gazelle acaiae) as mentioned by Joseph Saragusty, *et al*, also Cuvier gazelle (*Gazella cuvieri*) by Garde, J., *et al* in 2003 and 2005, and sand *gazelles* (*Gazella subgutturosa marica*) as mentioned by (Al-eissa, M.S., *et a,al, b,c)* [16], [12, 13], [3,4].

**How Cryoprotectants Protect Cells**

Cryoprotectants are chemicals that dissolve in water and lower the melting point of water. For applications outside cryobiology, such chemicals are sometimes called "antifreeze". Common examples are glycerol, ethylene glycol, propylene glycol, and dimethylsulfoxide (DMSO). A cryoprotectant concentration of about 5% to 15% is usually all that is required to permit survival of a substantial fraction of cells after freezing and thawing from liquid nitrogen temperature.

Figure 2. shows the essential concept of cryoprotection during cell freezing. Growing ice compacts cells into smaller and smaller pockets of unfrozen liquid as the temperature is lowered. The presence of cryoprotectants makes these pockets larger at any given temperature than they would be if no cryoprotectant were present. Larger unfrozen pockets for cells reduces damage from both forms of freezing injury, mechanical damage from ice and excessive concentration of salt [6].

**Diluents and Dilution**

Freezing of undiluted semen with some success is possible only with human semen [24]. In early studies with bull semen, the expression "glycerol equilibration time" was coined [20]. It is possible that the long time necessary between dilution and freezing in bull semen had very little to do with glycerol penetration of the spermatozoa. Also, the dilution effect may represent instability in the phospholipid bilayer of the sperm membrane, possibly caused by movement of the surface proteins [10, 11].

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