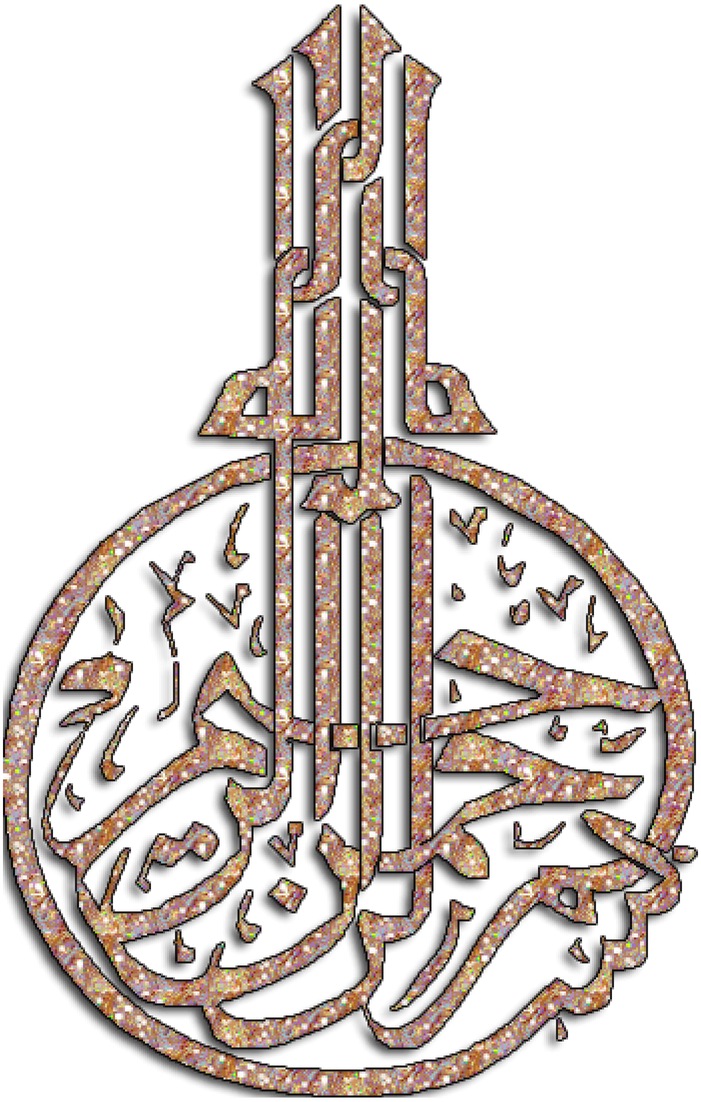

(وما أوتيتم من العلم إلا قليلاً)

صدق الله العظيم

سورة الإسراء ... الآية (٨٥)

Factors affecting cryopreservation of buffalo semen

A Thesis presented to the
Faculty of Veterinary Medicine; Alexandria University
In Partial fulfillment of the
Requirements for the Degree

Of
Ph . D. in veterinary sciences

In
Theriogenology

By

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تأثير بعض الإضافات على تجميد السائل المنوي الجاموسي

رسالة علمية

مقدمة إلى الدراسات العليا بكلية الطب البيطري – جامعة الأسكندرية

استيفاء للدراسات المقررة للحصول على درجة
دكتور الفلسفة في العلوم الطبية البيطرية

في

تخصص الولادة وأمراضها والتلقيح الاصطناعي

مقدمة من

ط.ب / زاهر محمد إبراهيم رواش

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List of abbreviations

8-OHdG	8-hydroxy-2-deoxyguanosine
(-S11)	Sulfydryl group
AB	Aniline blue
AI	Acrosomal Index
AO	Acridine orange
ART	Assisted reproduction technology
ASMA	Automated sperm morphology analyzer
ATP	Adenosine triphosphate
BC	Before Christ
CASA	Computer-aided sperm analysis
CMA3	Chromomycin A3
CV	Coefficients of variation
DBD-FISH assay	DNA breakage detection-fluorescent in situ hybridization assay
DF	Degree of freedom
DNA	Deoxyribonucleic acid
dsDNA	Double standed DNA
dUTP	Biotinylated deoxyuridine teiphosphate
FISH analysis	Fluorescence in situ hybridization analysis
FP	Forward progression
HPLC	High-performance liquid chromatography
ICSI	Intracytoplasmic sperm injection
IUI	Interauterine insemination
IVF	In-vitro fertilization
IVF-E.T	In-vitro fertilization- embryo transfer
LPO	Lipid peroxidation
MS	Mean squares
Mt	Million tons

mtDNA	Mitochondrial DNA
NCA	Neutral comet assay
NS	Non significant
NT	In situ nick translation
PHGPX	Phospholipids Hydroperoxide glulalhione peroxidase
PR	Pregnancy rate
PUFA	Poly unsaturated fatty acids
PVC	Poly vinyl chloride
ROS	Reactive oxygen species
SCD test	Sperm chromatin dispersion test
SCSA	Sperm chromatin structure assay
Sn-GPX	Sperm nucleus glulalhione peroxidase
SOD	Superoxide dismutase
ssDNA	Single stranded DNA
TB	Toluidine blue
TdT	Terminal deoxynucleotidyl transferase
TUNEL assay	Terminal deoxynucleotidyl transferase- mediated deoxyuridine triphosphate- nick end labeling assay
WHO	World Health Organization

Introduction

The Egyptian buffalo is kept as a draft animal and for milk production. They are grey-black with short curved horns. The varieties include Baladi (Lower Egypt) and Saidi in Upper Egypt. Water buffalo have been responsible for more than 10 % of world milk production for several years. The word “buffalo” evokes a mixed response in North America, a large section of Europe, and in many other parts of the world.

The immense popularity of buffalo milk and meat products has ensured that buffalo production has followed in the path of the dairy cattle industry. Buffalo, although potentially excellent for both milk and meat production, still languish in obscure conditions of poor nutrition, breeding, management and welfare. This animal is called the water buffalo because of its natural instinct to wallow in ponds of water and muddy pools.

Archaeological findings and historical data point to the fact that buffalo were first domesticated around 2500 BC in the Indus Valley; present day India and Pakistan**.** Around 600 AD, Arab traders brought water buffalo from Mesopotamia towards the Near East; modern day Syria, Israel and Turkey. During the Middle Ages the animal was brought to Europe by pilgrims and crusaders. Buffalo are now found in Italy, Hungary, Romania, some Balkan countries, Greece and Bulgaria. The domestic water buffalo has also been introduced into South America, the United States and Australia.

World milk production has doubled in the last few decades and it is noteworthy that in the last few years, buffalo have supplied about 12% of the total world milk production. India and Pakistan have produced respectively 60 and 30% of the world's buffalo milk. In India buffalo milk contributes 55%, and in Pakistan 75%, of their total milk production. Dairy buffalo production has been a tradition in parts of the world like the Caucasian countries, Asia and Egypt.

There are 170 million buffalo in the world today; 97% in Asia, 2% in Africa – mainly in Egypt, and 0.2% in Europe – mainly in Italy**.** India has 56%, Pakistan 14% and China 13% of the world buffalo population. Buffalo contribute 72 million tones (Mt) of milk and three Mt of meat annually to world food, much of it in areas that are prone to nutritional imbalances.

The domestic water buffalo Bubalus bubalis, belongs to the family Bovidae, sub-family Bovinae, genus bubalis and species arni or wild Indian buffalo**.** Buffalo are classified into two distinct classes: swamp buffalo and river buffalo.

Swamp buffalo are found in China, Thailand, the Philippines, Indonesia, Vietnam, Burma (Myanmar), Laos, Sri Lanka, Kampuchea and Malaysia. They are mainly used as draught animals, particularly in rice cultivation. Swamp buffalo produce relatively small quantities of milk – 1.0 to 1.5 liters per day, so they are not heavily used in milk production**.** The swamp buffalo might however be used in meat production**.** The name ‘swamp’ has probably arisen from their preference for wallowing in stagnant water pools and mud holes.

Riverine breeds of the Indian sub-continent are mainly raised for milk production. Their milk yield is about six to seven liters per day. Twelve of the 18 major breeds of buffalo are kept primarily for milk production. The main milk breeds of India and Pakistan are the Murrah, Nili-Ravi, Surti, Mehsana, Nagpuri and Jaffrabadi.

Beheri (Egypt):

Weight: male – 450 kg, female– 350 kg

Production: 1 800 to 2 000 kg in 300 days

The water buffalo is an important beast of burden in Asian farming. It is widely used to plough, level land, plant crops, puddle rice fields, cultivate field crops, pump water, haul carts, sleds and shallow-draft boats.

Buffalo have been used as draught animals for centuries. This has lead to exceptional muscular development, some animals can weigh more than 1000 kg. Though buffalo are a major source of meat. In general, a buffalo carcass has a higher proportion of muscle and a lower ratio of bone and fat than a cattle carcass.

Buffalo are the second largest source of milk supply in the world. In 2004, Organization **(FAO**) the world production of buffalo milk was 75.8 million tones (Mt). This volume is with according to statistics from the United Nations' Food and Agriculture, an average fat and protein content of 4% and 3.5% respectively. The average fat content in buffalo milk is about 7 to 8% while protein content in buffalo milk ranges from 4.2 to 4.5%. So in terms of energy corrected milk, buffalo milk is making a greater food contribution than the actual volume of buffalo milk suggests. The riverine breeds produce more milk than the swamp types.

Buffalo milk is high in total solids, fat, proteins and vitamins compared to cow's milk. Buffalo milk also contains less cholesterol and more tocopherol, which is a natural anti-oxidant. The peroxidase activity is two to four times higher in buffalo milk than in cow's milk, which means that buffalo milk has better natural keeping qualities**.** Buffalo milk appears to be whiter than cow's milk because it lacks the yellow pigment carotene, a precursor of vitamin A. But buffalo milk contains even more vitamin A than cow's milk.

Buffalo have a number of anatomical and physiological similarities with the other species in the Bovidae family. Cattle have 60 diploid chromosomes. Riverine type (50 diploid chromosomes) Breeds such as Murrah, Nili Ravi, Jaffrabadi, Mediterranean. Swamp type (48 diploid chromosomes) For example Carabaos. While the two types of buffalo can be mated to produce a fertile offspring which has 49 diploid chromosomes, buffalo cannot be successfully mated with any other species in this family. The buffalo has an exceptionally long productive life. A normal healthy female buffalo could have as many as nine to ten lactations.

Buffalo are less tolerant of extremes of heat and cold than various breeds of cattle. The body temperature of a buffalo is lower than that of a cow in spite of the fact that its black skin absorbs much heat and its skin has only one-sixth the density of sweat glands that a cow skin has. This explains why buffalo like to wallow in water when the temperature and humidity are high**.** Regulation of body temperature in this way influences feed intake, reproduction and milk production **(www.delaval.com Introduction to dairy buffalo production.mht).**

Today, the worldwide cattle industry is based on artificial insemination and frozen semen. Cryopreservation has allowed exploitation of superior sires and achieved rapid, large – scale genetic improvement in cattle stocks coupled with a reduction in disease transmission **(Foote, 1999)**. This impact would not have been possible without successful freezing of bull spermatozoa **(parkinson and whitfield, 1987; Foote and parks, 1993; Woelders, 1997; Vishwanath and shannon, 2000 and Foote, 2002).**

The process of cryopreservation represents an artificial interruption of the progress of the spermatozoon toward post – ejaculation, capacitation and fertilization (**Graham, 1978; Hammersted et al., 1990).** They found that cryopreservation induces partially irreversible damage to sperm membrane as a result of membrane destabilization.

The lipid peroxidation of the unsaturated fatty acids in the sperm plasma membrane causes the loss in membrane fluidity and flexibility and as a consequence the sperm cells lose their function **(Aitken and Sawyer 2003).** Also, lipid peroxidation can damage DNA by causing chromatin cross-linking, base changes and DNA strand breaks **(Twigg et al., 1998b)**. It was observed that frozen thawed bull spermatozoa are more easily peroxidized than fresh spermatozoa **(Trinchero et al., 1990).** Some studies showed that cryopreservtion enhances lipid peroxidation process which in part could be mediated by the loss of superoxide dismutase (SOD) activity occurring during the process **(Alvarez and Storey, 1992).**

Studies performed on buffalo spermatozoa showed that they are more susceptible to hazards during freezing than cattle spermatozoa **(Raizada et al., 1990).**

A variety of laboratory tests are used in routine evaluation of fresh or frozen-thawed bull spermatozoa such as sperm motion, acrosomal status, sperm viability and morphology. During last decades, various assays were developed to assess different aspects of the chromatin structure and DNA integrity of sperm cells that use specific and complex interactions between compounds and DNA **(Boe-Hansen et al; 2005a).** These include the single-cell gel electrophoresis assay (comet assay) **(Boe-Hansen et al; 2005b).** The sperm chromatin structure assay (SCSA) which has been used on semen from a number of different species including the bull **(Karabinus et al., 1990)** and a relationship between bull fertility and the SCSA parameters has been described by **Ballachey et al., (1987) and Bocheneck et al ., (2001).**

The examination of semen after handling and processing is used to investigate sperm quality and DNA integrity by using a different measurements that gives us information on semen handling and minimizing damage and increasing fertility.

The present work was undertaken to determine the effect of cryopreservation on viability, integrity of sperm DNA and fertility of buffalo spermatozoa as well as minimizing hazards effect of freezing procedures by adding certain additives to semen in order to establish a better viability and fertilizing capacity of cryopreserved buffalo semen.