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Effect of Seasonal Variations on Semen Quality and Fertility of Egyptian Water Buffalo (*Bubalus Bubalis*) Bulls.

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ABSTRACT

The objective of this study was to determine the effect of seasonality on semen quality and fertility of Egyptian water buffalo (Bubalus bubalis) bulls. The semen quality was investigated by studying sperm physical characters; motility, plasma membrane integrity, viability and morphology. In addition, level of lipid peroxidation (thiobarbituric acid reactive substances; TBARS), DNA integrity and fertilizing ability of semen specimens collected in winter and summer seasons. Results indicated that there was no significant variation in total motility and the percentage of sperm abnormalities between the two seasons. Meanwhile, Sperm membrane integrity and viability of winter specimens were increased significantly than that of summer. Meanwhile, the results indicated that the increase in climate temperature in summer season increased the oxidative stress that represented as increase in the concentration of lipid peroxidation in summer. In addition, the percentage of sperm DNA damage increased in summer. With regard to in vitro fertilization, the percentage of sperm binding oocytes and fertilization rate has been decreased in summer's specimens than that in winter. Based on the results presented here, it can be concluded that sperm quality of Egyptian water buffalo is affected by the elevated temperature during summer season that might be related to the increased production of reactive oxygen species (ROS).

Keywords: Buffalo, Seasonality, Sperm Physical characteristics, Sperm DNA integrity, lipid peroxidation, *in vitro* fertilization

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INTRODUCTION

Buffaloes constitute a significant part of the domestic stock in Egypt, as it represents the main source of meat and milk. Thus, the increasing importance of buffaloes in the dairy industry of Egypt has dictated breeding strategies, which necessitate extension of modern technologies such as artificial insemination (AI), embryo transfer and *in vitro* fertilization as breeding tools. Thus, semen usually collected from selected sires and processed for production of high quality cryopreserved semen for successful AI of domestic livestock [1].

Standard classification of semen for cryopreservation is based primarily on morphological features without any specific consideration of the time or season in which the semen was collected. Although, many studies have considered that season is one of the major factors, which influence the reproductive performance of these animals, and it exerts its effect through macro and micro climatic factors like temperature, humidity, rainfall, photo-period [2]. Many researcher reported that the quality of semen i.e. sperm motility, viability, concentration, etc. was variably affected by breed and seasonality differences [3-6]. However, conflicting reports have been published about semen quality and volume during various seasons of the year. Koivisto et al. [4] reported that the ejaculate volume, sperm concentration, gross-motility, progressive sperm motility, vigor and morphological sperm defects were significantly influenced by season and genotype when comparing between Bos indicus and Bos taurus bulls in Brazil.

Routine semen analysis that includes sperm morphology, motility, sperm cells concentration and volume per ejaculate are common criteria for evaluating semen quality at most artificial insemination (AI) stations. However, Sperm DNA integrity is an important criteria for the success of natural or assisted fertilization including normal development of the embryo, fetus or offspring, transmission of genetic information and therefore, the maintenance of good health in future generations [7].

Sperm with compromised DNA integrity appear to have the capacity to fertilize oocytes at the same rate as normal sperm. However, the embryos produced by fertilization of an oocyte with DNA damaged sperm cannot develop normally [8]. Therefore, the evaluation of sperm DNA integrity in addition to routine sperm parameters could add further information on the quality of spermatozoa and reproductive potential of males [9].

As a variety of factors, including season have been reported to affect the quality of extended buffalo bull semen but effect of these factors on sperm DNA integrity has not been adequately assessed [10,11]. Therefore, the present study was conducted to evaluate the effect of seasonal variations on quality of Egyptian water buffalo bulls' spermatozoa. In particular, the study aims to determine the routine sperm parameters including motility percentage, morphology, viability, plasma membrane integrity. In addition, evaluations of buffalo oxidative stress levels and spermatozoa DNA integrity as well as fertilizing ability of spermatozoa during different seasons.

MATERIALS AND METHODS

Semen Specimens:

In this study, 300 frozen semen AI doses (0.25 mL plastic straws) were prepared and collected from ejaculates of seven Egyptian AI buffalo bulls housed at Animal Production Research Institute, Sakha, Kafr El sheikh, Egypt. Semen from all the experimental bulls was collected during summer and winter seasons of the period from July to February. Samples were kept in liquid nitrogen tanks in embryology laboratory, cell biology department, biotechnology and genetics engineering division, NRC Cairo, Egypt. The averages of maximum and minimum ambient temperature in both seasons were 23.1 ºC and 15.4 ºC in winter (January - February), versus 37.3 ºC and 28.4 ºC in summer (July- August), while the averages of relative humidity were 80.1 % in winter and 89.6 % in summer.

Assessment of Sperm Characteristics:

This experiment was conducted by using three replicates of each bull for both seasons to evaluate sperm motion characteristics, plasma membrane integrity (PMI), sperm viability and sperm morphology assay to study association between seasonality and sperm physical characters.

Sperm motility using computer-assisted sperm analysis (CASA):

To evaluate the motility of semen, a drop of semen sample (5μL) was placed on a pre warmed (37ºC) Makler chamber and analyzed for sperm kinematics using a computerized sperm motion–cell motion analyzer (Sperm Vision™ software minitube Hauptstraße 41. 84184 Tiefenbach, Germany)[12].

Sperm plasma membrane integrity:

Integrity of the sperm membrane was tested by adding 10ul of semen sample to 100 µl of a 100 mM sucrose solution (hypoosmotic swelling test, HOST). Response of the sperm was based on the degree of swelling of the plasma membrane, determined by coiling or curling of the sperm tail [13]. Using phase contrast microscopy at X 400, 200 cells were classified as coiled or straight tail.

Sperm viability and morphology:

Sperm viability was assessed by using Nigrosin- Eosin staining according to the method described by [14]. The Nigrosin- Eosin stained semen smears as used in live sperm counts were also utilized in determining the percentage of morphological abnormal spermatozoa. The morphological abnormalities were classified as head, mid-piece and tail types, according to Ahmad et al. [14]**.**

Measurement of Semen Lipid Peroxidation:

Lipid peroxidation was measured by determining the production of thiobarbituric acid–reactive substance (TBARS), according to the method described by Laudat et al. [15].

Evaluation of Sperm DNA damage by comet assay:

To assess sperm DNA strand breaks, the alkaline comet assay will be carried out as described by Donnelly [16]. The fluorescent labeled DNA was visualized using an automated fluorescence microscope with an excitation filter of 520-590 nm and the images were captured on a computer equipped with Comet Score software (Komet IV). The parameters for the DNA damage analysis include: tail length (TL, in um), tail moment (TM) and % tail DNA (% DNA). TL is the maximum distance that the damaged DNA migrates from the centre of the cell nucleus. % DNA is the total DNA that migrates from the nucleus into the comet tail. TM is the product of the TL and % DNA, which gives a more integrated measurement of overall DNA damage in the cell.

Evaluation of Sperm DNA Integrity by Acridine-orange:

Acridine-orange (AO) staining assay was used for determination of sperm DNA integrity as described in Hammadeh et al. [17]. Air-dried slides were fixed overnight in freshly prepared Carnoy's solution (three parts methanol and one part glacial acetic acid) and allowed to air dry for a few min. Dried slides were stained for 3 min. with AO. The stained slides were evaluated immediately under fluorescence microscope with 490 nm excitation light. A total of 200 spermatozoa were observed, normal DNA showed green fluorescence over the head region, while DNA abnormalities showed varying fluorescence (from yellow-green to red).

Evaluation of fertilizing ability of buffalo bulls:

Collection of oocytes and in vitro maturation:

Buffalo ovaries (n= 316) were collected from a local abattoir immediately after slaughter and were transported within 2–3 h from the slaughterhouse to the laboratory in Dullecco's phosphate buffered saline (DPBS) at 37°C. Cumulus-oocyte complexes (COCs) were recovered by aspiration of 2–8mm follicles using a 20-gauge. The COCs were evaluated on the basis of their morphology. Only oocytes with compact layers of cumulus cells and intact cytoplasm were used for the experiments [18]. Selected oocytes were washed three times in TCM-199 with Earl's salts and 25mM HEPES supplemented with 10% fetal calf serum (heat treated at 56 °C for 30 min) and 50 µg/ml gentamycin sulfate. Oocytes were cultured in 4-well plastic Petri dishes containing 100 µl of culture medium at 38.5°C for 24 h in a controlled gas atmosphere of 5% CO2 in humidified air.

In vitro fertilization (IVF) and culture:

The straws containing frozen-semen from both winter and summer were thawed separately at 37ºC for 30 sec, and motile semen was separated by swim-up method as previously described by Parrish et al.[19]. Progressively motile spermatozoa were placed in 100 µl droplets of BO medium [20] containing 20 mg/ml bovine serum albumin to adjust the concentration of spermatozoa to 12.5×10^6 sperm/ml. After removing the cumulus cells, matured oocytes were washed three times in BO medium containing 10 mg/ml BSA and introduced into 100 µl droplets of sperm suspension (5–10 oocytes/droplet). The sperm and oocytes were co-cultured for 18 h under the same culture conditions: 5% CO2, 38.5 °C. After fertilization, part of the oocytes were washed by pipetting mainly at least three times in IVC medium, then *in vitro* cultured in TCM-199 +10% calf serum cultured medium. Cleavage and embryo developmental rates (based on morphological characters) were checked on after 48 h using an inverted microscope (Nickon, Japan). The percentage of cleavage rate was calculated by dividing the number of oocytes cleaved out of the total number of oocytes inseminated.

Sperm Zona pellucida Binding:

The other part of fertilized oocytes were rinsed 10 times in DPBS containing 0.5% BSA to remove loosely attached sperm. Oocytes were placed in the refrigerator at 4 °C until fixation. Groups of (5–10) oocytes were placed on glass slides fixed in a solution of methanol/glacial acetic acid (3:1 v/v), then stained with 1% acetio–orcin stain. The number of spermatozoa bounded to the zona pellucida was counted by phase contrast microscopy (X400). For each group, the sperm number attached per ova was calculated by dividing the total number of spermatozoa on all ova by total number of ova inseminated [21].

Statistical analyses:

Statistical analysis was determined using SPSS software (V 16; Lead Technologies, Inc., IL USA) by performing paired T-Test. Student's paired (t) test was used to detect statistical significance among the different groups. The data were expressed as means ± standard error of the mean. Multiple range test with $P<0.05$ being considered statistically significant [22].

RESULTS

Sperm physical characteristics:

Computer assisted sperm analysis (CASA) instrument was used to evaluate sperm motility parameters during different seasons on buffalo bull as presented in Table (1). The results revealed that there was no significant variation in total motility among seasons. While, a highly significant ($P < 0.01$) difference in the percentage of the progressive motility were recorded between winter 43.7 ± 1.64 % (range from 21.24 - 61.98 %) and summer 22.89 ±1.13 % (range from 13.11±1.29 to 35.61%) bulls. The analysis of CASA parameters showed a significant difference ($P < 0.01$) among different seasons in the velocity values; average path velocity, the curvilinear velocity, straight-line velocity and the amplitude of lateral head displacement.

Sperm Seasonal variations on semen characteristics of Egyptian water buffalo bulls collected throughout the year presented in Table (2). Data showed that seasonal variation has a significant ($P < 0.05$) effect on the buffalo sperm plasma membrane integrity. The average mean of plasma membrane integrity from sperm buffalo bull during summer seasons were in the range of 58.63-79.9 with overall mean percentage 68.92 ± 2.11. In winter, the mean of plasma membrane integrity ranged from 59.45- 68.72 with overall mean percentage 63.79 ± 1.4. The study demonstrated that seasonal variation has a significant effect on the sperm viability as illustrated in Table (2). An examination of sperm viability during winter revealed an increase of viable sperm 78.8 ±17.84 (range from 73.66 - 81.71) as compared to those which examined on summer seasons 72.42±14.66 (range from 66.22- 78.9).

Data in Table 2 represent the relation between seasonality and sperm morphology. In the present study, the mean percentages of normal sperm were < 82%, and the overall total mean percentages of sperm abnormalities of buffalo bull spermatozoa were < 18%, being (16.45 ± 0.58%) in winter and (17.52 ± 0.84%) in summer . Association between seasonality and various sperm abnormalities revealed no significant effect $P > 0.05$. The mean percentage of head defects were ranged between 1.19 -2.00% during winter *vs* 1.32 -2.73 in

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summer season. In addition, mid-piece defects among bulls were 3.50-6.60 % *vs*. 3.93- 7.64 % in winter *vs* summer seasons. Tail defects of all bulls were in average 8.53- 11.47 % in winter season *vs* 8.6- 13.03 % in summer.

Table 1: Seasonal effect on sperm motility parameters of Egyptian water buffaloes (*Bubalus bubalis*) bulls by using CASA instrument.

Parameters	Winter		Summer	
	Mean	Range	Mean	Range
Total Motility%	58.31±1.45	74.39-43.31	57.73 ±0.63	62.66-49.23
Prog. Motility (%)	43.7 ±1.64**	21.24- 61.98	22.89 ±1.13	13.11 35.6
VAP(micron/s)	43.78 ±0.35**	39.24-48.17	33.26 ±0.54	13.11-35.61
VCL (micron/s)	70.88 ±0.72**	63.67- 79.18	54.66 ±1.03	43.74- 66.52
VSL (micron/s)	32.29 ±0.23**	29.49-35.2	26.21 ±0.43	19.77- 32.95
ALH (µm)	4.11±0.05**	5.00 - 3.53	3.16±0.07	2.39- 4.23

Data were presented as mean ± standard Error of the mean (SEM) for three samples from each bull (n=7) per season (winter and summer), Significance (**) at $P < 0.01$. (VAP)Average path velocity, (VCL) curvilinear velocity, (VSL) straight-line velocity, (ALH) amplitude of lateral head displacement

Table 2: Seasonal effect on characteristics of sperm Egyptian water buffaloes (*Bubalus bubalis*) bulls.

Parameters	Winter		Summer	
	Mean	Range	Mean	Range
Membrane integrity	68.92±2.11*	58.63- 79.90	63.79±1.4	59.45- 68.72
Viability%	78.8±17.84*	73.66- 81.71	72.42±14.66	66.22- 78.9
Head Abnormality %	1.58±0.09	1.19 - 2.00	1.93±0.17	1.32 - 2.73
Midpiece Abnormality %	5.12±0.47	3.50 - 6.60	5.81 ± 0.40	3.93 - 7.64
Tail Abnormality%	9.75±0.47	8.53 - 11.47	9.78± 0.47	8.6 -13.03
Total Abnormal%	16.45 ± 0.58	18.50-14.92	17.52 ±0.84	22.77- 13.43

Data were presented as mean ± standard Error of the mean (SEM) for three samples from each bull (n=7) per season (winter and summer), Significance (*) at $P < 0.05$.

Sperm lipid peroxidation:

Seasonal variations on sperm lipid peroxidation of buffalo bulls collected through winter and summer season was presented in Figure (1). The study showed that seasons affected the sperm TBARS concentration significantly ($P < 0.01$) on all studied bulls that collected during summer season as compared with those which collected during the winter with an overall mean 0.184±0.006 (range from 0.16- 0.23) and 0.125±0.005 (range from 0.10- 0.14) nmol/10^7, respectively.

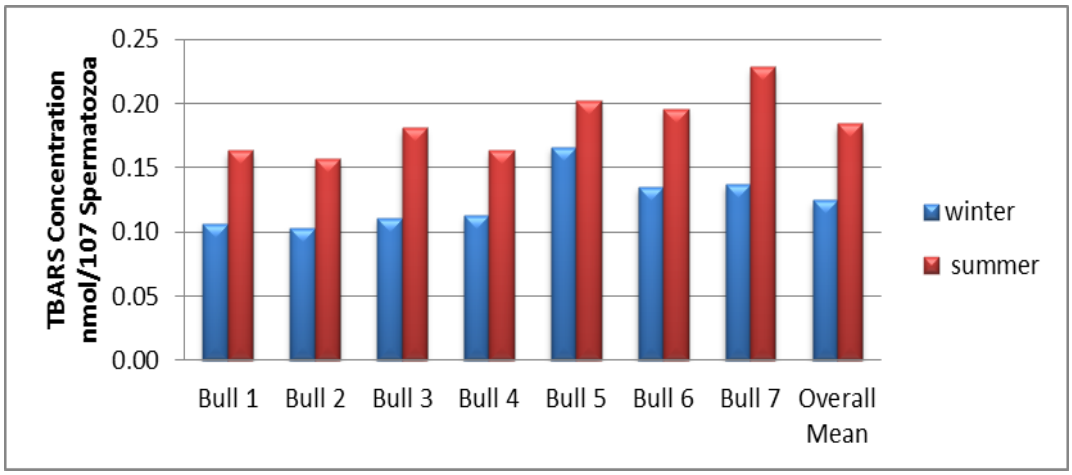

Figure (1): Relation between seasonality and spermatozoa lipid peroxidation of Egyptian water buffaloes *(Bubalus bubalis)* bulls.

Effect of seasonal variation on sperm DNA integrity:

The effect of seasonality on sperm DNA integrity of buffalo bull spermatozoa was detected by using acridine orange (AO) and comet assay are given in Table 3. The acridine orange (AO) stain has the metachromatic shift from green fluorescence (acridine orange intercalated into double stranded nucleic acid) to red fluorescence (acridine orange associated with single stranded DNA). The results illustrated that the percentage of orange-red fluorescence spermatozoa was significantly higher ($P < 0.01$) during the summer season 5.44±0.27 % range from (3.83 - 7.16) than those of winter 2.97±0.09 % range from (2.50 to 3.41).

An increase in the mean value of comet cells (DNA damage cells) percentage was observed in buffalo sperm collected during summer season 12.71±0.37 % , which were in the range 12.00- 13.67 % when compared with winter group ($P < 0.01$).whose mean comet value was 7.57±0.45 % , with a range 6.00 - 8.67%. A significant increase in all comet assay parameter (TL, tail DNA % and TM) on buffalo spermatozoa collected during summer season as compared with winter season.

Seasonal Effect on Fertilizing Ability of Buffalo Bulls

To test the effect of seasonality on sperm fertility, 539 oocytes that classified as excellent and good quality were incubated in maturation medium. Around 320 oocytes were matured with (60.58%) maturation rate. Matured oocytes were divided to winter group inseminated with spermatozoa of winter specimens and summer group inseminated with spermatozoa of summer specimens. Table 4 illustrates the fertilization rate and the mean number of spermatozoa binding oocytes in winter and summer seasons. The results showed that the mean number of spermatozoa binding oocytes in winter group was (13.48 ±1.94) which increased significantly ($P < 0.05$) than those of summer group (8.22 ±1.32). In addition, the fertilization rate was increased significantly ($P < 0.01$) in winter collected group with a mean percentage (33.06 ± 0.41%) when compared to those of summer group (19.96± 0.7 %).

Table (3): Seasonal changes in sperm DNA integrity of Egyptian water buffaloes (*Bubalus bubalis*) bull, using acridine orange and comet assays.

Parameters	Winter		Summer	
	Mean	Range	Mean	Range
AO%	2.97±0.09	2.50-3.41	5.44±0.27**	3.83- 7.16
Tailed (%)	7.57±0.45	6.0-8.6	12.71±0.37**	12-13.67
Tail Length (µm)	2.91±0.11	2.67-3.20	3.67±0.11**	3.08-4.08
Tail DNA (%)	3.41±0.10	3.08-3.55	3.84 ±0.12**	3.55-4.43
Tail moment (%)	9.89±0.48	9.08-11.33	13.39±0.58**	10.39- 15.98

Data were presented as mean ± standard Error of the mean (SEM) for three samples from each bull (n=7) per season (winter and summer), Significance (**) at $P < 0.01$.

Table (4): Relation between seasonality and fertilization assay of Egyptian water buffaloes *(Bubalus bubalis*) bulls.

Parameters	Winter	Summer
Spermatozoa Binding-Oocyte	13.48 ±1.94*	8.22 ±1.32
Fertilization Rate (%)	33.06± 0.41**	19.96± 0.7

Data were presented as mean ± standard error of the mean (SEM), Significance (*) $P < 0.05$ and Significance (**) $P < 0.01$.

DISCUSSION

Semen producing ability and quality of bull are essentials to ensure the supply of superior quality germplasm for maintaining the production performance in future progeny of individual breed [22]. Fluctuations in bull sperm output can be influenced by many factors, such as breed, age, nutrition, environmental effects, health status and frequency of use. These factors result in great variations of semen characteristics such as volume, concentration, motility, morphology and viability that depend on accessory sex gland secretion and epididymal function [23].

In the present study, clear seasonal variation in sperm quality of buffalo bulls was recorded. It has been evident that semen analysis is probably the most relevant procedure in evaluating male fertility potential

[24]. Motility is one of the most important characteristics believed to be associated with the fertilizing ability of spermatozoa. Several groups have reported a significant correlation between total [25] and progressive [26] motility of bull semen and its associated field fertility. Vincent et al. [27] demonstrated that sperm motility evaluations by using CASA are important for the assessment of semen quality and its relationships with fertility. Our observation illustrated that there was subtle changes in spermatozoa total motility between two seasons. Whereas, there was a significant decrease in sperm progressive motility, average path velocity (VAP), curvilinear velocity (VCL), straight-line velocity (VSL) and the amplitude of lateral head displacement (ALH) in sample processed during summer season than those processed in winter season. This result was consistent with the report of Koivisto et al. [4] that the gross-motility, progressive sperm motility, vigor and morphological sperm defects were significantly influenced by season and genotype when comparing between Bos indicus and Bos taurus bulls in Brazil. In addition, Orgal et al. [28] reported that sperm examined post-thawing showed lower velocity, motility and progressive motility in summer vs. winter samples. The reduction in sperm motility could be has been attributed to the reduction of total protein concentration and absence of specific protein from seminal plasma, due to seasonal affect, was associated to the lower sperm motility in frozen semen [29].

Plasma membrane integrity is crucial to sperm survival inside the female reproductive tract, and it plays an important role in several events taking place during fertilization e.g. capacitation, acrosome reaction, sperm fusion with the oocyte [30]. In our study, hypo-osmotic solution test (HOST) showed a marked decrease in plasma membrane integrity on samples collected at summer season compared to winter. These results were in agreement with those reported by (Koonjaenak et al [23] and Mandal et al. [31]. Several literatures reported that the exposure of spermatogenic cells to heat stress is known to produce reactive oxygen species (ROS) [3, 32]. These reactive molecules tend to affect unsaturated fatty acid rich cell membranes in mammal spermatozoa, which are considered especially susceptible to peroxidation [33, 34]. The activity of the cytoplasmic antioxidant enzymes in sperm cells is very low [35], makes them susceptible to oxidative stress [36]. Thus, the spermatozoa plasma membrane loses its fluidity and structure that related to poor sperm motility and functions [37, 38].

In addition, our results showed a marked decrease in viable buffalo bull sperm at summer season when compared to winter season. This is in agreement with Nandre et al. [39], who suggested that the composition in the seminal plasma would play an important role in sperm membrane stability and subsequent sperm viability. This lowered in sperm viability related to ambient temperature that effects on semen quality to be dependent on the period of exposure to heat stress [40]. Pérez-Crespo et al. [41] revealed that spermatocytes present within the testes at the time of heat stress resulted into a lower concentration of spermatozoa with reduced viability and low motility.

Regards to sperm morphological abnormalities, results revealed that there were non-significant increases in the percentage of sperm abnormalities in bulls collected in summer season than that of winter. The absence of any significance in sperm morphology percentage between bulls may be due to the fact that most of the artificial insemination centers utilize bull with nearly the same percentage of sperm morphological and motility to determine semen quality for cryopreservation and subsequent use. Seasonal influence on semen quality was investigated in Bos taurus and Bos indicus bulls where Bos taurus bulls were found more heat susceptible in terms of producing high number of abnormal heads followed by cytoplasmic droplets [4]. Zhang et al. [42] found that the percentage of normal morphology spermatozoa in middle and late summer was lower as compared to the sperm morphology on the four periods.

The present study showed that the total spermatozoal thiobarbituric acid reactive substance (TBARS) concentration of semen bull significantly affected by seasonal variation. This indicated that the antioxidant system of the sperm cells was damaged by heat exposure during summer season that goes in parallel with previous study of Heise et al. [43]. Who found that upon heat exposure to critical and higher temperatures an increase in oxidative damage markers such as TBARS and a more oxidized cellular redox potential, combined with reduced activities of the antioxidant enzyme superoxide dismutase. Nichi et al. [3] found an increase in lipid peroxidation in Simmental bulls that is consistent with their higher percentages of defective sperm in summer. In another study, Balic et al. [6] observed that the summer heat stress declined semen quality parameters in Bos taurus bulls and they could relate this finding to the presence of high lipid peroxidation. It was reported that testicular hyperthermia leads to excess generation of oxygen radicals, alteration in

antioxidant capacity of the stressed tissue and increase in lipid peroxidation resulting in events associated with male infertility [44].

Sperm DNA integrity is critical for the development of viable embryos following the completion of fertilization process [45]. Severe damage to the spermatozoa DNA integrity reduced its fertilization potential. It is interesting that sperm with apparently normal motion characteristics, intact plasma membrane and organelles could fertilize the oocytes and produced embryos successfully. But development of the embryos was impaired at four to eight cell stage and apoptosis initiated, if sperm DNA fragmentation had occurred due to any reason [46, 47]. Our results of spermatozoa DNA estimation using acridine orange showed that the overall mean abnormal DNA integrity increased in summer specimens than in winter. As regarding to the comet assay, the results showed that the mean tail length, tail DNA % (TDNA) and tail moment (TM) of bull semen collected in summer were significantly higher relative to percentage of semen collected in winter. The reason for this decrease of spermatozoa DNA integrity during summer season related to heat stress during spermatogenesis. This finding was in agreement with the study of Nandre et al. [48] they concluded that significant difference in DNA damage was observed among Surti buffalo bulls spermatozoa during summer season as compared to the winter season. In another study, a correlation found between poor semen quality and higher levels of spermatozoa DNA damaged detected by using the comet assay [49]. Comet extent and percent DNA in the comet tail were associated with a decline in sperm concentration and motility [50]. Others revealed that DNA integrity of mice spermatozoa compromised by heat stress. The higher degree of DNA damages found among spermatozoa resulting from spermatids present within the testis at the time of heat stress [41]. Elevated environmental temperature during the summer can increase testicular temperatures, metabolic rate and oxygen requirements [51]. The continued rise in ambient temperature may leads to reduce the blood flow, testicle tissue becomes hypoxic that results in excessive ROS production [3, 51]. ROS is also able to stimulate the sulfhydryl radical group oxidation in protein molecules as well as DNA fragmentation by increasing the frequency of single- and double-strand DNA breaks, thereby altering the structure and function of spermatozoa [52, 53]. A significant correlation recorded between DNA fragmentation and levels of ROS molecules in buffalo semen [54].

Fertilization success could not attribute to the absolute number of vital, motile, morphologically normal spermatozoa inseminated but more importantly to their functional competence [55]. Sperm cells ability to bind the homologous zona pellucida (ZP) is prediction of spermatozoal fertilizing ability [56]. In mammals, during fertilization sperm must bind to and penetrate the zona pellucid to fuse with the egg plasma membrane [57]. In our study, spermatozoa binding oocytes was positively affected by seasonal variation; it decreased significantly in summer season collected specimen than those of winter. In addition, the present study revealed that the fertilization rate decreased significantly in the group of summer-collected specimen as compared with winter collected specimen group. These results may be due to the increment of ROS during hot season that considered playing a key role in male infertility [46]. It is noteworthy that ROS can induce DNA fragmentation in spermatozoa that it is associated with loss of sperm motility, premature acrosome loss and failure of zona penetration [58]. In turn, increased DNA damage is linked with poor semen quality including sperm count, morphology and motility [59], low fertilization rates, impaired pre-implantation development, increased abortion and elevated disease levels (including cancer) in offspring [46] as well as potential infertility in offspring [59]. Kasimanickam et al. [34] reported that some deleterious effects of sperm lipid peroxidation are related to impairment in sperm DNA, which may also reduce the bull fertilizing potential. In another study, Rahman et al. [60] reported that heat stress significantly affects the potential of spermatozoa to penetrate oocytes, as well as subsequent embryo development and quality.

CONCLUSION

In conclusion, the data obtained in this study revealed that sperm quality of Egyptian water buffalo affected by the elevated temperature during summer season, which could be due to the increased production of ROS. The increment of oxidative stress can cause damage of sperm plasma membrane and may affect the DNA integrity not only cause DNA fragmentations but also blocked embryos development. In addition, Oxidative damage has detrimental effect on plasma membrane, which has an important role in motility and fertilization process. Therefore, fertilization rate using sperm collected during winter season was better than those in summer season were. Finally, all result confirmed that summer thermal stress could result in compromised reproductive performance of Egyptian water buffalo bulls and that collection of semen for AI would likely be most successful if it occurs during winter season.

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