ASSOCIATION OF CHROMOSOMAL ABERRATIONS AND SEMEN QUALITY IN EGYPTIAN BUFFALO BULLS

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SUMMARY

Milk production and processing in Egypt is a basically function of buffalo and cattle. Various reasons justify sterility and low reproductive performance in livestock, among them chromosomal aberration which is highly considered. The present study was undertaken to investigate the association between semen characteristics and cytogenetic structure as an early indicator of fertility in Egyptian buffalo bulls. Data and samples were collected during September and October, 2015 on 116 ejaculates of 29 randomly selected bulls (age from 22 to 26 months and body weight from 250 to 300 kg). Animals were classified into two groups based on semen quality: group 1 (G1) composed of 10 animals with low semen quality characteristics and group 2 (G2) that included 19 bulls with normal semen quality characteristics. Polyploidy accounted for 9.6% of total numerical aberrations in G1 compared to 1.2% in G2, while aneuploidy represented 5% and 0.4% in G1 and G2, respectively. G1 and G2 differed significantly (P < 0.05) in the percent of polyploidy and aneuploidy. Structural chromosomal aberrations identified were deletion, break, gap, fragmentation, ring, centromeric attenuation and centric fusion, which varied significantly (P<0.05) between G1 and G2. Such aberrations in metaphase chromosomes may have a direct relevance on fertility as they were consistent. All estimates of genetic correlation were significant (P<0.05), except that were related to centromeric attenuation. Chromosomal aberrations correlated negatively with motility, live sperm, hypo-osmotic swollen (%) and testosterone level; whereas their correlation coefficients with sperm abnormality were positive. Genetic correlations ranged from -0.391 between motility and polyploidy to 0.341 between sperm abnormality and polyploidy. The results obtained suggest screening buffalo bulls for chromosomal aberrations prior to their use in natural or artificial insemination.

Keywords: Buffalo, breeding bulls, correlation; chromosomal abnormalities, semen quality

INTRODUCTION

The global population of water buffalo has increased 121% during the last few decades, from 88 million in 1961 to 195 million in 2014 (FAOSTAT, 2016). Buffalo plays a vital role in agriculture and food security, where they are extensively used for milk, meat, fuel and fertilizer production, as well as for draught power, especially for the poorest rural families in developing countries. With this large population of buffalo which is very well adapted to the tropical and subtropical environments and has a competitive potential to meet the protein requirement of our growing human population, it appears quite appropriate to call it "black gold".

Buffalo contributes 70% of the total milk production in Egypt. In spite of Egypt's position as the fourth highest producer of buffalo milk in the world preceded only by India, Pakistan and China, productivity per animal is poor and not yet suitable for intensive commercial dairy farming systems, as compared to dairy cattle (FAOSTAT, 2016; http://faostat.fao.org). This is mainly due to low genetic potential for milk production, absence of confident farm records, using animals in agricultural works, poor level of nutrition and health care as well as limited reproductive performance (Galal, 2007). Fortunately, there is still a large room to genetically improve buffalo productivity through information produced from genomic studies and scientific research.

Traditionally, genetic improvement of livestock has widely laid on selection of animal candidates to be parent to next generations. Selection based on chromosomal morphology composes the first step to implement schemes and strategies for the genetic improvement of quantitative traits in Egyptian buffaloes. Chromosomal aberrations, detected by cytogenetic studies, are usually associated with reproductive disorder in livestock (Jamir *et al*., 2015). Therefore, animal cytogenetics provides a powerful basis for decision-making process towards culling pedigree-selected animals for breeding with chromosomal aberrations at an earlier age, which subsequently saves time, reduces cost of rearing, feeding and health care of animals, and also increases herd's profitability (Yimer and Rosnina, 2014). On the basis of these consequences, screening breeding bulls for chromosomal aberrations is recommended.

Several studies on clinical cytogenetics have been carried out in cattle suggesting chromosomal abnormalities as a reason of sterility and low reproductive performance due to unbalanced gametes during meiosis division (Patel *et al*., 2012; Jamir *et al*., 2015 and Zhang *et al*., 2015). Also, different chromosomal aberrations affecting fertility have been reported in buffaloes (Vijh *et al.,* 1994; Ahmad *et al*., 2004; Chauhan *et al*., 2009; Raudsepp and

Chowdhary, 2016 and Mughal *et al*., 2017). Fertility is a multifactorial phenomenon which is influenced by several factors that often overlap including nutrition, disease, hormonal interactions, reproductive functions, sperm quality, physical soundness, environment, management, genetic factors and abnormal karyotypes that lead to lower reproductive performance of farm animals through decreasing or forbidding capability to produce functional gametes as well as death of early- and late-maturity embryos (Yimer and Rosnina, 2014). The authors explained that those types of aberrations were gap formation (the lost area is shorter than diameter of chromatid), chromosome break (the lost area is longer than the diameter of chromatid or equal to it), chromosome deletion (one chromatid shorter than its sister), centromeric attenuations (the arm of one chromatid is separated from its sister), centric fusion (one chromosome is fused with another chromosome by centromers) and ring chromosome (the ends of the two chromosome's arms are joined together). However, information associating chromosomal morphology and structure with fertility of Egyptian buffalo bulls is scarce.

Therefore, the objectives of the present study were to identify chromosomal abnormalities in breeding bulls of Egyptian buffalo and to evaluate their possible association with semen quality characteristics.

MATERIALS AND METHODS

Animals and semen collection:

Resource population of the present study was based on 29 sexually mature bulls of Egyptian water buffalo. Age of animals was between 22 and 26 months old and their live body weight ranged from 250 to 300 kg. All bulls involved were free of any clinical diseases with a healthy appearance. Semen collection was conducted by means of an artificial vagina set up at optimal conditions to induce a good ejaculatory thrust. At the time of semen collection, another buffalo bull was used as a teaser for sexual preparation. Ejaculates were obtained from each bull one time per week at early morning (7:00am) for 4 consecutive weeks during September and October, 2015, resulted in a total number of 116 samples. The collected ejaculates were taken immediately to the laboratory in water bath at 37°C for semen evaluation processes.

Progressive sperm motility (%):

The percentage of motile spermatozoa was assessed using light microscope supplied with a hot stage adjusted to 37°C. Aliquots of evaluated semen were placed on the slide and covered by a warmed cover slip and were immediately examined under a high power magnification (400x) according to Amann and Hammerstedt (1980).

Live sperm (%):

A smear from semen was made on a glass slide and stained by 1.67% eosin (E 8761) and 10% nigrosin (N 4763), a mixture stain proposed by Hackett and Macpherson (1965). Except mentioned differently, all experimental reagents used were purchased from Sigma-Aldrich (S.A., Egypt). About 10 g Nigrosin and 1.67 g Eosin were dissolved in distilled water up to 100 ml for the preparation of Eosin-Nigrosin stain at 37°C. One drop of the prepared stain was added to one drop of the fresh semen and was mixed on glass slide at 37°C. Then, a thin smear was made by drawing of a second slide across the stained semen. The slide was allowed to dry on the hot stage and then examined under a high power magnification (400x). At the examination time, the live spermatozoa (unstained ones) and dead spermatozoa (stained ones) were counted in field of a total of 200 spermatozoa.

Sperm abnormalities (%):

During the examination of live/dead sperm percentage at a high power magnification (x400), the morphological screening of spermatozoa was carried out per 200 spermatozoa according to the classification adopted by Blom (1983).

Hypo-osmotic swelling test (HOST):

The response of spermatozoa to HOST was assessed using solution prepared with 0.21% lactose (L7401) and 0.48% sodium citrate (N3269) in a distilled water to give osmolarity of 50 ml osmol using a freezing-point depression Osmometer (Osmett A, Model 5002, Fisher Scientific, Pittsbury, PA, USA). Immediately after thawing, semen aliquots of 0.1 ml were added to 0.9 ml a mixture of sodium-citrate-lactose solution. The mixture was incubated at 37°C for 30 min. A drop from the mixture was placed on a warmed glass slide at 37°C and cover slipped. At least 200 spermatozoa were examined for calculating the percentage of curled spermatozoa, according to the method suggested by Azam *et al*. (1998).

Hormone measurement:

Blood samples were collected through jugular vein of each animal into heparinized vacutainer tube. After an hour at room temperature, samples were centrifuged at 2000 rpm for 20 min, and then plasma was collected and immediately kept frozen at -20 °C until the hormone assay. Concentration of the testosterone hormone was determined by radioimmunoassay according to the method described by Smith and Hafs (1973).

Chromosomal preparation and analysis:

Under hygienic condition, a blood sample of three ml was collected from the jugular vein of each animal into a sterilized sodium-heparinized-vacutainer tube with special care in handling. Chromosomal preparations were performed using standard whole blood culture in tissue culture 199 medium (TCM 199) (M4531) supplemented with antibiotics (Streptomycin and pencillin) (S2522), 20% fetal calf serum (F2442), 0.1% phytoheamagglutanin and 0.1% L-Glutamin (G7513) were added to stimulate mitotic activity of the lymphocyte cells and to facilitate the identification of

chromosomes. The blood culture was incubated at 37°C for 72 hours. To increase the relative frequency of prometaphase chromosomes, Colchicine (C180) 1 μg/ml was added to the culture for two hours prior to the harvesting. The cells were collected by centrifugation at 1500 g for seven min, followed by hypotonic treatment with 0.56% potassium chloride (P1302) for 20 min at 37°C and then fixed in 3:1 ratio of methanol (M0789) and glacial acetic acid. Finally, cell suspension was dropped on slides and air dried. Slides were conventionally stained in 10% Giemsa stain (G9641) for screening under the Nikon compound microscope attached with photographic system. About 50 metaphase spreads were screened to detect the chromosomal aberrations and prepare the karyotype (Halnan, 1977 and Iannuzzi and Di Berardino, 2008).

Statistical analysis:

Based on semen quality, breeding bulls were classified into two groups: group 1 (G1) composed of 10 animals with low semen quality characteristics and group 2 (G2) that included 19 bulls with normal semen quality characteristics. T test model was performed with SPSS 16.0 software package (SPSS Inc., Chicago, Illinois, USA, 2006). Results are expressed as means ± standard error of the mean. The level of significance adopted was set at P < 0.05.

Pearson's correlation coefficients were applied to examine the relationship between semen characteristics and chromosomal aberrations. Estimates of genetic correlation were considered statistically significant at P < 0.05.

RESULTS AND DISCUSSION

Semen characteristics:

Physical characteristics of buffalo semen are illustrated in Table (1). In general, means of the semen parameters obtained in the present study are comparable to those reported in the Egyptian river buffalo bulls by Hafez (2012), Khattab *et al*. (2015) and Mahmoud *et al*. (2015) and also in swamp buffalo as mentioned by Koonjaenak *et al*. (2007) and Bhakat *et al*. (2015).

Table 1. Physical semen characteristics, hypo-osmotic swelling test and testosterone hormone level in Egyptian buffalo bull semen (mean ± S.E.)

Group	No. of animals	Semen characteristics (%)			Hypo-osmotic swollen (%)	Testosterone level (ng/ml)
		Motility	Live	Abnormality		
G1	10	53.37 ± 0.70[a]	57.57 ± 0.52[a]	20.35 ± 0.27[a]	50.42 ± 0.69[a]	1.55 ± 0.06[a]
G2	19	72.96 ± 0.49[b]	77.43 ± 0.36[b]	12.42 ± 0.31[b]	71.29 ± 0.42[b]	6.00 ± 0.13[b]
Overall mean	29	66.20 ± 0.95	70.58 ± 0.93	15.15 ± 0.42	64.09 ± 0.99	4.46 ± 0.22

G1, low semen quality group; G2, normal semen quality group
Values with different superscripts within the same column differ significantly (P < 0.05)

Group 2 (G2) showed significantly (P<0.05) higher estimates in all studied semen traits than group 1 (G1), except abnormalities (%). Percentages of motility and live sperm increased (P<0.05) by 36.71 and 34.50% in G2 compared with G1, respectively. In the same trend, hypo-osmotic swelling test (%) and testosterone concentration (ng/ml) were also incremented by 41.39 and 287.1% in G2 in comparison to G1, respectively. On the other hand, abnormalities (%) were decreased significantly (P<0.05) by 38.97 in G2 than G1.

The percent of motility obtained in the current study is very similar to the one found by Koonjaenak *et al*. (2007), who mentioned that the initial motility tended to be higher during winter rather than summer or rainy season. On the contrary, the overall mean of abnormalities (%) detected in the present study was higher than the corresponding estimate reported by Bhakat *et al*. (2015) in Murrah buffalo (15.15 vs. 9.47%, respectively). This finding may be caused by the collection of semen ejaculates during apparently hot months (September and October) along 4 consecutive weeks per bull, which resulted in increased abnormalities (%). In summer season, extreme heat stress result in physical exhaustion, which might reduce the eagerness of bulls for copulation causing subsequently an increase in the reaction time and the total time for successful ejaculation, thus having a final effect on quantity and quality of sperms produced (Mandal *et al.*, 2000).

Viability of sperms is related to the proportions of spermatozoa with an intact and osmotically functional plasma membrane (plasma membrane integrity, PMI) as mentioned by Barkawi *et al.* (2006) and Mughal *et al*. (2017). In the present study, PMI was measured by a hypo-osmotic swelling test (HOST). The percent of HOST obtained in the present study (64.09%) is the same as that reported in Murrah buffalo by Koonjaenak *et al*. (2007), but it is higher than the value obtained for Nili-Ravi buffalo by Rasul *et al*. (2001). This difference could be attributed to the difference in animal species, environment and, most likely, to the method of measurement applied. The average PMI recorded using the HOST was significantly (P<0.05) higher in G2 compared to G1 (71.29 *vs* 50.42 %, respectively). Koonjaenak *et al*. (2007) reported seasonal variation in semen characteristics of swamp buffalo, where HOST estimates and the relative proportion of morphologically normal spermatozoa were significantly higher in summer compared to winter and rainy seasons. Although the proportions of PMI were influenced by season, they were always under what is considered refused for AI sires. In general,

HOST is an effective and inexpensive technique to determine PMI of ejaculated spermatozoa. Variation in semen characteristics from month to another was also reported by Hafez (2012), Bhakat *et al.* (2015) and Saraswat and Purohit (2016). The evaluation of PMI holds promise as an early predictor of fertility as it has been stated that semen with negative HOST values had a poor fertilizing capacity (Capra *et al.*, 2017). In addition, Jamir *et al.* (2015) reported significant differences between high and low motility groups in HOST scores; however, the HOST results did not differ significantly between cattle and buffalo. The results obtained in the present study suggest that the HOST can be used to judge the reproductive performance of breeding bulls and to differentiate the acceptable and unacceptable semen ejaculates. Recently, Mughal *et al.* (2017) concluded that osmotic pressure of the solution used in extender preparation influenced considerably post-thaw quality of cryopreserved buffalo bull semen.

In order to determine properly semen quality after sperm motility assessment, evaluation of sperm morphology is usually performed to identify sperm abnormalities which can reflect testicular, epididymal and accessory gland affections, and can also indicate any mishandling of the ejaculates during semen processing (Rodriguez-Martinez, 2003). Koonjaenak *et al.* (2007) found that the overall mean percentage of sperm abnormalities in swamp buffalo bulls was 15%. The overall sperm morphology did not vary statistically across the year. Although the tail abnormalities were affected by season, they were always below what is considered unacceptable for AI bulls. On the other hand, age of bull had a significant (P<0.001) effect on the incidence of total pathological head shapes, acrosome defects, proximal cytoplasmic droplets and total tail defects. Generally speaking, the sperm defects identified in our study are similar to those that have been described in other livestock species. Different reasons have been proposed as justifications for sperm abnormalities, including genetic factors (Chenoweth, 2005; Mahmoud *et al.*, 2015; Zhang *et al.*, 2015; Dhanoa *et al.*, 2016 and Capra *et al.*, 2017) and temporal impairment of either testicular or epididymal function (Rodriguez-Martinez, 2003). Recently, Bhakat *et al.* (2015) found different types of chromosomal abnormalities in Murrah buffalo including acrosome integrity as well as head, tail and mid-piece abnormality which averaged 70.10, 2.30, 1.62 and 5.50%, respectively.

Testosterone concentration in peripheral blood demonstrated similar trend to that recorded for semen characteristics, where the estimate recorded for G2 was significantly (P<0.05) higher than G1 (Table 1). The concentration of testosterone hormone in the present study averaged 4.46 ng/ml, which is lower than that of 5.62 reported by Hafez (2012). The authors found that almost all studied semen traits (semen volume, sperm concentration, total sperm output/ejaculate and live sperm output/ejaculate) varied significantly (P<0.05) along the experimental

period prolonged from April to September due to changes in heat temperature. As the sexual activity of each animal species is highly affected by the surrounding environment, the variation in these parameters was quite obvious on all semen parameters studied by Bhakat *et al.* (2015). Reasons for good quality seminal ejaculates during winter compared to summer season might be due to the congenial weather conditions which influence the activity and secretions of accessory reproductive glands, since the secretions are dependent on testosterone liberated by interstitial cells during winter which might have favored the spermatogenesis process (Mandal *et al.*, 2005). Semen characteristics and testosterone concentration seem to follow a positive relationship as confirmed by Mandal *et al.* (2005) and Hafez (2012).

Chromosomal aberrations:

Table (2) shows different types of chromosomal aberrations detected in blood samples collected from the Egyptian buffalo bulls. Generally speaking, all estimates measured for G2 were significantly (P<0.05) lower than those recorded for G1 indicating possible relationship between the observed reproductive performance of the buffalo bulls and the proportion of chromosomal abnormalities they carry.

Within each group of animals, the total percent of numerical aberrations were considerably inferior to their counterpart of structural aberrations. This finding is in agreement with that found by Vijh *et al.* (1994) in Murrah buffalo, El-Bayomi *et al.* (2011) in cattle and each of Patel *et al.* (2012) and Jamir *et al.* (2015) in Holstein Friesian cattle and Murrah buffalo. Considering numerical aberrations of chromosomes, two types of abnormalities were identified. Polyploidy accounted for 1.2% of total numerical aberrations in G2 compared to 9.6% in G1. The second type was aneuploidy that represented 0.4% and 5% in G2 and G1, respectively. The two studied groups differed significantly (P < 0.05) from each other in the percentages of polyploidy and aneuploidy. El-Bayomi *et al.* (2011) found that the percent of total numerical aberrations for repeat breeder group in Holstein Friesian cattle was 19.95% including 9.5% polyploidy and 10.45% aneuploidy. The karyotypic profile of normal fertile cows showed that the diploid number of chromosomes is 2n = 60 chromosomes (29 pairs of acrocentric autosomes and one pair of submetacentric X chromosomes). On the contrary, the water buffaloes (*Bubalus bubalis*) possess 50 chromosomes, divided into 24 pairs of autosomes and one pair of sex chromosomes (Patel *et al.*, 2012).

In regards to structural chromosomal aberrations, multiple profiles of chromosomal abnormalities were observed. The results obtained in the current study indicated that the total percent of structural aberrations were predominant over the numerical ones and recorded 6.2% and 45% in G2 and G1, respectively. Figure (1) shows some of the chromosomal aberrations of structural type identified

in the present study. The structural aberrations included deletion, break, gap, fragmentation, ring, centromeric attenuation and centric fusion. Breeding bulls belonging to G1 differed significantly (P<0.05) from those of G2 in all aspects of structural aberrations detected. Except centric fusion, all chromosomal abnormalities of structural origin detected in our study were also identified by El-Bayomi *et al.* (2011) in cattle. Our results are in harmony with that reported by Koonjaenak *et al.* (2007) in swamp buffalo in Thailand, El-Bayomi *et al.* (2011) in Holstein Friesian in Egypt and Bhakat *et al.* (2015) in Murrah buffalo in India. On the other hand, the metaphase plates did not reveal any numerical or structural chromosomal anomaly in Holstein Friesian cattle and Murrah buffalo raised in India (Jamir *et al.*, 2015).

Table 2. Numerical and structural chromosomal aberrations in Egyptian buffalo bulls

Items	G1 (low semen quality)			G2 (normal semen quality)		
	No. cells	Aberration (%)	Mean±S.E.	No. cells	Aberration (%)	Mean±S.E.
Numerical aberrations						
Polyploidy	48	9.6	0.128 ± 0.015^{a}	12	1.2	0.012 ± 0.003^{b}
Aneuploidy	25	5.0	0.040 ± 0.03^{a}	4	0.4	0.001 ± 0.001^{b}
Total	73	14.6		16	1.6	
Structural aberrations						
Deletion	61	12.2	0.136 ± 0.015^{a}	17	1.8	0.014 ± 0.004^{b}
Break	48	9.6	0.124 ± 0.015^{a}	6	0.6	0.004 ± 0.002^{b}
Gap	35	7.0	0.120 ± 0.015^{a}	12	1.3	0.013 ± 0.004^{b}
Fragmentation	19	3.8	0.106 ± 0.014^{a}	5	0.5	0.005 ± 0.002^{b}
Ring	22	4.4	0.052 ± 0.009^{a}	6	0.6	0.002 ± 0.001^{b}
Centromeric Attenuation	15	3.0	0.036 ± 0.008^{a}	9	0.9	0.003 ± 0.002^{b}
Centric Fusion	25	5.0	0.132 ± 0.015^{a}	5	0.5	0.007 ± 0.003^{b}
Total	225	45		60	6.2	

G1, low semen quality group; G2, normal semen quality group
Values with different super scripts between columns for the same item differ significantly (P < 0.05)
A total of 50 cells were examined per each animal
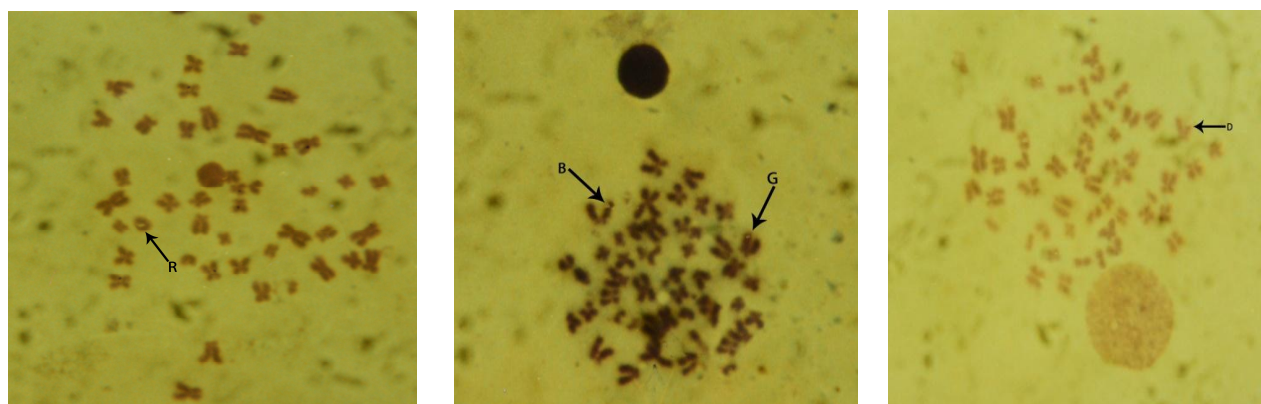
| (a) | (b) | (c) |

Figure 1. Metaphase spreads of buffalo bulls: (a) ring, (b) break & gap and (c) deletion

The Y- and X-chromosome in mammalian species have evolved from autosomes and are involved in sex determination and reproductive traits. The Y-chromosome plays major role in male fertility and is suitable to study the evolution, speciation and male infertility and/or subfertility due to its unique features such as long non-recombining region, abundance of repetitive sequences and holandric inheritance pattern (Dhanoa *et al.*, 2016).

In the present study, buffalo bulls were initially classified into two groups according to the semen quality. Obviously, animals of G2 had better semen characteristics than those of G1, and also G2 bulls demonstrated to have lower cases of chromosomal aberrations in comparison to G1 bulls. Consequently, it is expected that the data available on semen quality and chromosomal abnormalities would depict the future reproductive performance of those young buffalo bulls. Almost all the sex chromosome abnormalities shown in river buffalo were correlated to sterility (Patel *et al.*, 2012 and Yimer and Rosnina, 2014). The results obtained in the current study encourage the incorporation of the clinical cytogenetics for investigation of chromosomal anomalies in young buffalo bulls prior to being used in artificial insemination and breeding programs.

Unlike cattle, systematic cytogenetic screening programmes for chromosomal aberrations in both river and swamp buffaloes are not common and most of the reports are based on individuals suffering from reproductive problems. Autosomal chromosome abnormalities have been less reported in river buffalo compared with cattle; however, the most common chromosome aberration reported in river buffalo is sex chromosome abnormality (Yimer and Rosnina, 2014).

Genetic correlation:

Correlation coefficients between semen characteristics and chromosomal abnormalities are given in Table 3. All estimates of genetic correlation were significant ($P < 0.05$), except that related to centromeric attenuation. Motility, live sperm, hypo-osmotic swollen (%) and testosterone level had negative correlation coefficients with those traits associated with numerical and structural chromosomal aberrations. These findings indicate that selection that favors one trait can cause a decrease in other traits due to their negative correlation. The estimates obtained in the present study are generally lower than those reported in the Egyptian buffalo by Khattab *et al.* (2015) and Mahmoud *et al.* (2015).

Table 3. Coefficients of genetic correlation between semen-related traits and chromosomal aberrations in Egyptian buffalo bulls

	PO	AN	D	B	G	F	R	CA	CF
MO	-0.391	-0.236	-0.322	-0.187	-0.232	-0.250	-0.238	-0.092	-0.250
LI	-0.350	-0.230	-0.317	-0.171	-0.181	-0.279	-0.224	-0.103	-0.238
AB	0.341	0.242	0.334	0.221	0.176	0.251	0.251	0.078	0.261
HOS	-0.372	-0.261	-0.322	-0.195	-0.215	-0.250	-0.220	-0.126	-0.235
TEST	-0.294	-0.263	-0.262	-0.192	-0.272	-0.254	-0.183	-0.125	-0.175

PO, polyploidy; AN, aneuploidy; D, deletion; B, break; G, gap; F, fragmentation; R, ring; CA, centromeric attenuation; CF, centric fusion; MO, motility; LI, live sperm; AB, abnormality; HOS, hypo-osmotic swollen (%); TEST, testosterone hormone

In this respect, Ostermeier *et al.* (2001) studied the relationship between sperm nuclear shape and fertility using two groups of bulls differing in fertility based on lifetime non return rates. The results indicated that mean nuclear shape of high-fertility sperm was more elongated and tapered than those of low-fertility sperm. Also, a comparison of sperm chromatin structure analysis and harmonic amplitudes found that various measures of sperm nuclear shape were correlated significantly ($P < 0.05$) to chromatin stability. The authors suggested that sperm nuclear shape is a better predictor of bull fertility because some of the differences observed in sperm nuclear shape between the high- and low-fertility bulls may be due to different levels of chromatin stability.

In contrast, sperm abnormalities showed positive estimates of genetic correlation with chromosomal aberrations, ranging from 0.078 to 0.341 for centromeric attenuation and polyploidy, respectively. Correlation occurs when one trait is selected for, and another trait is changed simultaneously. The positive coefficients between sperm abnormalities and all chromosomal abnormalities would result in a correlated response when selecting for one trait only. Therefore, early selection for normal karyotype solely may ensure good semen characteristics at maturity. Our results are in consistency with those of earlier studies, in which chromosome profile was correlated with sperm morphometric values (Ostermeier *et al.*, 2001; Ahmad *et al.*, 2004; Katragadda *et al.*, 2013 and Mahmoud *et al.*, 2015). In swamp buffalo, Koonjaenak *et al.* (2007) reported very low correlations between sperm chromatin

integrity and sperm head morphology, where the most significant one was between DNA fragmentation index and loose abnormal heads.

Many studies indicated that different forms of chromosomal aberrations influenced fertility of buffalo and cattle (Chauhan *et al.*, 2009; Patel *et al.*, 2012; Katragadda *et al.*, 2013; Yimer and Rosnina, 2014 and Dhanoa *et al.*, 2016). As reported by Mahmoud *et al.* (2015), motility and live sperm (%) correlated significantly ($r = 0.482$, $P < 0.05$). Also, they observed an opposite trend concerning motility and sperm abnormalities ($r = -0.64$, $P < 0.05$) as well as motility and DNA damage ($r = -0.68$, $P < 0.05$), whereas a significant positive correlation ($r = 0.59$, $P < 0.05$) was reported between sperm abnormalities and DNA fragmentation. The authors concluded that motility may be a physiological marker of choice for semen quality because significant correlations were obtained between motility and other sperm parameters including sperm abnormalities and DNA damage. Moreover, on the light of the significantly positive correlation ($r = 0.59$, $P < 0.05$) found between sperm abnormalities and DNA fragmentation, the authors concluded that evaluating DNA damage can offer a simple description of DNA integrity and chromosomal aberrations which can predict the quality of sperms used in artificial insemination. Similar findings were reported by Ramos and Wetzels (2001) and Capra *et al.* (2017). According to Irvine *et al.* (2000), sperm motility is considered as an indicator of semen quality and conception rate when artificial insemination used due to motility reflects chromatin integrity and DNA fragmentation. The results indicated that sperm

samples with low motility tended to carry higher loads of DNA damage.

In a study to assess reasons of poor reproductive performance in buffalo and cattle bulls, Jamir *et al.* (2015) indicated that metaphase plates did not show any structural or numerical chromosomal abnormalities in both species. High and low motility groups within both buffalo and cattle revealed significant (P < 0.05) differences in HOST. However, the two species did not differ significantly from each other. In spite of not detecting any chromosomal abnormalities, the authors recommend testing future breeding bulls for cytogenetic profile and sperm membrane integrity.

Interestingly, Schmutz *et al.* (1996) found that about 10.7% of 55 aborted fetuses and stillborn calves belonging to 12 different dairy and beef cattle breeds had an abnormal chromosome complement. In about 5.5% of such cases, the abnormalities were probably the cause of death that usually happened during the last month of gestation period. This finding supports the adoption of chromosomal analysis as a cost effective tool to diagnose expected malformed fetuses and newborns. Because of seasonal suppression of fertility repeat breeding in buffaloes should be limited to the breeding season. Spring and winter calving, first parity, periparturient disease and lactation are significant risk factors for repeat breeding in buffaloes (Saraswat and Purohit, 2016). Patel *et al.* (2012) studied the relationship between chromosomal abnormalities and fertility problems in cattle and water buffalo breeding bulls. They found light incidences of chromosomal abnormalities in a few samples, including polyploidy, premature centromeric division, chromosomal fragmentation, aneuploid cells, fragile site, chromatid gaps or breaks in both species. As the detected aberrations were not consistent features in a particular animal; therefore, those aberrations could not have great effects on fertility. However, the authors suggested applying a regular screening to score such chromosomal aberrations which might cause a reduction in animal fertility. More recently, Mughal *et al.* (2017) found that the spermatozoa motility, acrosomal integrity and DNA integrity were significantly (P < 0.05) influenced by varied osmotic pressures.

Heritability of semen quality traits was estimated by Khattab *et al.* (2015). They found that heritability estimates for volume of semen, livability of sperm and motility of sperm were 0.30, 0.38 and 0.35, respectively. Moreover, estimates of genetic correlations between each pair of these traits were positive and highly significant (P < 0.05). The expected genetic change per generation ranged from 0.45 to 1.50 ml for volume of semen, from 6.19 to 10.50 % for livability and from 6.21 to 7.22 for motility of sperm. The authors suggested incorporating volume of semen in a selection index that involves motility and/or livability of sperm.

The relationship between chromosomal aberrations and reduced fertility in livestock has been widely studied for decades (Koonjaenak *et al.*, 2007; Saraswat and Purohit, 2016 and Dhanoa *et al.*, 2016). According to Raudsepp and Chowdhary (2016), chromosomal abnormalities directly affect meiosis, gametogenesis, and the viability of zygotes and embryos. In some instances, balanced structural rearrangements can be transmitted, causing fertility problems in subsequent generations.

Traditionally, the chromosomal abnormalities were reported basically by conventional methods (banding of chromosomes) by which it was difficult to detect specific causes of aberrations and to predict accurately bull fertility. The new molecular technologies like probe-based fluorescence in situ hybridization (FISH), sequencing and microarray may be applied to identify minor chromosomal abnormalities so that they can be correlated with infertility problems. In this respect, a method based on deep sequencing of sperm microRNA (miRNA) and Piwi-interacting RNA (piRNA) from individual bulls was recently developed by Capra *et al.* (2017). The specific miRNA expression was differentially represented in low versus high motile sperm, indicating an alteration of cell functions and increased germ cell apoptosis in the low motile fraction (Raudsepp and Chowdhary, 2016).

Currently, traditional cytogenetics is interfaced with advanced genomics approaches, such as array technologies and next-generation sequencing, to enrich our knowledge about animal genomes and consequently improve our understanding of connections between gross structural or molecular chromosome variations and reproductive disorders in domestic animals.

Generally speaking, significant differences in chromosomal aberrations and semen quality were found in the present study between low and normal semen quality categories. It can be concluded the possible association of semen characteristics with chromosomal aberrations, and subsequently the ability to incorporate it in early selection schemes for fertility in the Egyptian buffalo bulls. The breeding buffalo bulls kept for semen production have to be screened for cytogenetic profile and the sperm membrane integrity measured as HOST.

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إرتباط التشوهات الكروموسومية وجودة السائل المنوى فى ثيران الجاموس المصرى

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يعتمد إنتاج وتصنيع الألبان فى مصر بشكل أساسى على الجاموس والأبقار. توجد أسباب عديدة تبرر العقم وإنخفاض الأداء التناسلى فى الماشية، حيث تعتبر التشوهات الكروموسومية أحد أهم الأسباب. أجريت هذه الدراسة لبحث مدى الإرتباط بين خصائص السائل المنوى والتركيب الوراثى الخلوى كمؤشر مبكر للخصوبة فى ثيران الجاموس المصرى. تم جمع البيانات والعينات خلال شهرى سبتمبر وأكتوبر ٢٠١٥ لعدد ١١٦ قذفة منوية مُجمعة من ٢٩ ثور تم إختيارها عشوائياً (العمر ٢٢ ـ ٢٦ شهرا، وزن الجسم ٢٥٠ ـ ٣٠٠ كجم). صُنيفت الحيوانات إلى مجموعتين على أساس جودة السائل المنوى: المجموعة الأولى (ج١) تألفت من ١٠ حيوانات ذات خصائص سائل منوى منخفضة، بينما إشتملت المجموعة الثانية (ج٢) على ١٩ ثور ذات خصائص سائل منوى عادية (متوسطة). كما تم فحص عدد ٥٠ خلية فى طور الانقسام الميتوزى لكل حيوان على حده. شكل تضاعف عدد الكروموسومات ٢.١٪ من التشوهات العددية الإجمالية فى ج٢ مقابل ٩.٦٪ فى ج١، فى حين مثل عدم توازن عدد الكروموسومات نسبة ٠.٤٪ و٥٪ فى ج٢ و ج١، على الترتيب. إختلفت ج١ و ج٢ معنوياً (٠.٠٥ > P) فى النسبة المئوية لتضاعف عدد الكروموسومات وعدم توازن عدد الكروموسومات. إشتملت التشوهات الكروموسومية التركيبية التى تم تحديدها على كل من الحذف، الكسر، الفجوة، التجزئة، الحلق، الإنفصال السنترومبرى، الإتحاد السنترومبرى، والتى إختلفت معنوياً (٠.٠٥> P) بين ج١ و ج٢. هذه التشوهات الكروموسومية فى طور الإنقسام الميتوزى قد تكون لها صلة مباشرة بالخصوبة، حيث كانت متسقة وثابتة. كانت كل تقديرات الإرتباط الوراثى معنوية (٠.٠٥ > P)، بإستثناء تلك المتعلقة بالإنفصال السنترومبرى. إرتبطت التشوهات الكروموسومية سلبياً مع الحركة (٪)، الحيوانات المنوية الحية (٪)، الإنتفاخ الإسمورى الضعيف (٪) ومستوى هرمون التستوستيرون، فى حين كانت معاملات إرتباط التشوهات الكروموسومية مع شذوذ الحيوانات المنوية إيجابية. تراوحت معاملات الإرتباط الوراثى من ٠.٣٩١- بين الحركة وتضاعف عدد الكروموسومات إلى ٠.٣٤١ بين شذوذ الحيوانات المنوية وتضاعف عدد الكروموسومات. تشير النتائج المتحصل عليها إلى أهمية فحص ثيران الجاموس لتحديد التشوهات الكروموسومية قبل إستخدامها فى التلقيح الطبيعى أو الإصطناعى.