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# Characteristics, Yield, Flow Cytometer, DNA Fragmentation, and Comet Assay Parameters of Goat Spermatozoa in Semen of Zaraibi and Baladi Bucks at Young and Old Ages

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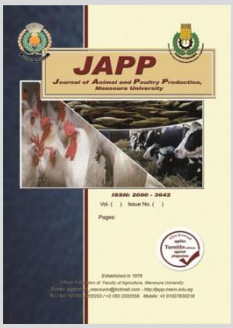


## ABSTRACT

The aim of this study was to determine goat breed (Zaraibi and Baladi) or buck age (young and old) effect on characteristics, production, flow cytometer, DNA fragmentation and comet assay parameters of spermatozoa. Twelve Baladi (BG) and Zaraibi (ZG) bucks, 3 old (2-4 years) and 3 young (8-12 months) from BG or ZG used for semen collection for 24 weeks. Volume (EV), sperm progressive motility (PM), livability (LS), abnormality (SA), and concentration (SCC) were evaluated, sperm outputs (SO) were calculated. Sperm apoptosis, DNA fragmentation and comet assay were analyzed. Results revealed that PM, LS, SA, SCC, total, motile, live, normal, and functional SO per ejaculate were significantly higher in ZG than BG and in old than young bucks in each breed. Both EV and LS were significantly higher, while SCC was significantly lower in old than young ZG. Each of EV, PM, and SCC was significantly higher, while SA was significantly lower in old than young BG. Viable sperm, haploid and cell cycle percent was significantly higher, while early apoptotic, apoptotic, and necrotic spermatozoa percent was significantly lower in old than young bucks of each breed. Spermatid percent in ZG and diploid percent in BG were significantly higher in old than young bucks. Tailed sperm, tail length, tail DNA percentage, and tail moment were significantly lower in ZG than BG, and in old than young bucks of each breed. It can be stated that Zaraibi bucks has a good potential for semen production than Baladi breed.

***Keywords:*** Semen, goats, breed differences, sperm function, apoptosis, DNA damage.

## INTRODUCTION

For centuries, goat was not only an economic animal for people living in arid regions, but was also valued as a good source of meat and milk. In developing countries, goat provides is an important source of milk and meat. Goat reproductive, genetic and environmental factors are factors affecting the productive efficiency (Hossain *et al.*, 2004; Song *et al.*, 2006). Reproductive performance male goat before mating is important to achieve breeding success (Ford *et al.*, 2009). Libido and semen characteristics are limited factors for male reproduction, being different in relation to breed (Karagiannidis *et al.*, 2000) and age (Mahal *et al.* 2013).

In goats, proper selection of breeding buck for the natural mating or artificial insemination (AI) is needing for goat breeding, especially for AI, which require in to increase and disseminate genotypes of quality breeding stock and improve a herd (Ngoma *et al.* 2016). The breeding buck examination is important to produce good fertility of a flock. This process includes semen evaluation as managerial practice in AI breeding program of goats (Gore *et al.*, 2020).

Genetic, hormonal, and spermatogenesis disorders, poor semen quality, and damage of DNA to spermatozoa are attributed to decreased male fertility (Esteves et al., 2012). The sperm DNA fragmentation routes and cell death were reported to be not fully caspase dependent (Cande et al., 2004). Variety of assays have been developed to detect DNA fragmentation levels including terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay (Mitchell et al., 2011), comet assay (Lewis et al., 2008), DNA breakage detection fluorescence in situ hybridization (DBD-FISH) (Fernández et al., 2000), potential of oxidative reduction (Agarwal et al., 2017) assays DNA damage and chromatin structure of spermatozoa (Fernández et al., 2005).

Both Egyptian Baladi (BG) and Zaraibi (ZG) goats are mostly found in Nile valley and delta (Galal et al., 2005). It is well known that ZG is related to the Egyptian Nubian breed, in the same time it considers as the most important native goat breed evaluated on the basis of milk and meat production (Marai et al., 2002; Galal et al., 2005). In Egypt, the Zaraibi goat, as a native breed, has a high genetic potential for production of milk (Dwidar et al., 2018). In developing countries, programs of genetic selection in dairy goats are still uncommon, but they have been great successful for improvement of breeding strategies for production of milk and meat from goat (Shrestha and Fahmy, 2007; Vakka et al., 2018).

The current study aimed to evaluate the effect of goat breed (Zaraibi and Baladi) or age of buck (young and old) on characteristics, production, flow cytometer, DNA fragmentation, and comet assay parameters of spermatozoa.

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## MATERIALS AND METHODS

**Site and location of the study:**

The experimental work was conducted at Animal Production Research Station, Sakha, Kafrelsheikh Governorate, located in the northern part of Nile Delta (latitude 31º 15'N and longitude 31º 45'E), belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt, during the period from January, 2021 until June, 2021.

**Animals and feeding system:**

Twelve bucks of Egyptian local goat breed Baladi (BG) and Zaraibi (ZG) goat bucks were used in this study. Baladi goats (n=6) included 3 old bucks (2-4 years of age and 50.66±1.36 kg LBW) and 3 young bucks (8-12 months of age and 23.66±1.36 kg LBW), while ZG included 3 old bucks (2-4 years of age and 53.33±1.36 kg LBW) and 3 young bucks (8-12 months of age and 26.33±1.36 kg LBW). All bucks were used for semen collection by artificial vagina.

Requirements of feeding were determined after to the recommendations of APRI, Ministry of Agriculture, Egypt. Each buck was fed Concentrated Feed Mix, including crude protein (14%), total digestible nutrients (%), plus four kg of fresh Egyptian alfalfa (*Trifolium alexandrinum*) during the period from December to February or one kg of alfalfa hay through March-June, with free access to mineral salt blocks and drinking water all the time.

**Semen collection and evaluation**

For semen collection period of 24 weeks, semen was collected once/week before feeding (7-8 a.m.) by a conventional artificial vagina. Semen ejaculates were transferred immediately after collection to the laboratory, and then placed in water bath at 37ºC.

Ejaculate volume was determined and semen was assessed for percentage of progressive sperm motility in each semen sample (10 µL diluted semen) using a phase contrast microscope supplied with a hot stage adjusted to 37 °C. Smears were prepared using nigrosin/eosin staining for determining the percentages of live and abnormal spermatozoa. The concentration of spermatozoa was determined with a Neubauer haemocytometer.

Sperm outputs (SO) were calculated ($x10^9$/ejaculate), in terms of total (TSO), motile (MSO), live (LSO), normal (NSO) and functional (FSO) as the following:

**TSO = ejaculate volume (ml) x sperm concentration ($x10^9$/ml)**

**MSO = progressive sperm motility (%) x TSO**

**LSO = live sperm (%) x TSO**

**NSO = (sperm abnormality (%) – 100) x TSO**

**FSO = TSO x sperm motility (%) x sperm livability (%) x normal sperm (%)**

**Sperm apoptosis:**

Phosphatidylserine (PS), a protein ordinarily found in the inner leaflet of the plasma membrane of healthy cells, travels to the outer layer of the plasma membrane during the early stages of apoptosis, where it is exposed to the cell's outer surface. Anexin-V binds to PS with a high affinity, and annexin staining with fluorochrome V is utilised to detect apoptosis (Plesca *et al.*, 2008).

For acquisition and analysis, flow cytometry was performed on an Accuri C6 cytometer (BD Biosciences, San Jose, CA) with Accuri C6 software (Becton Dickinson) (Masters and Harrison, 2014). The sperm subpopulations were classified as percentage of viable sperm (A-/PI-), early sperm (A+/PI-), apoptotic sperm (A+/PI+), and apoptotic sperm (A-/PI-) based on flow cytometry results. Spermatozoa that have died (A-/PI+) (Chaveiro *et al.*, 2007).

**Cellular morphology and DNA fragmentation (Cell Cycle method):**

The capacity of flow cytometry to determine cellular DNA concentration is reliant on the fluorometry of dyes that bind to DNA in a stoichiometric manner. Ploidy DNA is a measure of the DNA content of the cells under inquiry as a percentage of the normal (diploid) control. Due to the fact that DNA content repeats before cell division, mathematical models have been developed to predict the percentage of cells in various phases of the cell cycle (G0/1, S-phase, G2/M), diploid and aneuploid cycles, CV, and death ratio. Apoptotic cells, DI, diploid percentage, and anueploid percentage are all terms used to describe cells that have reached the end of their life cycle (Spyratos, 1993).

The sub-G1 approach for diagnosing cell death is based on the idea that during extended fixation, fragmented low molecular weight DNA is liberated from cells after DNA internal cleavage. This would result in a population of cells that bind the quantitative DNA pigment, PI, to a lesser level than G1 cells; G1 is the longest phase of the cell cycle, and as a result, the majority of cells are in G1. As a result, a cluster of cells to the left of the G1 apex will form.

One cc of ice-cold 100% alcohol per tube was used to fix lymphocytes or tissue cells, which were then stored at +4 °C until analysis. The material was centrifuged again at least 12 hours after fixing to eliminate the excess ethanol (Vindelov, 1977). In a 15-ml (2095) Falcon Compound tube, the cell suspension (200 l) was put in citrate solution. During preparation, storage, and staining, the propidium iodide solution was shielded from light with foil. In another 5 ml tube, the solution was combined and the sample was filtered through a 30 mm pore diameter nylon mesh filter to remove nuclear masses (12 x 75 mm, cat. no. 2058, Falcon Compounds). Within 1 hour of adding propidium iodide, samples were run using a flow cytometer.

MODFIT DNA analysis programme (verity software house, Inc. Po Box 247, Topsham, ME 04086 USA, version: 2.0 power Mac with 131072 KB Registration number: 42000960827-16193213) was used to analyse the data. Manufacturing date: September 16, 1996). This software calculated the coefficient of variation (CV) around the G0/G1 peak, the percentage of apoptosis, the DNA index (DI), and the percentage of cells in each Phase (G0/G1, S, and G2/M) of the DNA cell cycle for each sample, as well as DNA ploidy (diploid cycle and aneuploid cycle percent).

**The comet assay analysis:**

Comet assay based on the method of Badawy et al. (2018) was conducted. A fluorescent microscope with a 40x magnification and a 420-490 nm excitation filter was used to examine the migration patterns of DNA fragments from 100 cells/sample. Kinetic Imaging Ltd.'s Komet 5 image analysis software was used. (Liverpoo1, UK) used a CCD camera to measure the length of DNA migration and the proportion of migrating DNA to determine the quantitative

and qualitative amount of DNA damage in cells. The tails of the comets were measured from the middle of the nucleus to the tail's termination.

**Fertility trial:**

Totally, 126 healthy mature goats (71 Zaraibi and 55 Baladi) were naturally mated during September breeding season (from 1st to end of September). Conception rate (Number of lambed goats/number of inseminated goats) x100 and Litter size (LS)/ goat (Number of born lambs/number of lambed goats) according to Zonturlu *et al.* (2011) were considered as an index of fertility and reproductive performance.

**Statistical analysis:**

Data were statistically analyzed by T-test to compare between the two breeds, two ages, and two ages within each breed using computer program SAS (2004).

## RESULTS AND DISCUSSION

**Results**

**Physical semen characteristics:**

Physical semen characteristics of BG and ZG of young and old bucks are presented in Table 1. Results revealed that semen parameters including the percentage of progressive motility (PM), livability (LS), abnormality (SA), and sperm cell concentration (SCC) were better (P<0.0001) in ZG than in BG, but ejaculate volume (EV) was not affected (P≥0.05) by goat breed. As affected by age, EV (P<0.0001) and LS (P<0.05) were higher, while SCC was lower (P<0.05) in old than in young of ZG, but PM and SA were not affected by age. However, EV (P<0.001), PM, and SCC (P<0.01) were higher, while SA was lower (P<0.05) in old than in young of BG, but LS was not affected by age.

**Table 1. Effect of breed (Zaraibi and Baladi) and age (Young and old) on physical semen characteristics of goat bucks.**

| Sperm variable | Breed | | P-Value | Zaraibi | | P-Value | Baladi | | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | Zaraibi | Baladi | | Old | Young | | Old | Young | |
| EV (ml) | 0.82±0.05 | 0.70±0.05 | 0.11 | 1.03±0.05 | 0.62±0.04 | 0.0001 | 0.91±0.05 | 0.50±0.04 | 0.0001 |
| PM (%) | 80.33±0.75 | 67.83±0.74 | 0.0001 | 81.67±1.05 | 79.00±1.00 | 0.0771 | 70.00±0.84 | 65.67±0.95 | 0.0021 |
| LS (%) | 79.23±0.85 | 69.83±1.16 | 0.0001 | 80.93±1.03 | 77.53±1.24 | 0.0442 | 71.06±1.52 | 68.60±1.76 | 0.2997 |
| SA (%) | 11.16±0.63 | 20.36±0.82 | 0.0001 | 10.80±0.86 | 11.53±0.93 | 0.5702 | 18.53±1.21 | 22.20±0.92 | 0.0228 |
| SCC (x$10^9$/ml) | 2.48±0.68 | 1.85±0.80 | 0.0001 | 2.61±0.09 | 2.34±0.080 | 0.0410 | 2.07±0.11 | 1.63±0.08 | 0.0047 |

**Sperm outputs per ejaculate:**

Results in Table 2 show that sperm outputs in terms of total, motile, live, normal, and functional per ejaculate was higher (P<0.001) in ZG than in BG, and in old than in young bucks in each breed.

**Table 2. Effect of breed (Zaraibi and Baladi) and age (Young and old) on sperm outputs in ejaculate of goat bucks.**

| Sperm Output | Breed | | P-Value | Zaraibi | | P-Value | Baladi | | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | Zaraibi | Baladi | | Old | Young | | Old | Young | |
| Total | 1.98±0.98 | 1.35±0.13 | 0.0006 | 2.40±0.08 | 1.61±0.12 | 0.0001 | 1.93±0.17 | 0.78±0.06 | 0.0001 |
| Motile | 1.59±0.85 | 0.93±0.10 | 0.0001 | 1.92±0.08 | 1.27±0.09 | 0.0001 | 1.36±0.12 | 0.51±0.04 | 0.0001 |
| Live | 1.57±0.86 | 0.96±0.10 | 0.0001 | 1.90±0.08 | 1.25±0.09 | 0.0001 | 1.38±0.13 | 0.54±0.05 | 0.0001 |
| Normal | 1.76±0.91 | 1.09±0.11 | 0.0001 | 2.09±0.08 | 1.43±0.11 | 0.0001 | 1.58±0.14 | 0.61±0.05 | 0.0001 |
| Functional | 1.13±0.72 | 0.54±0.67 | 0.0001 | 1.40±0.08 | 0.87±0.06 | 0.0001 | 0.80±0.09 | 0.28±0.02 | 0.0001 |

**Sperm flow cytometry parameters:**

Sperm flow cytometry parameters of ZG and BG in young and old bucks are shown in Table 3 and illustrated in Fig. 1. Results revealed insignificant differences in the percentage of viable, early apoptotic, apoptotic, and necrotic spermatozoa between the two goat breeds. However, percentage of viable spermatozoa was higher (P<0.0001), while the percentage of early apoptotic, apoptotic, and necrotic spermatozoa was significantly lower in old than in young bucks of each breed.

**Table 3. Effect of breed (Zaraibi and Baladi) and age (Young and old) on sperm flow cytometer percentages of goat bucks.**

| Item | Breed | | P-Value | Zaraibi | | P-Value | Baladi | | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | Zaraibi | Baladi | | Old | Young | | Old | Young | |
| Viable | 81.95±1.44 | 79.70±1.47 | 0.28 | 87.74±0.39 | 76.16±0.53 | 0.0001 | 85.48±0.55 | 73.91±0.77 | 0.0001 |
| Early apoptosis | 4.15±0.38 | 4.26±0.40 | 0.84 | 2.85±0.23 | 5.45±0.39 | 0.0001 | 3.10±0.31 | 5.43±0.49 | 0.0011 |
| Apoptosis | 6.29±0.37 | 6.20±0.45 | 0.87 | 5.50±0.36 | 7.08±0.54 | 0.0279 | 4.70±0.33 | 7.70±0.46 | 0.0001 |
| Necrosis | 7.59±0.97 | 9.83±0.84 | 0.09 | 3.90±0.45 | 11.28±0.65 | 0.0001 | 6.71±0.41 | 12.95±0.64 | 0.0001 |

**Sperm DNA fragmentation:**

Sperm DNA parameters of ZG and BG in young and old bucks are shown in Table 4 and illustrated in Fig. 2. Results showed insignificant differences in the percentage of sub G1, haploid, spermatid, diploid, and cell cycle between the two goat breeds. However, percentage of sub G1 was lower (P<0.0001), while the percentage of haploid and cell cycle was higher in old than in young bucks of each breed.

Only, spermatid percentage in ZG, and diploid percentage in BG were significantly higher in old than in young bucks.

**Comet assay parameters:**

Comet assay parameters of sperm cells of ZG and BG in young and old bucks are shown in Table 5 and illustrated in Fig. 3. Results showed that percentage of untailed sperm cells was higher (P<0.001), while percentage of tailed spermatozoa, tail length, tail DNA percentage, and tail moment were lower (P<0.05) in ZG than in BG, and lower (P<0.001) in old than in young bucks of each goat breed.

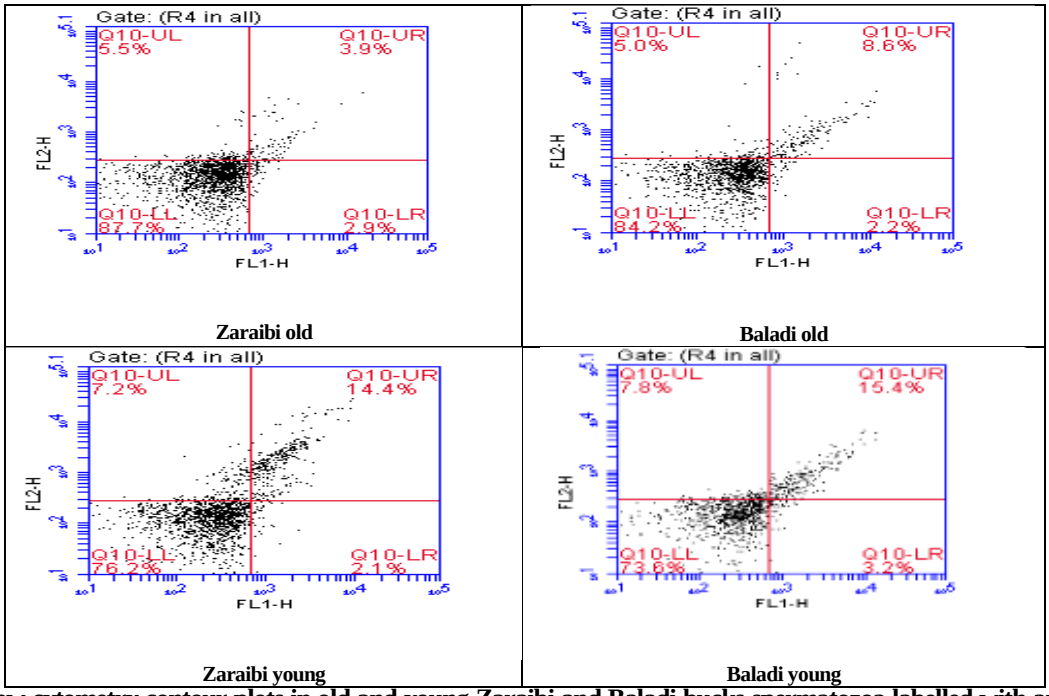


**Fig. 1. Flow cytometry contour plots in old and young Zaraibi and Baladi bucks spermatozoa labelled with annexin V-fluorescein isothiocyanate (FITC) fluorescence vs. propidium iodide (PI) fluorescence. The lower left quadrant (LL) contains viable spermatozoa which are negative for Annexin-V and exclude PI staining (A-/PI-). The lower right quadrant (LR) shows early apoptotic spermatozoa which bind Annexin-V but exclude PI (A+/PI-). The upper right quadrant (UR) represents apoptotic spermatozoa binding Annexin-V and PI (A+/PI+). The upper left quadrant (UL) represents necrotic cells excluding Annexin-V and binding PI (A-/PI+).**

**Table 4. Effect of breed (Zaraibi and Baladi) and age (Young and old) on sperm DNA fragmentation of goat bucks.**

| Parameter | Breed | | P- | Zaraibi | | P- | Baladi | | P- |
|---|---|---|---|---|---|---|---|---|---|
| (%) | Zaraibi | Baladi | Value | Old | Young | Value | Old | Young | Value |
| Sub G1 | 12.15±1.58 | 16.12±2.44 | 0.18 | 6.48±0.93 | 17.82±1.30 | 0.0001 | 6.53±0.91 | 25.72±1.24 | 0.0001 |
| Haploid | 79.10±1.03 | 77.45±1.61 | 0.39 | 81.74±1.01 | 76.45±1.34 | 0.0063 | 83.66±0.52 | 71.24±1.06 | 0.0001 |
| Spermatid | 4.88±0.80 | 4.17±0.76 | 0.52 | 7.15±0.93 | 2.61±0.78 | 0.0018 | 5.41±0.94 | 2.93±1.09 | 0.1066 |
| Diploid | 3.86±0.78 | 2.25±0.58 | 0.10 | 4.61±0.66 | 3.11±1.42 | 0.3551 | 4.40±0.54 | 0.10±0.02 | 0.0001 |
| Cell cycle | 87.84±1.58 | 83.87±2.44 | 0.18 | 93.51±0.93 | 82.17±1.30 | 0.0001 | 93.47±0.90 | 74.27±1.24 | 0.0001 |

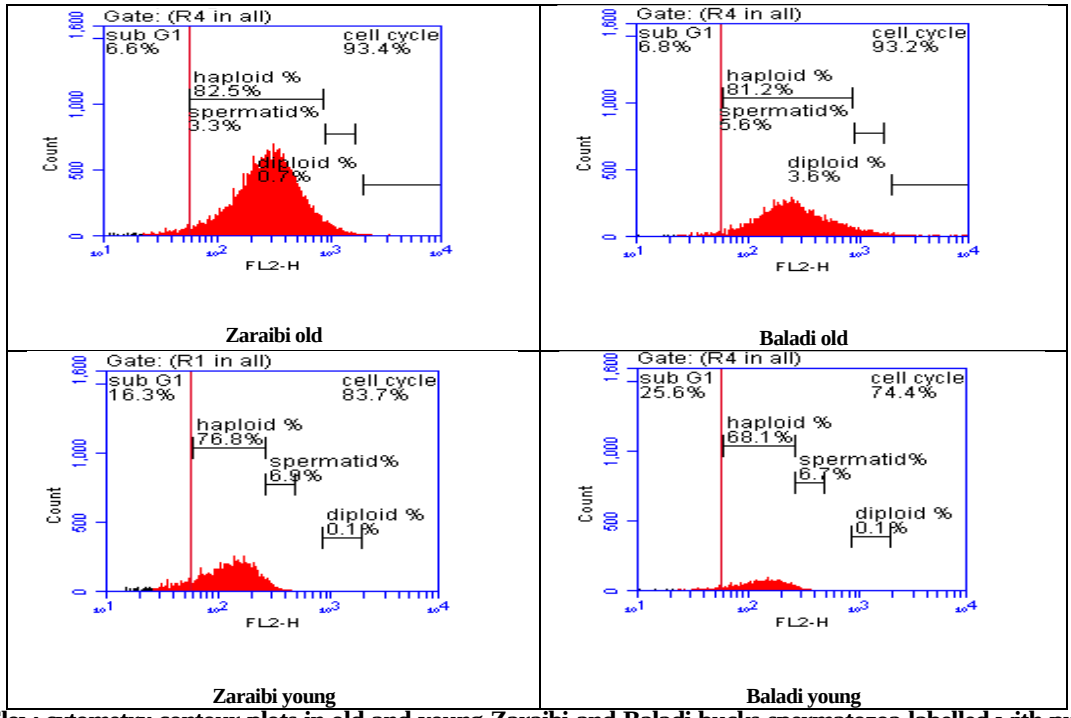


**Fig. 2. Flow cytometry contour plots in old and young Zaraibi and Baladi bucks spermatozoa labelled with propidium iodide (PI) fluorescence. Analysis of cell number % of each cell cycle phase relative to total phases, Sub-G1: represents the percentage of DNA fragmentation according to the staining of apoptotic bodies or fragments of necrotic cells in the sample. Haploid%: represents the percentage of (IN) haploid sperm (DNA content). Spermatid%: represents the round spermatid and very closed in the content of DNA (IN). Diploid%: represents three types of cells: diploid sperm (2N) (DNA content), diploid cells lymphocyte (2N) and two sticky sperm (N) represents as one sperm.**

**Table 5. Effect of breed (Zaraibi and Baladi) and age (Young and old) on sperm comet assay parameters of goat bucks.**

| Items | Breed | | P-Value | Zaraibi | | P-Value | Baladi | | P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | Zaraibi | Baladi | | Old | Young | | Old | Young | |
| Tailed (%) | 15.34±1.56 | 30.41±0.88 | 0.0001 | 9.02±0.40 | 21.66±0.52 | 0.0001 | 27.04±0.56 | 33.77±0.42 | 0.0001 |
| Untailed (%) | 84.65±1.60 | 69.58±0.90 | 0.0001 | 90.97±0.40 | 78.33±0.52 | 0.0001 | 72.95±0.56 | 66.22±0.42 | 0.0001 |
| Tail length (µm) | 5.46±0.46 | 9.64±0.32 | 0.0001 | 3.79±0.33 | 7.13±0.33 | 0.0001 | 8.69±0.35 | 10.60±0.32 | 0.0011 |
| Tail DNA (%) | 4.80±0.39 | 8.28±0.23 | 0.0001 | 3.40±0.29 | 6.21±0.29 | 0.0001 | 7.83±0.29 | 8.74±0.31 | 0.0514 |
| Tail moment | 29.32±4.45 | 80.60±4.24 | 0.0001 | 13.53±2.29 | 45.12±4.09 | 0.0001 | 68.53±4.82 | 92.67±4.10 | 0.0015 |

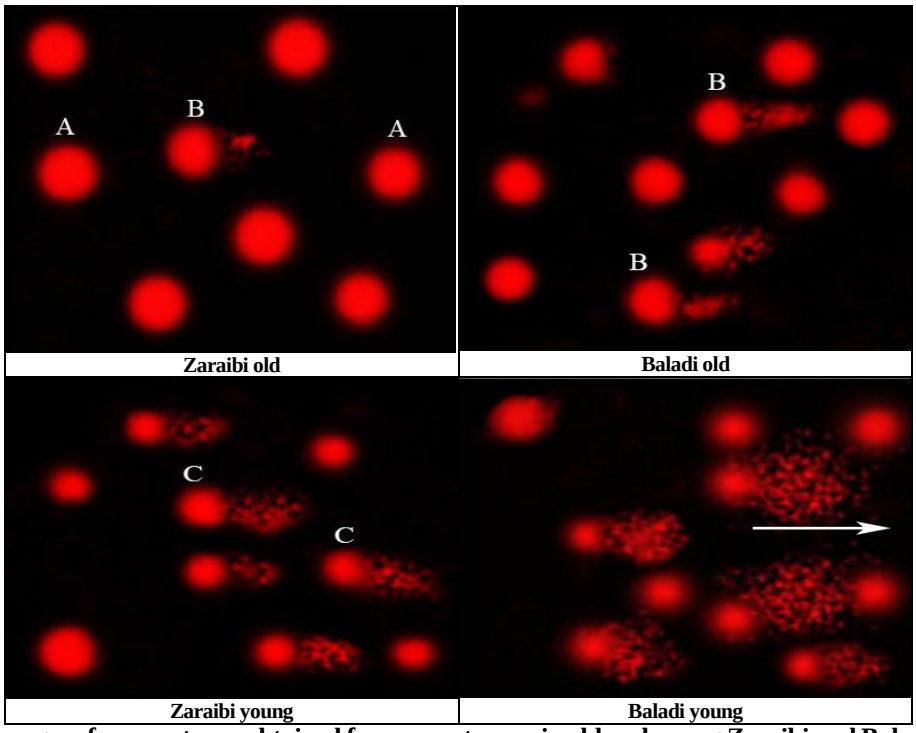


**Fig. 3. Typical images of spermatozoa obtained from comet assay in old and young Zaraibi and Baladi bucks semen. (A) Spermatozoa with intact nuclei, undamaged comet (without DNA fragmentation). (B) Spermatozoa with low damaged comet (low DNA fragmentation). (C) Spermatozoa with high damaged comet (high DNA fragmentation). Arrow (→) pointed to a degree of tail length (Ethidium bromide stain, x 400).**

**Conception rate and litter size:**

Results in Table 6 showed that conception rate and litter size of goat does mated with old bucks in the same breed were higher in ZG than in BG.

**Table 6. Conception rate and litter size of goats mated with old Zaraibi and Baladi bucks.**

| Goat Breed | Buck No. | Mated goat does | Pregnant goat does | Conception Rate | No. of Lambs | Litter size |
|---|---|---|---|---|---|---|
| Zaraibi | 1 | 23 | 21 | 91.30 | 46 | 2.19 |
| | 2 | 23 | 19 | 82.61 | 38 | 2 |
| | 3 | 25 | 21 | 84.00 | 42 | 2 |
| Total | | 71 | 61 | 85.9 | 126 | 2.07 |
| Baladi | 1 | 17 | 15 | 88.24 | 31 | 2.06 |
| | 2 | 20 | 15 | 75.00 | 29 | 1.93 |
| | 3 | 18 | 13 | 72.22 | 23 | 1.76 |
| Total | | 55 | 43 | 78.2 | 83 | 1.93 |

**Discussion**

Semen quality, in terms of semen volume, sperm cell concentration and sperm motility, are among the most important variables estimated for male fertility centers (García-Ferreyra, 2015). In our study, there were insignificant differences in EV between ZG and BG EV. In old BG bucks, EV was 0.91±0.05 ml, being nearly similar to the ejaculate volume of Baladi bucks (0.81±0.01 ml) as reported by El-Ruby *et al.* (2010). This value is close to those reported by Furstoss *et al.* (2009) and Barkawi *et al.* (2006). Also, EV of ZG old bucks is within a range from 0.92 to 1.27 ml in different goat breeds were recorded by Al-Ghalban *et al.* (2004). In accordance with the present results, Aller *et al.* (2012) indicated that breed of ram had no effect (P >0.05) on the semen volume. However, Hassanin *et al.* (2013) reported significant (P<0.05) breed effect, for Najdi and Harri breeds, on EV (1.02±0.01 and 0.96±0.01ml, respectively). Although the present study indicated significant effect of goat age on EV, being higher with old than young bucks, some authors found that age did not influence semen volume of bucks (Gore *et al.,* 2020) or rams (Tabbaa *et al.,* 2006). Age is a phenomenon, being in paralleling in association with different reproductive risks such as decreased testis volume, changes in testicular tissue, lower B/FSH ratio compatible with reduced Sertoli cell mass (Mahmoud et al., 2003). Semen quality including PM and SCC are important variables for normal fertilization, serving as an indirect measure of metabolic activity and sperm vitality (Marcus-Braun et al., 2004). Several studies showed that very low sperm motility was a good predictor of poor fertilization in IVF or ICSI (Freeman *et al.,* 2001). The differences observed in sperm motility parameters between ZG and BG were also reported between goat breeds (Nelson, *et al.* 1987), sheep breeds (Kubovičová *et al.,* 2011). Also, Ayoub *et al.* (2013) found that a significant difference in SCC between Awassi (imported from Syria) and local Awassi in Egypt. However, mass motility and SCC were not significantly affected by breed for Najdi and Harri breeds, respectively (Hassanin *et al.,* 2013). In agreement with the present results, buck age affect PM and SCC, bucks aging 1-2 years showed lower PM and SCC than bucks aging 3-6 years (Gore *et al.,* 2020). Moreover, SCC elevated with in

old bucks (Al-Ghalban *et al.* 2004; Mahal *et al.* 2013) and in old rams (Benia *et al.,* 2018). However, PM and SCC were not affected by age. Generally, age, breed, nutrition and production system were found to affect semen quality (Kridli *et al.,* 2005; Tabbaa *et al.,* 2006).

In accordance with the breed differences between ZG and BG, Abdel-Rahman *et al.* (2000) found significant effect of breed on LS percentage in Najdi and Naemi breeds, being higher in than in Merino, Somalian and Sudanese breeds. Despite the significant effect of age on LS of BG bucks and SA in ZG bucks, some authors reported insignificant effect of age on live sperm cells and normal morphology in goats (Gore *et al.,* 2020) and rams (Tabbaa *et al.,* 2006). Total sperm output per ejaculate is very important factor affecting fertility and depends on both EV and SCC. Factors affecting the EV can also affect the sperm concentration and in turn total sperm output per ejaculate (El-Maghraby, 2007). Also, Kridli *et al.* (2005) reported negative correlation between SCC and EV. These relationships are pronounced in each breed and with advancing age of goat bucks. It is of interest to note that the results of sperm flow cytometry parameters were not affected by goat breed, bur are in parallel with physical semen characteristic in old and young bucks. Viable sperm percentage increased, while sperm early apoptosis and apoptosis, and necrosis percentages were lower in old than in young bucks. Both apoptosis and necrosis are two cell death types. Necrosis of injury cells causes swelling and membrane rupture of the cells, but apoptosis affects single cells without any accompanying inflammation in the surrounding tissues (Wyllie et al., 1980).

Various divisions (mitotic and meiotic), mature haploid and polarized sperm cells from a diploid spermatogonium occurred during spermatogenesis as spermatocytogenesis and spermiogenesis (Kumaresan *et al.,* 2020). During the latter part of spermatogenesis, the Our results indicated slight differences in spermatogenesis within the seminiferous tubules between both goat breeds. However, there are more development processes may be occurred by age advancing between old and young bucks in both breeds, being more pronounced in ZG than in BG bucks.

Results of comet assay parameters indicated higher sperm DNA fragmentation in BG than in ZG, and in young than in old bucks, in terms of decreasing percent untailed sperm and increasing percent tailed sperm, tail length, tail DNA, and tail moment. The sperm DNA integrity is central to the transmission of genetic information during reproduction and DNA damage can a result in paternal fertility problems (Evenson *et al.,* 2002; Agarwal and Said, 2003). The tail DNA percentage is a marker of the intensity of the relative fluorescent in head and tail of sperm cells (Collins, 2004), while tail moment is a measurement of the genetic material migration, the relative amount of DNA in the tail, and a product of the tail length and tail DNA percentage (Lovell and Omori, 2008). Tail length and comet length were positively correlated with the tail moment (Gliozzi *et al.,* 2011). Generally, Hourcade *et al.,* (2010), suggested that defects in DNA fragmentation are likely to inhibit sperm motility, as a result of changes in the overall sperm morphology, and eventually impede fertilization or subsequent embryo development (Fatehi *et al.,* 2006).

It has also been mentioned that DNA integrity of spermatozoa may be a more marked marker of sperm function in contrast to standard semen analysis (Saleh et al., 2003; Avdatek et al., 2010), and may be more reliable for predicting potential fertility. Semen fertility using a combination of laboratory tests to predict the characteristics of different sperm. It has been documented that loss of chromatin integrity is associated with sperm morphological abnormalities (Tavalaee et al., 2009), loss of viability and progressive motility (Ozmen et al., 2007), and decreased concentration and maturation of sperm (Virro et al., 2004).

Furthermore, there is a strong relationship between chromatin integrity loss and implantation or spontaneous abortion (Carrell and Liu, 2001). Therefore, sperm cells will later support the embryonic development pregnancy maintenance (Kumaresan *et al.,* 2020). DNA fragmentation is the hallmark of apoptosis (Nagata *et al.,* 2003), providing a basis for the development of two types of flow cytometric assays to identify apoptotic cells (Huang *et al.,* 2005). This finding was observed in each breed and by advancing age, whereas increasing DNA fragmentation was associated with elevating percentage of apoptotic spermatozoa. Since DNA fragments are lost from apoptotic nuclei and DNA content can be easily measured by flow cytometry, after staining DNA with specific fluorochromes (Nunez, 2001), methods have been developed to perform a quantitative assessment of apoptotic nuclei. At a late stage of apoptosis, DNA may be lost due to elimination of apoptotic bodies containing chromatin fragments (Halicka et al., 2000).

Simultaneous measurement of DNA and RNA in the cells makes it possible to distinguish whether apoptosis is preferable to G1 or G0 cells (Halicka et al., 2002). However, sub-G1 DNA content cannot be used as a single marker of apoptotic cells because DNA fragmentation into micro- or mononuclear fragments does not always occur during apoptosis (Darzynkiewicz et al., 2001). Consistent with the results obtained by García-Ferreyra et al. (2012), the DNA fragmentation, progressive motility, and morphology of sperm cells are associated with advanced paternal age. The effect of DNA integrity of spermatozoa on the fertility and semen quality has been studied in stallion (Neuhauser et al., 2019), pig (Khezri et al., 2019), and ram (Falchi et al., 2018; Peris-Frau et al., 2019). In this way, Belloc et al. (2009) found significant increase in DNA damage of spermatozoa with male age progress. A stronger inverse correlation was reported between DNA damage and SCC (Irvine *et al.,* 2000) or sperm motility (Saleh *et al.,* 2003). A higher fragmentation of DNA was found in younger than old animals. Fortes *et al.* (2012) found that Nellore bulls aging 1.8–2 y and 8–14.3 y were more susceptible to DNA integrity than bulls aging 3.5–7 y. The young bulls exhibited more defective protamination than old bulls and old bulls had more nuclear oxidative damage (Carreira *et al.,* 2017). Sperm DNA integrity had an important role in male fertility, which is in association with presence of undeveloped sperm cells, in terms of proximal cytoplasmic droplets and change in shape of sperm head (Boe-Hansen *et al.,* 2018). These findings revealed superiority of ZG bucks in sperm characteristics and cleared an indication that these semen characteristics improve with increasing age of bucks in each goat breed (Gore *et al.,* 2020). The difference may be attributed to breed variation (Nelson, *et al.* 1987) and advancing age (Al-Ghalban *et al.,* 2004) in terms of testicular volume, testosterone level, and spermatogenesis. The superiority of Zaraibi bucks in physical

characteristics of semen may be related indirectly to the role of testosterone on spermatogenesis (Massoud *et al.* (1991). Rate of sperm production was found to be affected mainly by testicular size. These effects may be attributed to alterations in the seminiferous tubules size and spermatogenesis efficiency (Abdel-Khalek et al., 2000). Effects on sperm function are accompanied by changes in the endocrine testicular function (testosterone or inhibin). Improving some physiological characteristics of semen may be related indirectly to the role of testosterone on spermatogenesis (Massoud *et al.* (1991).

Litter size is an important marker for the fertility and reproduction performances in sheep and goat (Cao *et al.* 2011; Tang *et al.* 2012; Dinçel *et al.* 2015). In comparing with other farm animals, goats have a high reproductive efficiency with the potential for increasing the litter size and shortening the interval between generations (Safari *et al.*, 2007). Viability, intact acrosome, and neutral comet assay variables pronouncedly affect male fertility (Ahmed *et al.*, 2016). Also, sperm DNA damage was lower in fertile males compared to infertile males (Kumaresan *et al.*, 2017). In bulls, infertile males showed >2-fold higher DNA damage than fertile bulls (Kumaresan *et al.*, 2017). Several assays of sperm DNA fragmentation was reported in human (McQueen *et al.*, 2019), bull (Waterhouse *et al.*, 2006), boar (Peña *et al.*, 2019), and stallion (Ferreira *et al.*, 2018). Based on the above mentioned findings, ZG does showed higher conception rate and litter size than BG does. This may be associated with the superiority in semen quality or breed differences in female of both breeds.

## CONCLUSION

Our results indicated breed and age differences in semen quality of Zaraibi and Baladi goat bucks. Comparatively, Zaraibi bucks showed better semen variables than Baladi bucks in terms of output, viability, apoptosis, and intact DNA of spermatozoa. All semen characteristics and sperm variables were developed in old than in young bucks, being showing more development in Zaraibi than in Baladi bucks. It can be stated that Zaraibi bucks has a good potential for semen production than Baladi breed.

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## خصائص وانتاجية ومقاييس التدفق الخلوي وتفتت الحمض النووي وفحص المذنب للسائل المنوى لذكور الماعز الزرايبى والبلدى فى الاعمار الصغيرة والناضجة

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هدفت الدراسة الحالية إلى تقييم تأثير سلالة الماعز (الزريبي والبلدي) أو عمر الذكر (عمر سنة وناضج) على الخصائص والإنتاج ومقاييس التدفق الخلوي وتفتت الحمض النووي ومقاييس فحص المذنب للحيوانات المنوية. تم استخدام اثني عشر من الماعز البلدي والزريبي في هذه الدراسة حيث تتضمن ثلاثة ذكور ناضجة من كل من الماعز البلدي والزريبي (من ٢ إلى ٤ سنوات) و ثلاثة ذكور صغيرة من كل من الماعز البلدي والزريبي (من ٨-١٢ شهرًا). تم استخدام جميع الذكور لجمع السائل المنوي مرة واحدة أسبوعياً عن طريق المهبل الصناعي لمدة ٢٤ أسبوعًا وتم الاحتفاظ بها تحت نفس الظروف الغذائية والبيئية. تم تقييم السائل المنوي من حيث الحجم و التركيز ونسب الحركة التقدمية للحيوانات المنوية والحى والميت والشواذ ثم تم حساب مخرجات السائل المنوى. تم فحص عينات السائل المنوي من خلال التدفق الخلوي (موت الخلايا المبرمج للحيوانات المنوية) و فحص التشكل الخلوي وتفتت الحمض النووي (طريقة دورة الخلية) وسلامة الحمض النووى (طريقة فحص المذنب). أظهرت النتائج أن نسب الحركة التقدمية و الحيوانات المنوية الحية والشواذ و كذلك التركيز كانت أفضل بكثير في الذكور الزرايبى من الذكور البلدى. كان كل من حجم القذفة و نسبة الحيوانات المنوية الحية أعلى بشكل ملحوظ بينما كان التركيز أقل بشكل ملحوظ في السائل المنوى لذكورالماعز الزرايبى الناضجة من الصغيرة. كان كل من حجم القذفة و نسبة الحركة التقدمية والتركيز أعلى بشكل ملحوظ بينما كان نسبة الشواذ أقل بشكل ملحوظ في السائل المنوى لذكورالماعز البلدى الناضجة من الصغيرة. كانت مخرجات السائل المنوى الكلى والحركي والحي والطبيعي والوظيفي لكل قذفة أعلى بشكل ملحوظ في الذكور الزرايبى من الذكور البلدى وأعلى في الذكور الناضجة من الصغيرة في كل سلالة. كانت النسبة المئوية للحيوانات المنوية الحية أعلى بشكل ملحوظ في حين كانت نسبة الحيوانات المنوية الميتة ومبكرة الموت وذات الموت المبرمج أقل بكثير في الذكور الناضجة من الصغيرة في كل سلالة. كانت النسبة المئوية للحيوانات المنوية فى شكل سبرماتيد في ذكورالماعز الزرايبى والنسبة المئوية للحيوانات المنوية ثنائية الحمض النووى في ذكورالماعز البلدى أعلى بشكل ملحوظ في الذكور الناضجة من الصغيرة. كانت النسبة المئوية لخلايا الحيوانات المنوية بدون مذنب أعلى بشكل ملحوظ بينما كانت نسبة الحيوانات المنوية ذات مذنب وطول الذيل ونسبة الحمض النووي فى المذنب أقل بكثير في الذكور الزرايبى من الذكورالبلدى وفي ذكورالماعز الناضجة فى كل سلالة. توجد اختلافات في جودة السائل المنوي لاختلاف السلالة والعمر في ذكور الماعز الزريبي والبلدي. يمكن القول أن ذكور الماعز الزريبي لديها إمكانيات جيدة لإنتاج السائل المنوي من ذكور الماعز البلدي.