Semen Quality and Testicular Ultrasound Features of Heat Stressed Bulgarian Murrah Buffalo Bulls Subjected to Fan and Sprinkler Cooling

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ABSTRACT

The study aimed to determine the monthly changes, and the effect of fan and sprinkle cooling on semen quality of Murrah buffalo bulls during the second and third quarter months of the year and to determine monthly changes and effect of fan and sprinkler cooling in testicular tissue using ultrasonography.

The effect of month of collection and treatment was tested between a group of 6 Murrah buffalo bulls subjected to fan and sprinkler cooling and a group of 6 Murrah buffalo bulls without fan and sprinkler cooling in a 6-month trial period. Semen quality parameters which included Semen Volume (SV), Live Sperm Count (LSC), Semen Mass Activity (SMA), Semen Consistency (SCo), Semen Initial Motility (SIM), and Sperm Concentration (SC) were measured monthly. The difference between rectal and scrotal surface temperatures was determined and related to semen quality after the 13.5-day sperm production cycle. The study also determined the cost effectiveness of the fan and sprinkler cooling intervention. Furthermore, testicular ultrasonographic Echomean (Em) and Testicular Covering Thickness (TCT) were measured and compared at the start, middle and end of the six-month period of fan and sprinkle cooling. Findings were related to sperm quality of Murrah buffalo bulls.

There were no effects of months and its interaction to treatment for all parameters. There were effects of treatment for SV, LSC, SMA, SCo, and SIM. There was higher LSC (95.18$\pm$0.58 vs 81.67$\pm$1.90 %), SMA (2.77$\pm$0.07 vs 2.28$\pm$0.10), SCo (2.72$\pm$0.06 vs 2.36$\pm$0.10), and SIM (53.78$\pm$1.54 vs 43.19$\pm$2.44) in the experimental group than in the control group. There was higher SV (5.62$\pm$0.37 vs 4.63$\pm$0.08 ml) in the control group than in the experimental group. There was no effect of treatment for SC. Rectal Temperature and Scrotal Surface Temperature Difference (RTSSTD) were 5.74^{o}C for the experimental group and 5.14^{o}C for the control group. There was a tendency (P=0.1166) for Em to be lower in the experimental group (101.81$\pm$6.58) compared to the Em of the control group (116.74$\pm$4.50). TCT was comparable among the collection months. TCT was greater in the experimental group than the control group (13.44$\pm$0.24 vs 11.44$\pm$0.47 mm).

Fan and sprinkler cooling was a cost-effective way to improve semen quality of Murrah buffalo bulls.

Keywords: *cooling, heat stress, semen quality, testicular echomean*

INTRODUCTION

In the tropical Philippines, semen production by Murrah buffalo bulls can be affected by heat stress which may depress semen quality. Heat stress has detrimental effect on the semen quality of bulls [1]. Testicular tissue damage caused by heat stress alters semen quality. Visualization of testicular damage of various causes can be done using several ways. One of them is ultrasonography. Ultrasonography is usually used in assessing reproductive capacities of female animals. Ultrasonography in male animals is not commonly done to assess reproductive functions. However, there are studies that looked into the usefulness of ultrasonography to evaluate the breeding soundness of male animals. [2] looked on the use of testicular ultrasound as a non-invasive tool to identify specific testicular and epididymal lesions. [3] found that testicular ultrasound may help evaluate reproductive soundness of status of sheep rams. Seminiferous tubule calcification affects the homogeneity of testicular parenchyma during the warmest season. Homogeneity changed but not reaching the levels compatible with testicular degeneration as confirmed by semen quality maintenance throughout the year.

Various studies on the provision of cooling using fan and sprinklers during hot and dry season significantly improve semen quality. However, these findings were validated on locations with different environmental conditions. The study determined the monthly changes, and the effect of fan and sprinkle cooling on semen quality of Murrah buffalo bulls during the second and third quarter months of the year. The difference between rectal and scrotal surface temperatures was also determined and related to semen quality after the 13.5-day sperm production cycle. The study also determined the cost effectiveness of the fan and sprinkler cooling intervention. Moreover, the study determined the monthly changes and effect of fan and sprinkler cooling on testicular tissue using ultrasonography.

MATERIALS AND METHODS

Study area

The Philippine Carabao Center (PCC) - University of the Philippines Los Baños (UPLB) Station Farm is located inside the UPLB campus, near the Dairy Training and Research Institute (DTRI). It is located on the foot of Mt. Makiling, Los Baños, Laguna, Philippines with a climate described as tropical monsoon. The bull barn is situated at 14º09'08.1" N 121º14'33.5" E. Near the barn is the PCC Artificial Insemination Laboratory.

Animal care and management

Animals were kept in enclosures with an area of 16.75 square meters [dimension of 5.03 m in length (shaded part is 3.48 m; unshaded part is 1.55 m) and 3.33 m in width]. Enclosures were installed with cemented water through which were always filled with water for drinking. Feeding was done twice a day with cut and carry forage which comprised of mixture of paragrass, stargrass and napier. The farm had an on-site veterinarian to maintain the general well-being and health of the animals and to attend to medical and surgical cases.

Ethical consideration

In accordance with the ethical use of animals in research, all procedures done in the study were reviewed and approved by the Institutional Animal Care Committee (IACUC) under the University of the Philippines College of Veterinary Medicine (UPCVM) with Assigned Protocol No. 2018-0008.

Experimental design

Twelve (12) apparently healthy Murrah buffalo bulls were used in the study. All were in their mature breeding ages of three years and above. Pair sampling was done by randomly assigning bulls with relatively close ages (in months) into the experimental (EXPT) and control (CTRL) groups. The bulls weighed from 440.5 kg to 680.5 and had body condition scores (BCS) from 3.0 to 4.0 at the start of the study.

The animals were divided into two groups of six, housed into a barn with individual enclosures. One group was assigned as the experimental group, with a mounted 26 inches industrial fan (190 m^3/minute air volume) and ½ inch water sprinkle head at approximately 2.5 m over the enclosures and the other was the control group with existing housing

ventilation and shade. Water sprinkler with a 0.19 cubic meter/minute water discharge had a 1.5-meter diameter in the enclosure floor. Water temperature was recorded at 31 to 33ºC. The treatment groups were housed in the opposite ends of the barn. Animals were marked accordingly. They were acclimatized in their respective enclosures for two weeks before the start of the experiment. The animals were maintained similarly based on existing farm husbandry practices.

The trial was performed from April to September 2018. This coincided with the second and third quarter periods of the year. The barn's Relative Humidity (RH) and Ambient Temperature (AT) were measured with EasyLog EL-USB-2-LCD® data logger at 15 minutes interval. The logger was retrieved to save data and re set up monthly. Monthly Average RH and AT was derived from the statistical tool of the data logger. The monthly Average Thermal Humidity Index (THI) was computed using the formula [2]: THI = db°C-{(0.31-0.31RH)(db°C-14.4)} [where db°C = monthly average dry bulb temperature in °C and RH = monthly average relative humidity percentage (RH%) / 100].

The fan and sprinkle were switched on automatically during the day with the following schedule: [1] 8:30 to 10:00 am (continuous fan), [2] 10:00 to 11:00 am (10 minutes sprinkler and 45 minutes fan), [3] 11:00 am to 12:00 noon (60 minutes continuous fan), [4] 12:00 noon – 1:00 pm (10 minutes sprinkler and 45 minutes fan), [5] 1:00 pm to 2:00 pm (60 minutes continuous fan), [6] 2:00 pm to 3:00 pm (10 minutes sprinkler and 45 minutes fan), and [7] 3:00 pm to 4:30 pm (continuous fan).

Semen Evaluation

Semen collection of the experimental animals was done twice a week (Tuesdays and Fridays starting 5:00am) using an artificial vagina for the 6-month trial period. Each bull had two collections per collection day. Semen Volume (SV) was measured using collection tube graduation. Semen Volume (SV) is the total volume derived from the two collections. After collection, ejaculates were brought to the PCC UPLB Station AI Laboratory for routine evaluation and processing. Experienced laboratory technicians evaluated the semen using one drop of semen into a glass slide. The parameters evaluated were: (1) semen mass activity (SMA), (2) semen consistency (SCo) and (3) semen initial motility (SIM).

The following criteria were used for mass activity: + - no movement, ++ - slow swirl, +++ - fast swirl, +++* - faster swirl, and ++++ - fastest swirl. The following criteria were used for consistency: + - thin, ++ - moderate thickness, +++ - thick. Initial motility was the combination of the evaluation using mass activity and consistency. Each plus (+) sign was equivalent to a score of 10 and each asterisk (*) sign was equivalent to a score of 5. Evaluated semen with initial motility score of <55 was declared discarded semen and semen with initial motility of $\geq$60 was declared good for processing.

Sperm Concentration (SC) was measured using IMV Technologies Bovine Photometer n°1248® after 40 ul semen dilution with 3960 ul NaCl solution.

Live Sperm Count (LSC) was done once in a month (last Friday of the month) using a Nikon Eclipse E200®. At 40 x, 200 sperm cells from slides with eosin-nigrosin stained semen smear were counted and the percentage of live sperms were computed.

All parameters were expressed as monthly averages and treatment averages. Baseline values for SV, SC, SMA, SCo and SIM were computed using semen evaluation data of March and was used as reference value for derived semen quality parameters. In addition, the average Rectal Temperature and Scrotal Surface Temperature Difference (RTSSTD) was computed from measured rectal and scrotal surface temperatures for both experimental and control groups (Chapter 3 Study 1). Considering the 13.5 day sperm production cycle, SV, SC, SMA, SCo and SIM were noted from two collections after a two week period of the RT and SST measurement. The averages for SV, SC, SMA, SCo and SIM were computed for the 6-month trial and related to the RTSSTD.

Cost and benefit analysis for maintaining the experimental and control groups was done. Using technical assumptions, costs were determined in maintaining the bulls. Profit was determined by the number of straws produced by the treatment groups multiplied by the prevailing costs of semen straw. Net profit difference between the treatments was expressed as profit per bull per month.

Ultrasonography

Six bulls were purposively selected from the total of 12 bulls based on their temperament and willingness to

be scanned. Three bulls from the experimental group and three bulls from the control group were subjected to testicular ultrasonography. Animals were scanned at a specified date at the start (April 4), middle (June 25) and last (September 27) weeks of the 6-month trial period. The left testicle was scanned using Honda Electronics HS 101V® ultrasound with a HLV 155 5.0MHz 50mm rectal probe. The left testicle was the only one scanned because the willingness of the bull to be scanned was considered. The probe was placed on the middle of the posterior side of the scrotum along the transverse plane of the left testicle.

Image analysis and biometric measurements were done using Adobe Photoshop CS3Extended version 10.0. Testicular echomean was measured in three areas of the ultrasonogram. Each area consists of 25 sq mm placed in a homogenous area of the image. Using the Adobe Photoshop ruler tab as reference, the areas were located uniformly for all the ultrasonograms. Area 1 was placed 6 cm from the side and 3 cm from the top. Area 2 was placed 5 cm from the side and 4 cm from the top. Area 3 was placed 5.5 cm from the side and 5 cm from the top. Adobe Photoshop automatically measured the echomean of the three areas using the luminosity analysis feature of the histogram. Testicular covering thickness (TCT) was measured using the ruler tool of Adobe Photoshop. Testicular diameter was not measured because some of the image did not capture the through and through image of the testicular tissue. The average Echomeans (Em) and Testicular Covering Thickness (TCT) of the treatment groups were computed and compared. Findings were related to sperm quality.

Statistical Analysis

Two Factorial in Randomized Complete Block Design (SAS version 9) where month is Factor A and treatment is Factor B was used to analyze hematologic response data. Three Factorial in Randomized Complete Block Design (SAS version 9) where month is Factor A, period is Factor B and treatment is Factor C was used to analyze physiologic response data. Mean differences were tested using Least Significant Difference (LSD). All means were expressed as mean±standard error of the mean (Mean±SEM).

RESULTS AND DISCUSSION

Thermal Humidity Index (THI)

The average Thermal Humidity Index recorded monthly. The highest THI was recorded in the Month of May at 28.32 and the lowest THI was recorded in the month of July at 26.80 (Table 1).

During the duration of the study, the animals were experiencing extreme severe heat stress conditions at THI>25.6 [4]. THI increase in the month of May was remarkable making the animals experience the highest consequence of the heat stress condition. Moderate degree of the heat stress condition was in the months of April, June, August and September. The least degree of the heat stress condition was in July.

Table 1. Average Ambient Temperature, Relative Humidity and Thermal Humidity Index (THI) during the experimental period

Collection Month	Average Ambient Temperature (°C)	Average Relative Humidity (%)	Average THI
April	28.10	78.20	27.17
May	29.30	78.80	28.32
June	28.00	84.60	27.35
July	27.30	87.40	26.80
August	27.80	85.00	27.18
September	27.50	89.00	27.05

Semen Volume and Sperm Concentration

Semen Volume (SV) differed between experimental and control groups (Table 2). Control group had higher SV than experimental group (Figure 1). This is inconsistent with [5] findings that there is increased semen volume with shaded and water sprinkled Murrah buffalo. However, [6] reported a non-significant difference in semen volume in Jersey cattle with and without shed and water sprinkle. [7] noted inconsistencies in the semen volume as affected by heat stress. [8] reported that during the hot dry summer season, semen volume marked the highest among Murrah buffalo bulls.

The average SV of the experimental group of 4.63 ± 0.08 ml was within its baseline value of 4.60 ± 0.21 ml while the average SV of the control group of 5.62±0.37 ml was within and slightly above its baseline value of 5.27 ± 0.29 ml.

There was no month interaction for SV. Monthly SV were comparable (Table 2).

Table 2. Semen volume (ml) of Murrah buffalo bulls with or without fan and sprinkler cooling and month of collection.

	Treatments		
Collection Month	Experimental	Control	Mean±SEM
April	4.37	5.89	5.13±0.44
May	5.20	6.78	5.99±0.68
June	4.55	5.48	5.01±0.51
July	4.48	5.57	5.03±0.44
August	4.53	5.04	4.78±0.35
September	4.66	4.98	4.82±0.45
Mean±SEM[s]	4.63±0.08	5.62±0.37	

s – significant at p<0.01

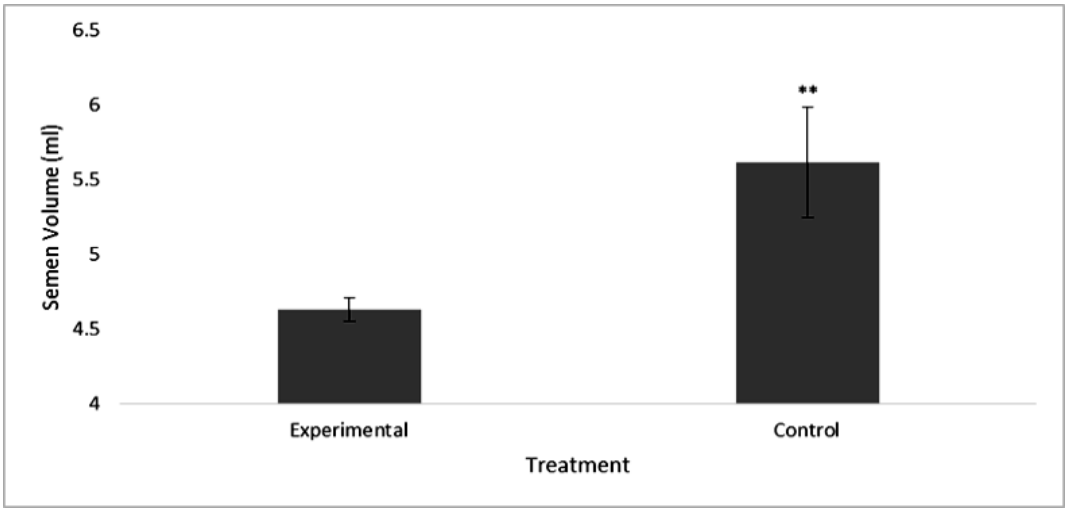

Figure 1. Semen volume (ml) of Murrah buffalo bulls with fan and sprinkler (Experimental) and without fan and sprinkler (Control). Mean values are different at p<0.01.

There was no month interaction for Sperm Concentration (SC). SC was comparable between experimental and control groups and among the months of collection (Table 3). Although not statistically significant, SC was higher in the experimental group than the control group.

Both the average SC of the experimental group of 176.39 ± 5.98 ($x10^7$/ml) and the average SC of the control group of 172.01 ± 5.19 ($x10^7$/ml) were within and above their baselines of 165.02 ± 10.84 ($x10^7$/ml) and 163.06 ± 10.51 ($x10^7$/ml), respectively.

Considering that semen volume was significantly higher in control and an apparent higher sperm concentration in the experimental group, semen concentration was affected by the cooling intervention. The role of the epididymis is to concentrate semen by absorbing fluid coming from the testis. In the control group, the increased semen volume was due to the detrimental effect of heat stress to the epididymal function to concentrate semen. The experimental group was able to ensure semen quality by storing a concentrated semen than an ejaculate full of fluids. Moreover, SV was lesser in the experimental group as a result of the mitigation of the increased watery secretion of the accessory sex glands as mediated by heat stress. It is possible that the accessory sex glands of the control group increased water composition of their secretions as a heat stress response. This response was mitigated by the cooling intervention in the experimental group.

Semen Quality

Live Sperm Count (LSC) differed between experimental and control groups (Table 4). Experimental group had higher LSC than the control group (Figure 24). There was no month interaction for LSC. Monthly LSC were comparable (Table 4).

Semen Mass Activity (SMA) differed between experimental and control groups (Table 5). Experimental group had higher SMA than control group (Figure 3). The average SMA of the experimental group of 2.77±0.07 was within and slightly above its baseline value of 2.73 ± 0.09 while the average SMA of the control group of 2.28±0.10 was below its baseline value of 2.72 ± 0.09.

There was no month interaction for SMA. Monthly SMA were comparable (Table 5).

Semen Consistency (SCo) differed between experimental and control groups (Table 6). Experimental group had higher SCo than the control group (Figure 4). The average SCo of the experimental group of 2.72±0.06 was within its baseline value of 2.74 ± 0.09 while the average SCo of the control group of 2.36 ± 0.10 was below its baseline value of 2.73 ± 0.09.

There was no month interaction for SCo. Monthly SCo were comparable (Table 6).

Table 3. Sperm Concentration ($x10^7$/ml) of Murrah buffalo bulls with or without fan and sprinkler cooling and month of collection

	Treatments		
Collection Month	Experimental	Control	Mean±SEM
April	187.49	170.44	178.96±7.98
May	173.81	169.99	171.90±8.99
June	172.87	180.93	176.90±9.09
July	179.52	177.87	178.69±10.25
August	165.49	169.50	167.49±12.63
September	179.15	163.34	171.25±9.98
Mean±SEM	176.39±5.98	172.01±5.19	

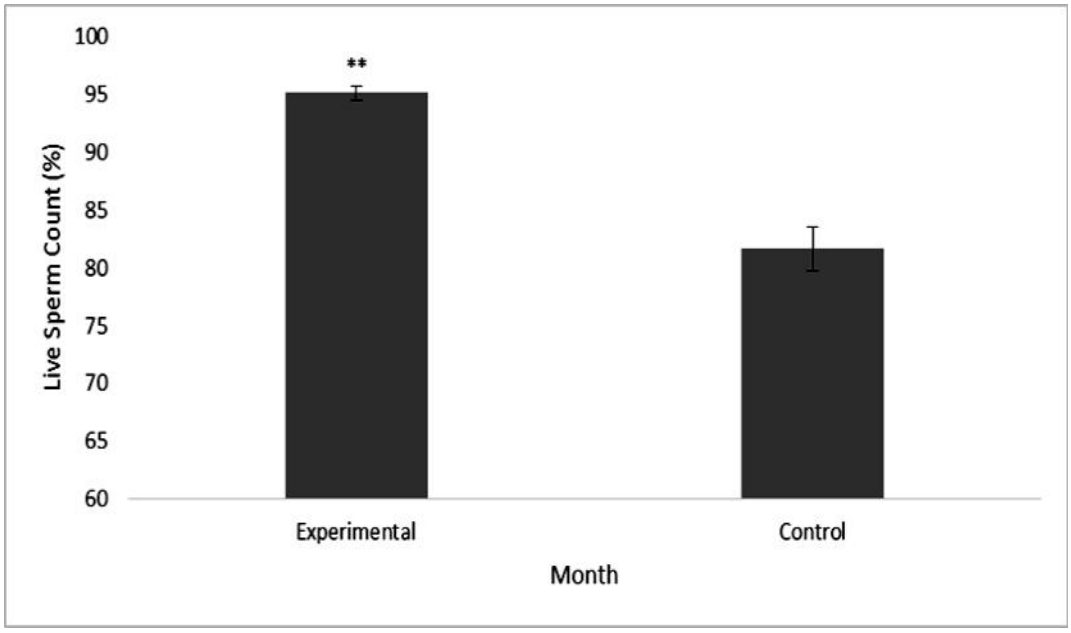

Figure 2. Live sperm count (%) of Murrah buffalo bulls with fan and sprinkler (Experimental) and without fan and sprinkler (Control). Mean values are different at p<0.01.

Table 4. Live Sperm Counts (%) of Murrah buffalo bulls with or without fan and sprinkler cooling and month of collection

	Treatments		
Collection Month	Experimental	Control	Mean±SEM
April	97.71	81.42	89.56±3.97
May	91.58	79.13	85.35±3.42
June	95.63	87.25	91.44±1.63
July	79.46	79.46	88.40±4.52
August	94.17	79.54	86.85±2.48
September	94.67	83.25	88.96±1.91
Mean±SEM[s]	95.18±0.58	81.67±1.90	

s – significant at p<0.01

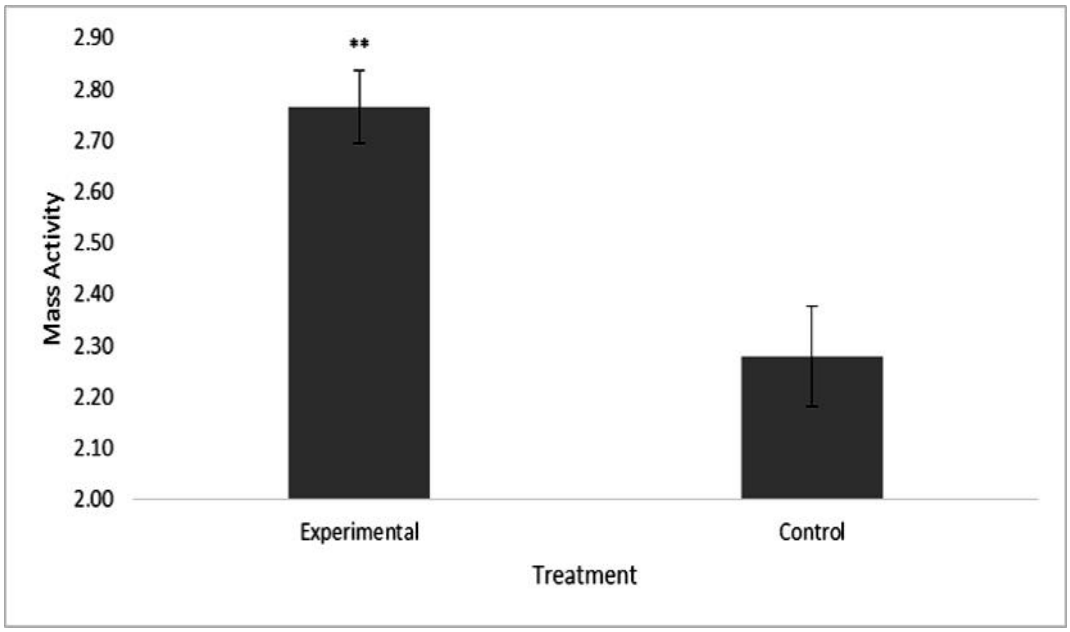

Figure 3. Semen Mass Activity of Murrah buffalo bulls with fan and sprinkler (Experimental) and without fan and sprinkler (Control). Mean values are different at p<0.01.

Table 5. Semen Mass Activity of Murrah buffalo bulls with or without fan and sprinkler cooling and month of collection

	Treatments		
Collection Month	Experimental	Control	Mean±SEM
April	2.62	2.54	2.58±0.09
May	2.88	2.34	2.61±0.17
June	2.80	2.34	2.57±0.18
July	2.71	2.15	2.43±0.16
August	2.76	2.05	2.41±0.21
September	2.82	2.24	2.53±0.17
Mean±SEM[s]	2.77±0.07	2.28±0.10	

s – significant at p<0.01

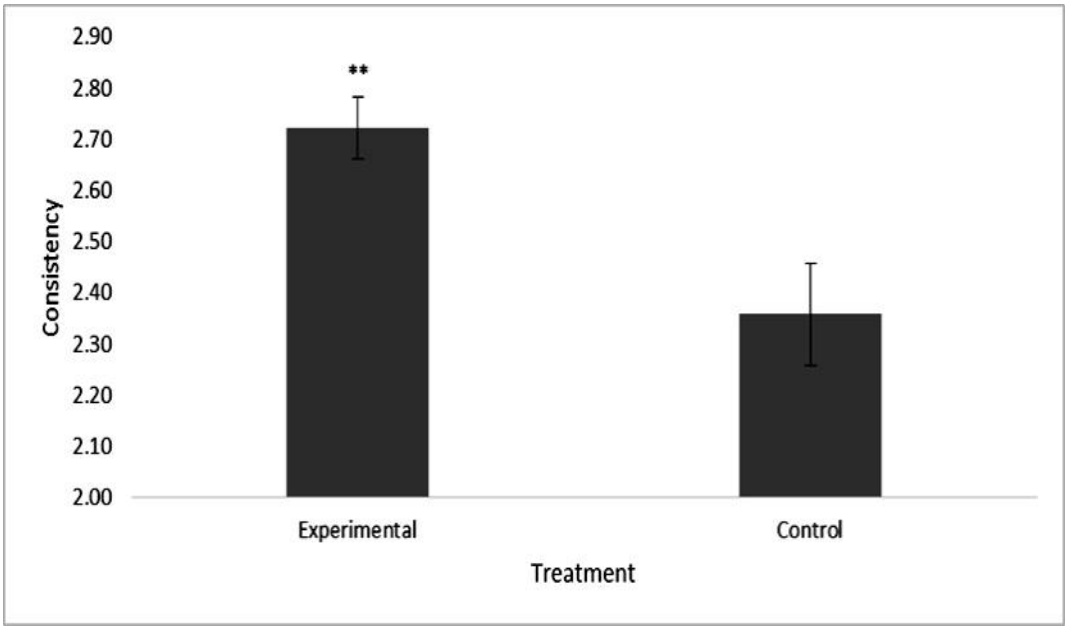

Figure 4. Semen Consistency of Murrah buffalo bulls with fan and sprinkler (Experimental) and without fan and sprinkler (Control). Mean values are different at p<0.01.

Table 6. Semen Consistency of Murrah buffalo bulls with or without fan and sprinkler cooling and month of collection

	Treatments		
Collection Month	Experimental	Control	Mean±SEM
April	2.60	2.59	2.60±0.09
May	2.83	2.45	2.64±0.16
June	2.73	2.39	2.56±0.15
July	2.69	2.20	2.44±0.15
August	2.67	2.17	2.42±0.19
September	2.81	2.34	2.57±0.17
Mean±SEM^{s}	2.72±0.06	2.36±0.10	

s– significant at p<0.01

Semen Initial Motility (SIM) differed between experimental and control groups (Table 7). Experimental group had higher SIM than the control group (Figure 5). The average SIM of the experimental group of 53.78±1.54 was within its baseline value of 53.67 ± 2.22 while the average SIM of the control group of 43.19 ± 2.44 was below its baseline value of 53.42 ± 2.19.

There was no month interaction for SIM. Monthly SIM were comparable (Table 7).

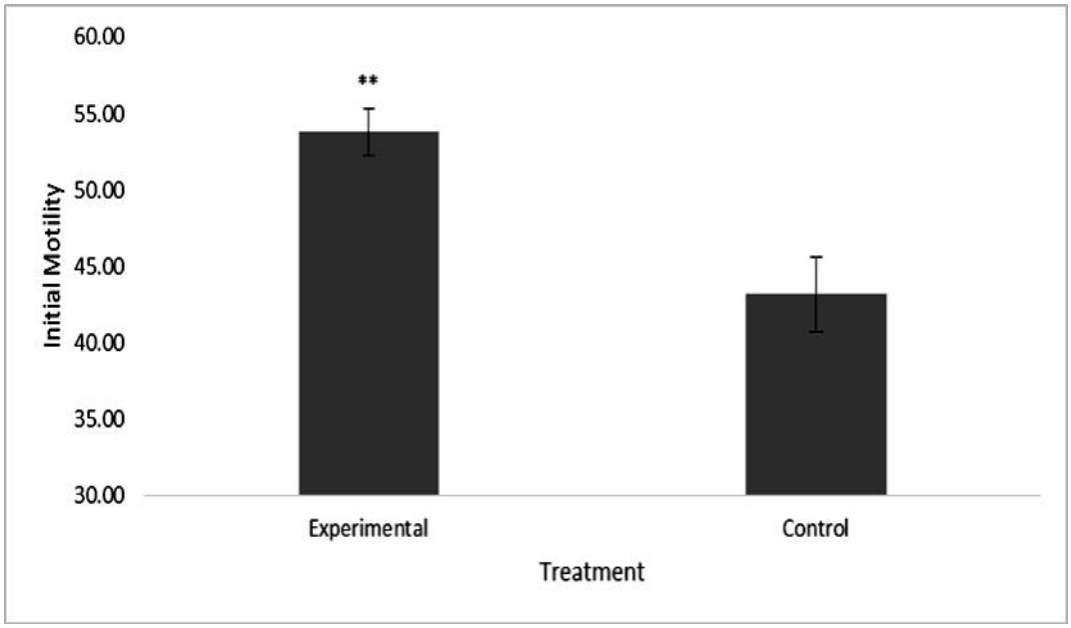

Figure 5. Semen Initial Motility of Murrah buffalo bulls with fan and sprinkler (Experimental) and without fan and sprinkler (Control). Mean values are different at p<0.01.

Table 7. Semen Initial Motility (%) of Murrah buffalo bulls with or without fan and sprinkler cooling and month of collection

	Treatments		
Collection Month	Experimental	Control	Mean±SEM
April	51.15	50.69	50.92±2.04
May	57.29	45.65	51.47±4.07
June	54.46	45.10	49.78±3.89
July	52.84	39.59	46.21±3.80
August	51.88	36.17	44.02±4.79
September	55.07	41.92	48.49±4.21
Mean±SEM[s]	53.78±1.54	43.19±2.44	

s– significant at p<0.01

The present intervention brought a general increase in sperm quality (live sperm count, mass activity, consistency, initial motility). Moreover, the intervention was effective in maintaining the sperm quality parameters within the baseline value for the experimental group. It was also evident that control group's sperm quality was below their baseline values. This is consistent with [5] in buffalo bulls and [6] in Jersey bulls. The present intervention improved semen qualities by reducing the effect of heat stress due to testicular degeneration [9]. Based on the result of Study 2, fan and sprinkler cooling improved red blood cell profile of the experimental group. A good supply of oxygen and nutrients is needed for spermatogenesis to proceed and animals with a better red blood cell profile are more able to sustain this process since red blood cells contain hemoglobin which carries oxygen to various body tissues including testicular tissues.

Rectal temperature and scrotal surface temperature difference and sperm quality

Rectal Temperature and Scrotal Surface Temperature Difference (RTSSTD) was 5.74^{o}C for the experimental group and 5.14^{o}C for the control group. There was apparently greater RTSSTD in the experimental group compared to the control group which may be more conducive for spermatogenesis to proceed.

Table 8 summarizes sperm quality of bulls which was taken after a two-week sperm production cycle following RTSSTD measurements.

The effect of a larger RTSSTD difference was significantly higher SV in control group compared to the experimental group. There was comparable SC between the treatments. As discussed previously, the cooling intervention affected the role of the epididymis to concentrate semen by absorbing fluid coming from the testis. In the control group, there was an increase in semen volume probably because heat stress was detrimental to the epididymal function to concentrate semen.

SMA, SCo and SIM were significantly higher in the experimental group compared to the control group as a consequence of a larger RTSSTD of the experimental group. Similar to the results in the previous section, the cooling intervention brought a general increase in sperm quality in terms of mass activity, consistency and initial motility. In most mammals, testicular temperature 2ºC to 6ºC lower than the body temperature is needed for the production of normal sperms [1]. Although the RTSSTD of the experimental and control group were within the aforementioned temperature range, the greater RTSSTD in the experimental group perhaps provided a more conducive environment for spermatogenesis to proceed.

Economic Analysis

The total number of semen straw produced by the experimental group was 26, 666 for the 6-month duration of the study while the straw produced by the control was 21 487. There were an additional 5 179 straws produced by the fan and sprinkler intervention. There was a profit difference of P 12,939.95 per bull per month added to the experimental group by the cooling intervention.

Testicular Ultrasound Features

Samples of the testicular ultrasonic appearance of Murrah bulls are shown in Figure 6. The testicular covering consists of the skin, the cremaster muscle, and the tunics. Topmost was the hyperechoic skin. The next layer which was presented as an hypoechoic area with interspersed hyperechoic areas were the cremaster muscles combined with some fats and the last hyperechoic line was the tunics. The testicular covering ranged from 9 mm to 15 mm. The testicular parenchyma was granular homogenous and moderately echogenic all throughout with no signs of fibrosis and calcifications in all samples. The parenchyma echomean ranged from 83.23 to 146.02 and its thickness ranged from 38 mm to 63 mm. The hyperechoic linear structure distinctively located at the bottom of the ultrasonogram is the testicular covering layer on the other side of the testis starting with the tunica albuginea.

All ultrasonograms (Image A1 to C6 in Figure 6) show homogenous hypoechogenic testicular parenchyma with dispersed hyperechoic areas giving the parenchyma a granular appearance. Image B3, C1 and C3 had almost round distinguishable hyperechoic area in the middle of the ultrasonograms which represent the mediastinum. Image C2 shows a mirror image, an artifact produced by air, in the bottom part of the image.

The present findings are consistent with [10] in Egyptian buffalo bulls where the testis was found with

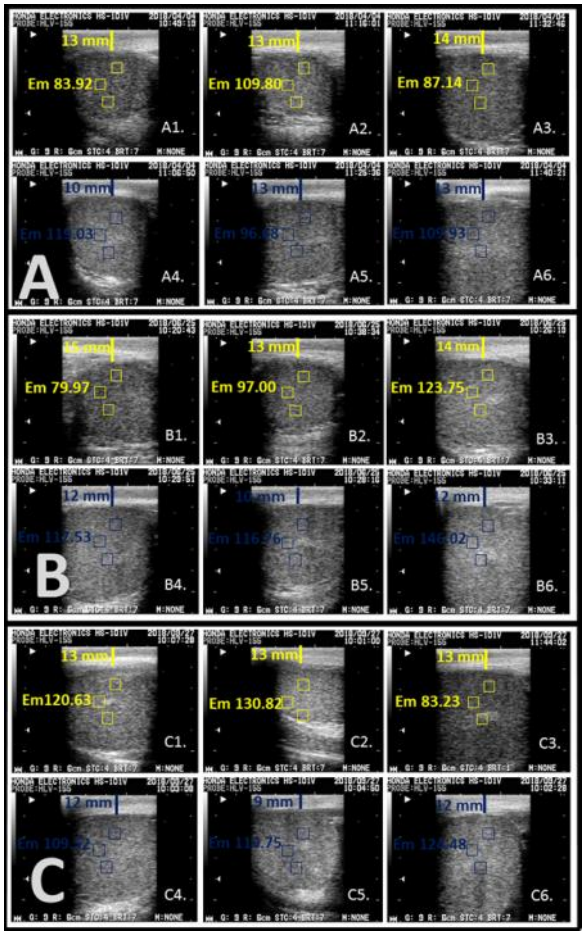

Figure 6. Samples of the testicular ultrasonic appearance of murrah bulls

Table 8. Semen Quality after a Two-week Sperm Production Cycle from Rectal Temperature and Scrotal Surface Temperature Difference Measurement of Murrah buffalo bulls with or without fan and sprinkler cooling

PARAMETER	EXPERIMENTAL (Mean ± SEM)	Control
Semen Volume (ml)	4.62 ± 0.18^{b}	5.66 ± 0.98^{a}
Sperm Concentration (10^7/ml)	162.91 ± 5.86^{a}	163.97 ± 14.28^{a}
Mass Activity	2.75 ± 0.09^{a}	2.35 ± 0.28^{b}
Consistency	2.71 ± 0.08^{a}	2.39 ± 0.28^{b}
Initial Motility (%)	53.56 ± 1.90^{a}	44.72 ± 6.74^{b}

Means followed by a different letter are different at $p<0.05$.

a homogeneous hypoechoic parenchyma with a centrally located hyperechoic mediastinum bounded by a distinct hyperechoic tunic. The shape of the mediastinum described in the present study is consistent with the findings of [11] in buffalo bulls where the mediastinum was described as an almost round hyperechoic structure which is centrally located in transverse images.

Ultrasound Echomean

Testicular parenchyma echomeans (Em) were comparable among the months of collection and between experimental and control groups (Table 9).

The present findings revealed no significant differences among the months of collection.

There was a tendency (P=0.1166) for Em to be lower in the experimental group compared to the Em of the control group (Figure 7). This finding shows a more vigorous spermatogenesis activity in the experimental group as evident with the increased fluid content within the testicular parenchyma. The active emptying of the Sertoli cells within the seminiferous tubules would mean that more sperm cells are being

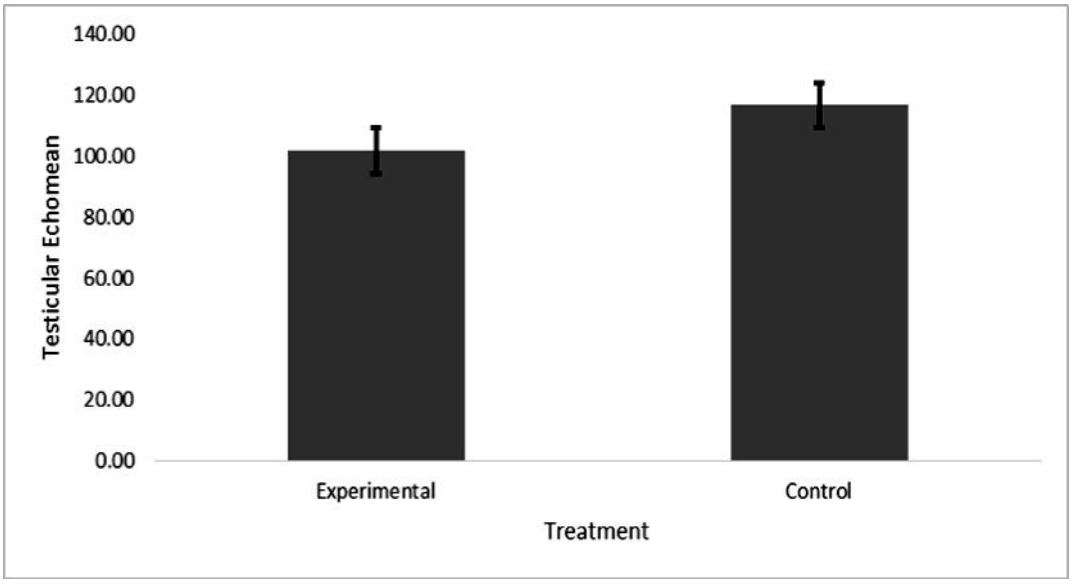

Figure 7. Testicular Parenchyma Echomean (Em) of Murrah buffalo bulls with fan and sprinkler (Experimental) and without fan and sprinkler (Control). Em of experimental group has a tendency to be lower compared to the Em of the control group (p=0.0116).

Table 9. Testicular Ultrasonogram Echomean of Murrah buffalo bull with or without fan and sprinkle cooling and month of blood collection

	Treatments		
Collection Month	Experimental	Control	Mean±SEM
April	93.62	101.08±5.73	101.08±5.73
June	100.24	113.51±9.28	113.51±9.28
September	111	113.24±6.86	113.24±6.86
Mean±SEM[s]	101.81±6.58	116.74±4.50	

transported in the seminiferous tubules and this process is facilitated by more fluids. If there were less spermatogenesis activity within the testicular parenchyma there would be less fluid produced and needed. The increased fluid content of the testicular parenchyma of the experimental group, producing less dense echoes in ultrasonograms, means that the testicular tissue is filled with more fluids signaling a more active sperm development. This finding proves the results of improved semen quality that the fan and sprinkler cooling is an effective way to improve semen quality. In the best of the author's knowledge, this is the first time that such findings are reported.

Testicular Covering Thickness

Testicular Covering Thickness (TCT) was comparable among the collection months (Table 10). TCT was greater in the experimental group than the control group (Figure 8).

Increase in the testicular covering in the experimental group was a consequence of the increased spermatogenesis activity within the testicular tissue. Obviously, if there is an increased activity there will be more blood supply to the testicular tissue and to the surrounding scrotal tissues increasing their sizes.

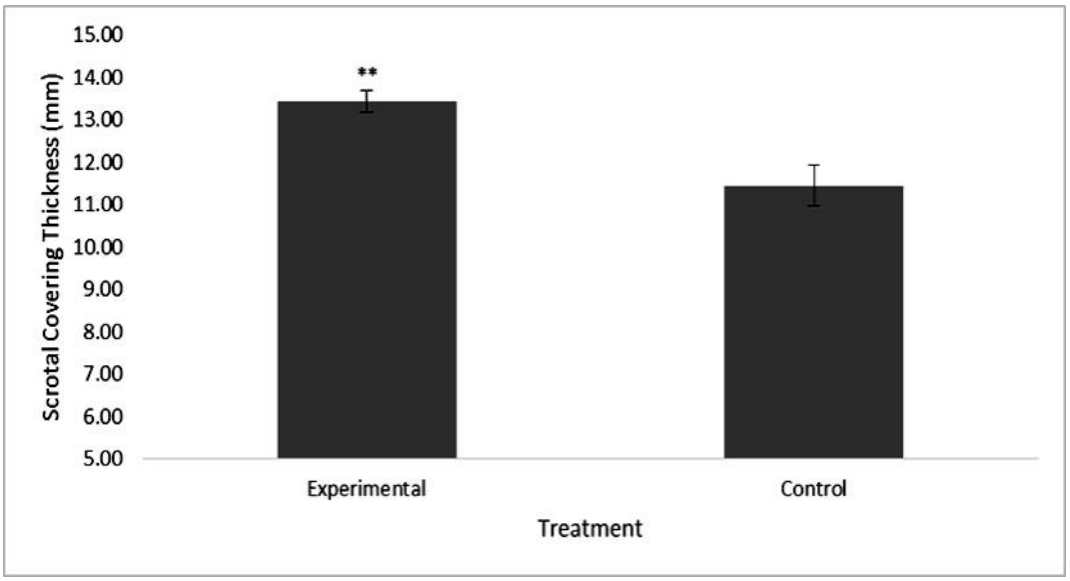

Figure 8. Testicular Covering Thickness (TCT) [mm] of Murrah buffalo bulls with fan and sprinkler (Experimental) and without fan and sprinkler (Control). Mean values are different at $p<0.01$.

Table 10. Testicular Covering Thickness (mm) of Murrah buffalo bull with or without fan and sprinkle cooling and month of blood collection

	Treatments		
Collection Month	Experimental	Control	Mean±SEM
April	13.33	12.00	12.67±0.56
June	14.00	11.33	12.67±0.71
September	13.00	11.00	12.00±0.63
Mean±SEM[s]	13.44±0.24	11.44±0.47	

s – significant at $p<0.01$

IMPLICATIONS

Fan and sprinkler cooling mitigates the increase in semen volume as a sign of the detrimental effect of heat stress to the functions of the epididymis and the accessory sex glands. Moreover, Increased Rectal Temperature and Scrotal Surface Temperature Difference provides a better condition to spermatogenesis to proceed improving semen quality. Fan and sprinkler cooling is a cost-effective way to improve semen quality of Murrah buffalo bulls. Ultrasonography may be used to assess the effect of heat stress in testicular tissue and to assess the effectiveness of cooling methods to mitigate the effect of heat stress in testicular tissue.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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