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Monitoring Energy Requirement and Weight Gain in Adult Cats after Ovariectomy.

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World Vet. J. 7(2): 36-39, 2017; pii:S232245681700006-7

ABSTRACT

To evaluate the effect of ovariectomy on food intake and body weight gain in adult cats fed according to their maintenance energy requirement (MER) and cats fed 1.5 times their MER. Eleven crossbreed adult female cats were monitored and weighed daily before undergoing ovariectomy, and their MER was determined once a stable body weight had been achieved. The ovariectomies were performed afterwards the cats were divided into two groups: group 1, composed of 6 cats, receiving the amount of food corresponding to their MER; and group 2, composed of 5 cats, fed 1.5 times their MER. No significant differences were found regarding food intake, alometric factor (kcal/kg$^{0.67}$) and Metabolizable Energy (ME) intake (kcal/day) ($P > 0.05$) for cats in group 1. However, these cats gained weight over the 30 days after ovariectomy ($P < 0.05$). Cats in group 2 presented significant difference for food intake, alometric factor, ME intake (kcal/day) and body weight ($P < 0.05$). Ovariectomy had influenced the MER. Spayed cats that continued receiving the same energy intake before ovariectomy had gained weight, showing that the MER for cats had decreased after the procedure. Cats in group 2 had gained more weight than those in group 1. In order to keep spayed cats within the optimal body weight, it is necessary to monitor their feed intake, considering that the MER changes after ovariectomy, and female cats tend to overeat and gain weight, which may lead to being overweight or obesity.

Key words: Spayed, Obesity, Food intake, Cat nutrition

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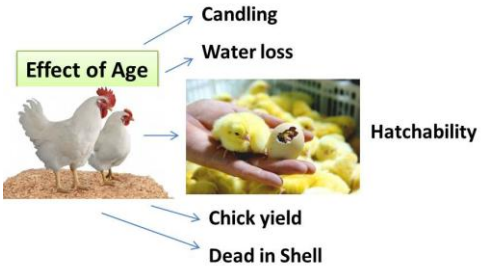
Research Paper



Effect of Broiler Breeders Age on Hatchability, Candling, Water loss, Chick yield and Dead in Shell.

Jabbar A and Ditta YA (2017).

World Vet. J. 7(2): 40-46, 2017; pii:S232245681700007-7

ABSTRACT

Water loss, chick yield and DIS analysis are all important factors in quality investigation of both the chick and hatchery performance. The age of breeders affect the hatchability, egg weight, chick weight water loss, Candling and DIS. In this experiment the broiler breeder were divided into three groups on the basis of age, young (24-30 weeks), prime (31-50 weeks) and old (51+ weeks) to investigate the effects of hatchability , egg weight, chick weight , Water loss, chick yield and DIS along mal-position and mal-formations. Hatchability (81.98±1.1, 88.44±1.6, 79.60±1.6), candling (10.25±1.25, 6.44±0.88, 10.73±1.25), DIS (7.7±0.4, 5.1±0.6, 9.6±0.6), water loss (11.29±0.11, 11.77±0.16, 12.13±0.16), egg weight (57.2±0.5, 64.2±0.7, 70.5±0.7), chick weight (39.4±0.3, 43.9±0.5, 48.15±0.5) were significantly ($P< 0.001$) different for young, prime and old groups respectively. For crack eggs (0.5 ±0.12, 0.5±0.17, 1.1±0.17) and contaminated eggs (0.63 ±0.09, 0.50±0.12, 1.31±0.12) young and prime were significantly ($P< 0.0001$) better then old. Young, prime and old were significantly different for early embryonic mortality (3.07±0.3, 3.46±0.2, 4.55±0.3) respectively, while for mid (1.04±0.13, 0.53±0.09, 1.0±0.13) and late embryonic mortality (3.77±0.4, 2.58±0.2, 3.28±0.4) prime was significantly better than young and old. Range of hatch window was 20-24 hours for prime and old while 20-22 hours for young. Mal-Position and Mal-Formation were significantly identical for all three groups. Mal-Position and Mal-Formation were 1.5% and 0.5% for total eggs set respectively.

Key words: Broiler, Breeders, Water loss, Chick yield, DIS, Mal-Formation/Mal-Position

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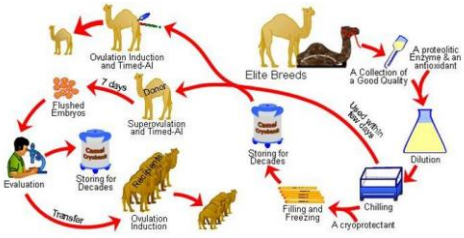
Review



Prospects of Improving Semen Collection and Preservation from Elite Dromedary Camel Breeds.

El-Hassanein EE.

World Vet. J. 7(2): 47-64, 2017; pii:S232245681700008-7

ABSTRACT

Low reproductive performance is one of the most important factors affecting camel productivity. The genetic improvement of dromedary camels is still less progressed compared with other productive farm animals. In order to improve productive and reproductive performance of camels, monitoring and increasing efficient selection strategies in concurrent with developing assisted reproductive technologies and establishing a semen bank for elite camel breeds is urgently needed. To this date,

semen preservation and its related techniques in dromedary camels has been slow due to the continual preference of natural breeding with elite males, the difficulty of semen collection, the highly viscous nature of camel semen and the low sperm resilience, unable to withstand processing for preservation. The present article is aimed in surveying of the fundamental challenges of semen collection, processing and preservation from dromedary camels. Developing a collection process, improving the rheological characteristics of delivered semen, modification of extenders and optimizations of the preservation protocols have also been discussed.
Key words: Breeding management, Electroejaculation, Camel-dummy, Semen viscosity, Processing, Chilling, Freezing.

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Research Paper

Sero-Prevalence of Contagious Bovine Pleuropneumonia in Three Senatorial District of Kaduna State, Nigeria Using Latex Agglutination Test.

Billy I La'ah, Balami Arhyel G, Sackey Anthony KB, Tekdek Lazarus B, Sa'idu Shehu NA and Okaiyeto Solomon O.

World Vet. J. 7(2): 65-73, 2017; pii:S232245681700009-7

ABSTRACT

A study was conducted to determine the sero-prevalence of Contagious Bovine Pleuropneumonia (CBPP) in cattle in 3 senatorial districts of Kaduna State, using Latex Agglutination Test (BoviLAT PA 6223). One Local Government Area (LGA) from each senatorial district was randomly selected for this study thus; Ikara (Northern), Chikun (Central) and Kauru (Southern). A total of 300 blood samples were each collected from the selected LGAs in each senatorial district using simple random sampling without replacement technique. An overall sero-prevalence of 26.0% (234/900) was recorded, with Kauru having a higher sero-prevalence of 46.0% (138/300), followed by Ikara (17.0%) (51/300) and Chikun (15.0%) (45/300) LGAs. The sero-prevalence was highest (30%) in the age group of >6 years old and lowest (19.3%) in the age group <1-3 years old (P=0.0027). Based on sex of the cattle, there was no statistical significant association (P=0.1424) in the sero-prevalence of CBPP between the female (27.1%) and the male (21.9%) cattle. Though the sero-prevalence showed some degree of variation, there was no statistical significant difference (P=0.0572) in infection among Sokoto Gudali (57.1%), Red Bororo (50%) and White Fulani (25.3%) breeds of cattle. The study revealed the sero-prevalence of CBPP in cattle in the study area. Although BoviLAT Latex Agglutination is not capable of differentiating between antibodies from vaccinated animals and those of natural infection, it however provides, a fast and easy to perform diagnostic technique in the field, and therefore, good for early detection of cattle with CBPP.

Keywords: Chikun, Ikara, Kauru, Latex Agglutination, *Mycoplasma mycoides*, Sero-prevalence.

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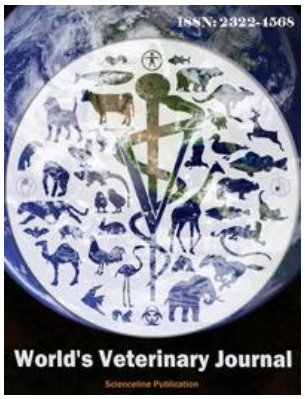

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Monitoring Energy Requirement and Weight Gain in Adult Cats after Ovariectomy

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ABSTRACT

To evaluate the effect of ovariectomy on food intake and body weight gain in adult cats fed according to their maintenance energy requirement (MER) and cats fed 1.5 times their MER. Eleven crossbreed adult female cats were monitored and weighed daily before undergoing ovariectomy, and their MER was determined once a stable body weight had been achieved. The ovariectomies were performed afterwards the cats were divided into two groups: group 1, composed of 6 cats, receiving the amount of food corresponding to their MER; and group 2, composed of 5 cats, fed 1.5 times their MER. No significant differences were found regarding food intake, alometric factor (kcal/kg$^{0.67}$) and Metabolizable Energy (ME) intake (kcal/day) ($P>0.05$) for cats in group 1. However, these cats gained weight over the 30 days after ovariectomy ($P<0.05$). Cats in group 2 presented significant difference for food intake, alometric factor, ME intake (kcal/day) and body weight ($P<0.05$). Ovariectomy had influenced the MER. Spayed cats that continued receiving the same energy intake before ovariectomy had gained weight, showing that the MER for cats had decreased after the procedure. Cats in group 2 had gained more weight than those in group 1. In order to keep spayed cats within the optimal body weight, it is necessary to monitor their feed intake, considering that the MER changes after ovariectomy, and female cats tend to overeat and gain weight, which may lead to being overweight or obesity.

Key words: Spayed, Obesity, Food intake, Cat nutrition

SHORT COMMUNICATION
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INTRODUCTION

Over the last 30 years, obesity in companion animals has been identified as an emerging problem, affecting the welfare and health of domestic dogs and cats. It has been estimated that obesity affects around 6-12% of domestic cats and 25-45% of the dogs population (Lazaroto et al., 1999; Bland et al., 2010; Brunetto et al., 2011). In the period from 2007 to 2011, in a general veterinary practice population in the USA, it was observed an increase in overweight and obesity in dogs and cats by 37% and 90%, respectively (Banfield, 2012). In most cases, it is related with excessive food intake, reduced physical activity, thereby increasing the predisposition of weight gain. Likewise, the spaying procedure has been correlated with weight gain. The chances of a castrated animal becoming obese is twice as often a regular cat, considering the hormonal changes caused by the absence of the gonads (Wolfsheimer, 1994).

Although weight control depends on a joint action involving both hormones and neurotransmitters, it is also influenced by many other factors, such as specie, breed, age, physical activity and nutritional management. The sexual hormones have an effect on body weight either by trigging the brain satiety and activity centers or indirectly by altering the cellular metabolism (Salmeri et al., 1991a; Salmeri et al., 1991b). The reduction of hormone production after castration and the following decrease of the basal metabolism rate affect the perception of satiety, bringing it to a higher threshold and leading to sedentariness (Nielson et al., 1997). The decrease concentration of estradiol observed in

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neutered cats may be a major factor influencing the increase in food intake (Larsen, 2016). It has been shown that administration of low-dose estradiol (0.5µg) to overweight neutered cats reduced food intake significantly (Cave et al., 2007). All of these metabolic changes provide greater chances to develop obesity in castrated animals. The aim of this study was to evaluate food intake and weight gain in adult cats fed their Maintenance Energy Rrequirement (MER) and 1.5 times their MER after spayed.

MATERIALS AND METHODS

Ethical approval

The Animals Ethics Committee at the Federal University of Rio Grande do Sul, Brasil approved all experimental procedures performed (Protocol number 23.953).

Animals and Requirements

Eleven crossbreed adult female cats were used in this study. All cats were healthy and with an average Body Condition Score (BCS) of 4.5 out of 9 (Laflamme, 1997). The cats were weighed daily and fed with a diet to maintain their BCS between 4 and 5 (Table 1). During 15 days previous to ovariectomy, the cats were monitored to determine their MER (kcal/day) (NRC, 2006), and the alometric factor (kcal/kg^0.67) was calculated to maintain each cat within its ideal body weight. After the ovariectomy procedure, cats were divided randomly into two groups: group 1, composed of 6 cats, fed the same MER achieved before ovariectomy; and group 2, composed by 5 cats, fed 1.5 times their MER. Over 30 days after the ovariectomy, food intake (g), body weight (kg) and BCS were recorded daily. Food was given twice a day (08:00 a.m. and 17:00 p.m hours), and the cats were fed individually. The cats were kept in individual cages located in a controlled environment, and between the two meals they were released into a collective area for exercise. During the night all cats were kept in an individual cage.

Table 1. Ingredients and chemical composition of experimental diet

Analyzed chemical composition at dry matter (DM) of diet	
Humidity, %	12.4
Ash, %	7.00
Crude Protein, %	31.7
Acid hydrolized fat, %	10.4
Crude fiber, %	2.30
Gross Energy, kcal/kg	4,949
Metabolizable Energy measured , kcal/kg	3,901

[1]Ingredients of basal diet: corn grain, 10.4 %; brewers rice, 30.5 %; corn gluten meal 10.0; full-fat rice bran 12,0 %; poultry byproducts meal 28.0 %; bovine meat and bone meal 3,5%; poultry fat 2,0 %; salt 0.7%; meat hydrolyzate 0.2 %; yucca extract 0.03 %; phosphoric acid 0.47%; vitamin/mineral premix 0.4 %. Premix composition per kg: vitamina A (10,800 IU), vitamin D3 (980 IU), vitamin E (60 mg), vitamin K3 (4.8 mg), vitamin B1 (8.1 mg), vitamin B2 (6.0 mg), vitamin B6 (6.0 mg) 12 vitamin (30 mcg), pantothenic acid (12 mg), niacin (60 mg), folic acid (0.8 mg), biotin (0.084 mg), manganese (7.5 mg), zinc (100 mg), iron (35 mg), copper (7.0 mg), cobalt (10 mg), iodine (1.5 mg), selenium (0.36 mg), choline (2400 mg), taurine (100 mg) antioxidant BHT (150 mg).

Statistical Analysis

The average values were compared according to the paired *t* test from SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with $P < 0.05$. The BCS values were analyzed by the nonparametric Kruskal-Wallis test ($P < 0.05$).

RESULTS AND DISCUSSION

Before the ovariectomy procedure, all cats maintained a stable body weight allowing the calculation of MER (kcal/day) for each cat, which was used to calculate their food supply in the next period, after the ovariectomy. In this period, cats from both groups had gained weight (Table 2). In group 1, the ovariectomy had affected neither the food nor ME intake and alometric factor, demonstrating that the food supply had been controlled efficiently. Nonetheless, the cats gained weight during the 30 days after ovariectomy, without changing the BCS. The cats had gained an average of 103 g, which represents an increase of 4% over their initial body weight, even under restricted food supply. This effect can be directly attributed to castration. However, this study does not allow us to estimate the ideal restriction of ME that had enabled spayed cats to maintain their initial body weight. Mitsuhashi et al. (2011) found a reduction on the MER of 25% of NRC recommendation for adult cats to maintain BW after being spayed.

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Table 2. Food intake, maintenance energy requirement (MER), alometric factor (AF), body weight (BW) and the average body score condition (BSC) from group 1 and 2 before and after ovariectomy

Items	Group 1[1]			Group 2[2]		
	Before spaying[3]	After spaying[4]	P-value	Before spaying[3]	After spaying[4]	P-value
Food intake (g)	53.8	52.8	0.3046	55	74.2	0.0008
MER (kcal/dia)	178	176	0.7367	185	237	0.0084
AF[5] (kcal/kg$^{0.67}$)	90.4	86.3	0.1641	87.5	105	0.0233
BW (kg)	2.80	2.9	0.0209	3.11	3	0.0022
BCS[6]	4.8	4.8	0.4500	5.2	5.1	0.4000

[1]The cats received the amount of diet corresponding to their MER before and after ovariectomy. (MER = alometric factor* PV^0.67), n = 6 cats; [2]The cats received the amount of diet corresponding to their MER and to 1.5 MER before ovariectomy and after ovariectomy, respectively, n = 5 cats; [3]Before ovariectomy = period of 15 days prior to ovariectomy; [4]After ovariectomy = period of 30 days after ovariectomy; [5]Alometric fator; [6] Kruscall-Wallis Test P > 0.05.

A significant difference had been observed in food intake, ME intake, body weight and alometric factor ($P < 0.05$) in cats fed 1.5 times their MER after ovariectomy. Cats gained an average of 230 g after ovariectomy, which represents an increase of 7.5% over their initial body weight, showing the importance of controlling energy intake in spayed cats. This result is in agreement with that found in male cats by Wei et al. (2014), who observed a post-castration weight gain in cats fed *ad libitum* due to higher food intake with no changes on energy expenditure after castration. Cats naturally gain weight after being spayed, and offering more food than the required will permit overconsumption, which increases the risk of obesity. Choosing a specific diet might be a path to reduce the energy intake when food intake is not controlled. Specific diets for spayed cats can be formulated by decreasing the energy density, using sources of fibers and low energy feeds. Also, another strategy for reducing dietary energy density could be via addition of water to dry food (Alexander et al., 2014). At the Nutritional Guidelines for Complete and Complementary Pet Food for Cats and Dogs (FEDIAF, 2013), it is possible to find current information about how to meet the animal nutrient requirement when alometric factor has been reduced, decreasing the risk of nutritional deficiencies. Increasing physical activity, when used in combination with a diet management, contributes to maintaining an optimal body weight.

CONCLUSION

Spayed cats gain weight when they keep being fed with the same amount of food after an ovariectomy. Furthermore, spayed cats have no control on food consumption, and, as a consequence, tend to overeat, if food is available. The weight gain is progressive, and cats tend to become overweight or obese if food consumption is not controlled.

Competing interests

The authors have no competing interests to declare.

Author`s contribution

The authors Geruza S. Machado and Júlia G. Pezzali were responsible for collection and tabulation of data, experimental management of the cats, as well as the article writing. The authors Carlos Ongaratto, Bruna Schoroeder e Lucas M. Villela were responsible for collection and tabulation of data, experimental management of animals and review of the manuscript. The author Luciano Trevizan guided the project, wrote and revised the manuscript.

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Effect of Broiler Breeders Age on Hatchability, Candling, Water Loss, Chick Yield and Dead in Shell

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ABSTRACT

Water loss, chick yield and DIS analysis are all important factors in quality investigation of both the chick and hatchery performance. The age of breeders affect the hatchability, egg weight, chick weight water loss, Candling and DIS. In this experiment the broiler breeder were divided into three groups on the basis of age, young (24-30 weeks), prime (31-50 weeks) and old (51+ weeks) to investigate the effects of hatchability , egg weight, chick weight , Water loss, chick yield and DIS along mal-position and mal-formations. Hatchability (81.98±1.1, 88.44±1.6, 79.60±1.6), candling (10.25±1.25, 6.44±0.88, 10.73±1.25), DIS (7.7±0.4, 5.1 ±0.6, 9.6±0.6), water loss (11.29±0.11, 11.77±0.16, 12.13±0.16), egg weight (57.2±0.5, 64.2±0.7, 70.5±0.7), chick weight (39.4±0.3, 43.9±0.5, 48.15±0.5) were significantly (P<0.001) different for young, prime and old groups respectively. For crack eggs (0.5 ±0.12, 0.5±0.17, 1.1±0.17) and contaminated eggs (0.63 ±0.09, 0.50±0.12, 1.31±0.12) young and prime were significantly (P<0.0001) better then old. Young, prime and old were significantly different for early embryonic mortality (3.07±0.3, 3.46±0.2, 4.55±0.3) respectively, while for mid (1.04±0.13, 0.53±0.09, 1.0±0.13) and late embryonic mortality (3.77±0.4, 2.58±0.2, 3.28±0.4) prime was significantly better than young and old. Range of hatch window was 20-24 hours for prime and old while 20-22 hours for young. Mal-Position and Mal-Formation were significantly identical for all three groups. Mal-Position and Mal-Formation were 1.5% and 0.5% for total eggs set respectively.

Key words: Broiler, Breeders, Water loss, Chick yield, DIS, Mal-Formation/Mal-Position

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INTRODUCTION

Poultry products are rich source of protein and income (Hussain, 2015). The poultry industry has engaged thousands of veterinarians for disease management and quality insurance of poultry products (Anonyms, 2011). Quality of egg hatching and incubation condition influences broiler hatch ability (Jabbar et al., 2017). Temperature and Humidity play a key role which is essential environmental factors during incubation (Lourens et al., 2005). During third week of incubation, the eggshell temperature increases due to the higher heat production of the embryo (Lourens et al., 2005). Both fertility and hatch ability of chicken had decreased with the age of breeders (King'ori, 2011).Different temperatures and humidity degrees are required for the embryo at different stages of ages (Lourens et al., 2005). Modern broiler chickens are extra sensitive to metabolic disorders such as Ascites as a result of their genetic selection for quick growth and high meat yield (Balog, 2003; Arce-Menocal et al., 2009), which result in decreased visceral organ development, ideal chick yield and water loss (Havenstein et al., 2003). Modern technology and incubation system are essential to improve the performance breeders meat production (Mehaffey et al., 2006). It was documented that water loss, chick yield, chick weight, hatchability and temperature are closely interrelated (Jabbar et al., 2017). Development of embryo in broiler breeder eggs do until the time of laying, as at the moment of laying, the egg temperature rises above 40°C (North and Bell, 1990). Temperature, humidity and water loss are the main critical aspects in egg hatching management as temperature affects embryo growth (Yousaf et al., 2017). The age based incubation profiles straightly influence embryo capability and water loss, as it might affect cell death especially when cell viability is reduced after prolonged storage (Jabbar et al., 2017). The purpose of this study is to investigate the hatchery performance through considering primary factors such as: Hatchability, Candling, DIS, water loss, Chick Yield and chick weight of broiler breeders according to ages.

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MATERIALS AND METHODS

Ethical approval

This experiment was a routine field work in hatchery considering all rules and regulations regarding animal rights and ethic, university of veterinary and animal sciences, Lahore, Pakistan.

Site selection

This study was carried out at Sadiq Poultry (Pvt) Limited, Chakri Hatchery Rawalpindi which is situated 5 km from chakri interchange on motorway (M2). The hatchery contains the latest Heating Ventilation and Air Conditioning (HVAC) automation, having ISO (International standard organization) 1900-2000 certified. This hatchery has the largest eggs capacity in south of Asia, which is producing the best quality of chicks through single stage incubation system (Avida G4, Chick Master USA).

Egg selection

High-quality hatching egg with good quality shells, without ridges or small lumps of calcified material (pimples) were selected. The eggs were graded on the basis of their quality and weight, all the hatch able eggs were graded through egg grading machine MOBA 9A. While the poor shell, crack, bloody stained, elongated eggs were rejected (Khan et al., 2016), only oval shape and good quality eggs were selected. Egg room temperature and humidity were kept at 75 ^{0}F and 65 respectively with fresh air 2 CFM/1000 eggs during the course of the study.

Group/Breed classification

Broiler breeders were classified into three groups young (age breeder eggs 24-31 weeks), prime (age breeder eggs 32-50 weeks), and old (age breeder's eggs 50+ weeks) according to breeder's age. Each experimental group was consisted of (n=8640, 000) eggs with sixteen replicates for each group (n=540,000) eggs.

Selected Flocks

The young group contains Sadiq Poultry (SP) flock no: 102-1 ross, 105 ross, 106-1 hubbard classic, 106-2 and Arsaln Poultry (AP) flock no: 22-1 ross, 22-2 ross. Prime group contains SP flock no: 103 Cobb, 94 cobb, 98ross, 99ross, AP flock no: 21-cobb-1, 21-cobb-2, Khan Poultry (KP-6 Arbor Acre) flock no: 6, Rahim Farm (RF-9 Cobb) flock no: 9. Old group contains SP flock no: 90-cobb-2 , 90-hubbard classic -2, 92-1 hubbard classic, 92-2 hubbard classic, 96 cobb, AP flock no: 22 Ross, Kaloo Farm (KRA, KRB) cobb, Attock Farm (ATK Ross), rose (RAC Ross) and Green land (Gl-Cobb-3).

Egg weight

Before setting the eggs weight of each individual group was calculated by the formula:

$$\text{Egg weight:} \quad \frac{\text{Full tray weight at Setting- Weight of empty tray}}{\text{Total No of eggs in tray}}$$

Egg fumigation

Before the weighing, the trial eggs were fumigated with 20 g $KMnO_4$and 40ml formalin (40%) and 40 ml of water for 100ft ^{3} areas for 15 minutes through automatic fumigation process provided by Chick Master.

Incubation programme

All the groups had been pre-heated at 82^{o}F for 5 hours inside incubators. After completion of the pre-warming the setter started automatically the incubation stage profile (Recommended by Chicks Master USA). Incubation duration for young, prime and old was 456 hours in setter (19th day) and 50 hours in hatchers.

Setter hall and hatcher hall

Environmental conditions in setter hall were at 75 ^{0}F temperatures and 40% Relative humidity; whereas in the hatcher hall temperature was at75 ^{0}F and relative humidity had been increased up to 60%. The positive pressure in setter and hatcher hall was 15 Pascal and 10 Pascal respectively, while negative pressure inside setter and hatcher plenum was -25 Pascal during the course study.

Candling

Fertility of eggs were performed through candling then shifted to hatchers for next 50 hrs. These entire incubation stage programs have been recommended by chick master USA.

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Egg's water loss

Before being transferred to hatchers water loss was measured from each group individually after 456hrs of incubation. Water loss was measure by given formula:

$$\text{Water Loss \%:} \quad \frac{\text{Full tray weight at Setting- Full Tray Weight at Transfer} \times 100}{\text{Full tray weight at Setting- Empty Tray Wight}}$$

Chick yield measure

After hatch out immediately, the chick's weight and yield were measured through electrical weight balance by using following formula:

$$\text{Chick Yield \%:} \quad \frac{\text{Weight of chicks} \times 100}{\text{Egg weight}}$$

Hatch window

Hatch window is the duration between the 1st chick hatches out up to last chick hatch out (Noiva et al., 2014). Range of hatch window is 20-24 hours. For prime and old it was 22-24 hours while 20-22 hours for young.

Chick grading

Grading of chicks was performed on conveyer, automatic grading table. Only stranded (shining eyes, soft legs and nose, healed naval and healthy chicks) were shifted to chick's box after counting, while under weight, weak, and unhealed naval chicks were removed as international standard as describe by (Yousaf et al., 2017).

Dead in shell (DIS) analysis

To investigate the reason of embryo's mortality inside the eggs, un hatched eggs were broken. For this purpose analysis of un-hatch eggs was performed as presented in table 2 and their details are presented in table 3, 4 and 5.

Statistical analyses

All data were analyzed by using Statistical Analysis System package software (SAS version 9.2, SAS Institute Inc., Cary, NC, USA). All means were compared using Duncan's Multiple Range test and results were presented as mean ± SEM (standard error of mean). Results were considered significant if $P<0.05$.

RESULTS

Hatchability (81.98 ± 1.1^a, 88.44 ± 1.6^b, 79.60 ± 1.6^c), candling (10.25 ± 1.25^a, 6.44 ± 0.88^b, 10.73 ± 1.25^a), DIS (7.7 ± 0.4^a, 5.1 ± 0.6^b, 9.6 ± 0.6^c), water loss (11.29 ± 0.11^a, 11.77 ± 0.16^b, 12.13 ± 0.16^c), egg weight (57.2 ± 0.5^a, 64.2 ± 0.7^b, 70.5 ± 0.7^c), Chick weight (39.4 ± 0.3^a, 43.9 ± 0.5^b, 48.15 ± 0.5^c) were significantly ($P<0.001$) different for young, prime and old respectively. For crack (0.5 ± 0.12^a, 0.5 ± 0.17^a, 1.1 ± 0.17^b) and contaminated eggs (0.63 ± 0.09^a, 0.50 ± 0.12^a, 1.31 ± 0.12^b) young and prime were significantly ($P<0.0001$) better then old as show in table1 The high percentage of crack eggs in older flocks is due to thin egg shell. Young, prime and old were significantly different for early embryonic mortality (3.07 ± 0.3^a, 3.46 ± 0.2^b, 4.55 ± 0.3^c) respectively, while for mid (1.04 ± 0.13^a, 0.53 ± 0.09^b, 1.0 ± 0.13^a) and late (3.77 ± 0.4^a, 2.58 ± 0.2^b, 3.28 ± 0.4^a) prime was significantly better than young and old as shown in table 3.Mal-Position and Mal-Formation were at 1.5% and 0.5% for total eggs set respectively as shown in tables 4 and 5. The age of breeders does not affect significantly the chick yield, chick waste, Mal-position and Mal-formation. Mal-position and Mal-formation are mostly related to flock health condition, hatchery management and some genetic disorder regardless of the age of breed. Prime flock was found to have better result in term of hatch ability, candling, DIS, contamination of eggs and crack eggs percentage.

Table 1. Effect of broiler breeder's age on eggs weight, crack eggs, contaminated eggs, chick weight, water loss, and chicks yield and egg waste in Sadiq hatchery Pakistan at March 2016

Parameters	Group A (Young)	Group B (Prime)	Group C (Old)
Incubation durations (h)	506.2 ± 0.4^a	506.2 ± 0.4^a	506.2 ± 0.4^a
Egg Weight (g) Day 1st	57.2 ± 0.5^a	64.2 ± 0.7^b	70.5 ± 0.7^c
Crack eggs (%)	0.5 ± 0.12^a	0.5 ± 0.17^a	1.1 ± 0.17^b
Contaminated eggs (%)	0.63 ± 0.09^a	0.50 ± 0.12^a	1.31 ± 0.12^b
Chicks Weight (g)	39.4 ± 0.3^a	43.9 ± 0.5^b	48.15 ± 0.5^c
Water Loss (%)	11.29 ± 0.11^a	11.77 ± 0.16^b	12.13 ± 0.16^c
Chick Yield (%)	68.99 ± 0.2^a	68.46 ± 0.4^a	68.72 ± 0.4^a
Egg Waste (%)	19.71 ± 0.3^a	19.75 ± 0.4^a	19.13 ± 0.4^a

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Table 2. Effect of broiler breeder's age on hatchability, candling and dead in shell in Sadiq hatchery Pakistan at March 2016

Parameters	Group A (Young)	Group B (Prime)	Group C (Old)
Hatchability (%)	81.98±1.1[a]	88.44±1.6[b]	79.60±1.6[c]
Candling (%)	10.25±1.25[a]	6.44±0.88[b]	10.73±1.25[a]
DIS (%)	7.7 ±0.4[a]	5.1 ±0.6[b]	9.6±0.6[c]

Table 3. Effect of broiler breeder's age on early, mid and late embryonic mortality in Sadiq hatchery Pakistan at March 2016

Parameters	Group A (Young)	Group B (Prime)	Group C (Old)
Early embryo dead (0-7 da) (%)	3.07±0.3[a]	3.46±0.2[b]	4.55±0.3[c]
Mild embryo dead (8-14 day) (%)	1.04 ±0.13[a]	0.53 ±0.09[b]	1.0±0.13[a]
Late embryo dead (14-21 day) (%)	3.77 ±0.4[a]	2.58 ±0.2[b]	3.28±0.4[a]

DISCUSSION

Results of sixteen successful hatches had been recorded for each individual group. Hatchability, candling, DIS Chick yield, water loss and chick weight were recorded for every group individually (Tables 1 and 2). For all three groups incubation duration was insignificant (P>0.05) 506 hrs table 1. Incubation duration of 506 hours is good for chicks in term of chick's quality, yield, water loss (Jabbar et al., 2017). The young flock which may hatch in (502-504 hours) while for old it may goes to (506-510 hours) (King'ori, 2011). Hatch window is directly related to pre-warming, incubation temperature and breeders age for good quality chicks (Yousaf et al., 2017). Fresh eggs were weighted (g) before being set to incubation. Fresh eggs were significantly different (P<0.001) for young, prime and old. Weight of young age breeder's eggs was less in weight due to small size while egg weight of prime and old age breeders was better due to age difference Table 1.The standard weight and size of egg are recommended for incubation according to breeders age but it may be affected due to multiple reasons, such as age and breed type, diseases in breeder (ND, IB, H9, EDS, MG etc.), and farm management issue (King'ori, 2011). Crack eggs depend on egg shell quality and breeders age. The crack eggs percentage is significantly better (P<0.001) in young and prime as compare to old Table 1. Egg's shell of young and prime breeder are thick and hard that is why crack percentage has not been recorded in such flocks, while it is recorded to the maximum in old flock (Khan et al., 2016) Table 1The contamination significantly (P<0.001) increases from young to prime then old. The contaminated eggs also depend upon flock health status and farm management as shown in table 1 (Reis et al., 1997). The weight of chicken significantly increased (P<0.001) from young to prime then old as shown in table 1. Chick weight is affected by multiple reasons, such as ideal water loss, contamination free eggs, ideal incubation duration (506 hours), short hatch window and ideal temperature and humidity in setter/Hatcher and chick hall (Jabbar et al., 2017). Water loss had significantly increases from young to prime then old as shown in table 1.Water loss from hatch able eggs during incubation is critical for good chick yield and chick weight. To ensure the good quality of chicks ideal water loss and chick yield are the key factor (Yousaf et al., 2017).

The chick yield is percentage of chick conversion from egg. Chick yield is non-significantly (P>0.001) for young, prime and old Table 1. Water loss was significantly different among breeder flock (young, prime, old), where as the chick yield will be remain same which will be not affected by age of flock (Tong et al., 2013). The percentage of chick conversion from egg remains the same in young, prime and old. Eggs waste was significantly (P<0.001) similar in percentage, but it be different by weight table 1. The older flocks will contain high waste as compare to young or prime (Løtvedt and Jensen, 2014)**.** Hatchability, candling and DIS were significant (P<0.001) in young to prime and old as shown in table 2. The young flock's fertility depends upon the time of male being introduced to the flock (Van de Ven, 2012), whereas prime flock goes peak hatchability (Yousaf, 2016) and then for older flocks there is decreasing trend for hatchability (Van de Ven, 2012). The decline in reproductive performance is well documented after 45 weeks of age (Van de Ven, 2012). The age of breeder flock had affected the reproductive performance (Yousaf, 2016). Candling of breeders flock (P<0.001) had increased significantly in young to prime and old respectively as shown in table 2. The candling, hatchability and DIS are related to each other. There are so many factors which affect the hatchability of flock such as health status of flock, diseases issues, spiking, induce molting and farm management (Jabbar et al., 2015). These factors are responsible for change in Hatchability, candling and DIS regardless the age of breeders (Vieira et al, 2005). DIS (dead in shell) (P<0.001) had significantly increased in young, prime and old respectively as shown in table 2. DIS

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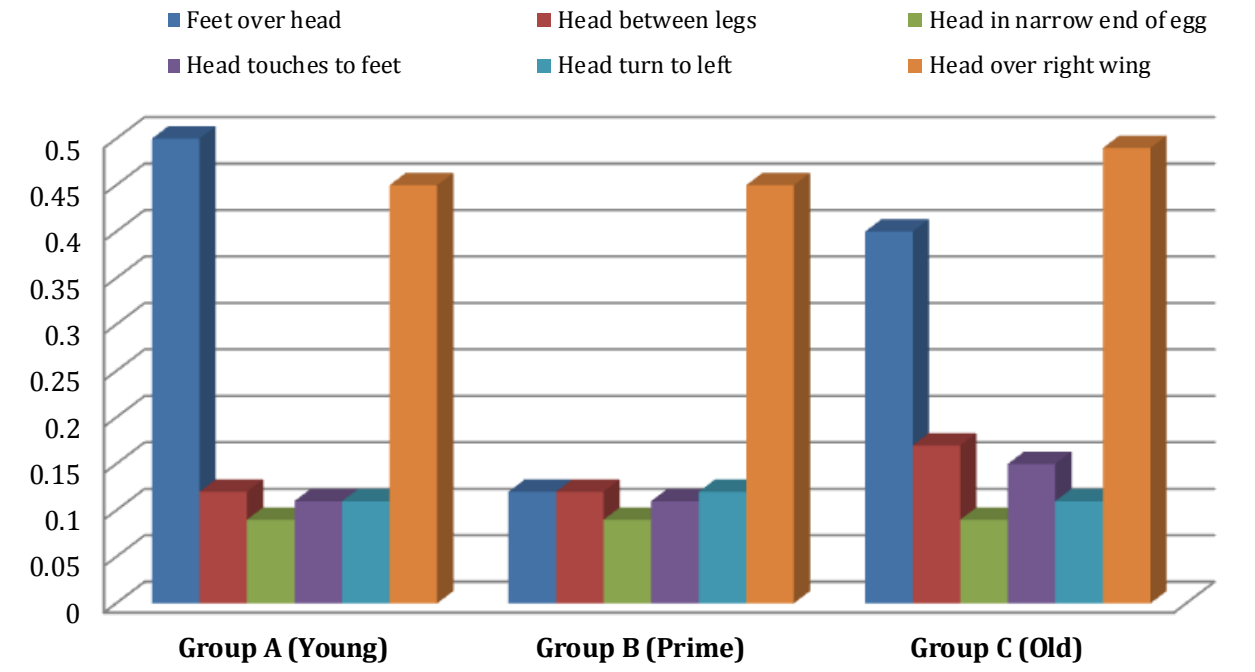
analysis is good tool to asses' hatchery performance. The early, mid and late embryonic mortality significantly (P<0.001) increase according to age of breeders as shown in table 3. The early embryonic mortality had also been affected by temperature shock, pre-warming, condensation, transportation condition, egg room temperature, storage duration and condition at the farm, fumigation, ventilation, turning and initial (0-7 days) temperature and humidity set points (Van de Ven et al., 2011). The mid (7-14 days) embryonic mortality occurs due to improper incubation condition in setters including temperature and humidity, turning, ventilation, over or under heat. The reason for late (15-21 days) embryonic mortality is improper incubation condition in setters and mostly in hatchers such as high temperature, low humidity, ventilation, turning, jerk during transfer, inadequate transfer temperature, improper hatch window and incubation duration (Løtvedt and Jensen, 2014).

The embryonic mortality at any stage early, mid and later may also affected by breeder health status and vertically transmitted diseases such as salmonella, MG and Adeno Virus (Vieira et al., 2005). Range of hatch window was 20-24 hours. For prime and old it was 22-24 hours while for young 20-22 hours. Older flock eggs required more heat in the early stage and produced more heat as compared to young flock eggs in later stages, which increases their hatch window. Mal-positions such as feet over head, head between legs, head touching to feet, head turn to left and head over right wing all these conditions are due to improper turning and low humidity, disease and vitamin deficiency specially Vitamin B complex (Tong et al., 2013). Head in narrow end of egg is due to eggs blend end were placed up during egg setting. All these mal-positions were non-significant for young, prime and old as shown in graph 1. Such kind of mal-positions should remain at 1.5% of total eggs set regardless the age of breeders (Tong et al., 2013)**.** Expose brain, ectopic viscera, extra limbs, unhealed navals, excessive albumin, Ascites, Hock swelling, extra eyes, scissor beak, curved toe, dry eggs contents and culling are common mal-formation that occur during incubation as shown in graph 2 (Yildirim et al., 2004). The malformations were insignificant (P>0.001) according to the age of breeders as shown in graph 2. The reason for exposed brain, excessive albumin, Ascites, ectopic viscera and extra limbs is mostly due to high incubation conditions (Yildirim et al., 2004). Hock swelling occurs due to hard egg shell of young and prime age breeder egg or it may be due to long time incubation in plastic Hatcher trays (Tong et al., 2013). Extra eyes, scissor beak and curved toe may be due to high incubation conditions and genetical disorders (Tong et al., 2013). Dry egg content is due to hairline crack due to which the egg content becomes dry. Un-heal naval are very common problem occurring due to being under heat high humidity or contamination inside machines. This condition may also develop due to vertical diseases (Vieira et al., 2005). Culling rate again depends upon the health condition of flock and incubation conditions of machines. The culling rate had increased in young, prime and old respectively. All these malformation should be less than 0.5% of total eggs set for a good hatch as shown in graph 2 (Tong et al., 2013).



Graph 1. Late embryo dead (14-21 days) due to Mal- Position of embryo

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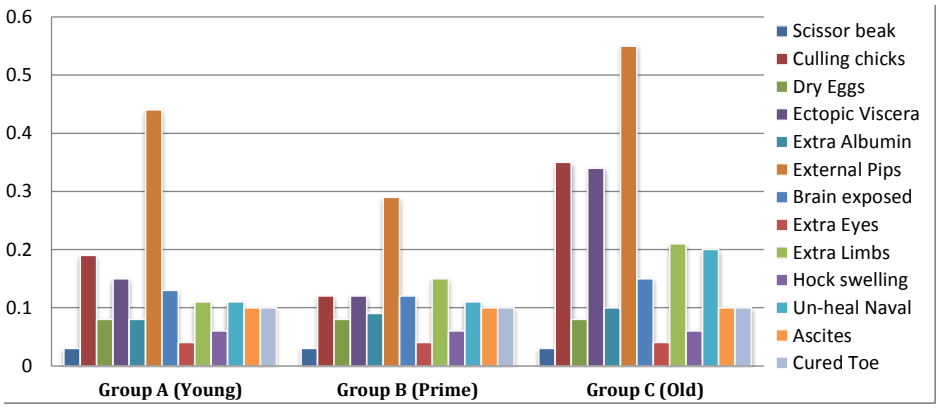

Graph 2. Late embryo dead (14-21 days) due to Mal-Formations of embryo

CONCLUSION

The age of broiler breeders affects the quality investigation factors of hatchery i-e hatchability, candling, DIS, water loss, egg weight, chick yield and Chick weight. The age of breeds does not affect Mal-position and Mal-formation. Prime flock was found to have better results in term of hatchability, candling, DIS, contamination of eggs and crack eggs percentage.

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Author's contribution

Both authors have equally contribution in this work.

Competing of interest

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article.

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Prospects of Improving Semen Collection and Preservation from Elite Dromedary Camel Breeds

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ABSTRACT

Low reproductive performance is one of the most important factors affecting camel productivity. The genetic improvement of dromedary camels is still less progressed compared with other productive farm animals. In order to improve productive and reproductive performance of camels, monitoring and increasing efficient selection strategies in concurrent with developing assisted reproductive technologies and establishing a semen bank for elite camel breeds is urgently needed. To this date, semen preservation and its related techniques in dromedary camels has been slow due to the continual preference of natural breeding with elite males, the difficulty of semen collection, the highly viscous nature of camel semen and the low sperm resilience, unable to withstand processing for preservation. The present article is aimed in surveying of the fundamental challenges of semen collection, processing and preservation from dromedary camels. Developing a collection process, improving the rheological characteristics of delivered semen, modification of extenders and optimizations of the preservation protocols have also been discussed.

Key words: Breeding management, Electroejaculation, Camel-dummy, Semen viscosity, Processing, Chilling, Freezing.

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INTRODUCTION

More than 70% of dromedary camels (C. *dromerdarius*) are raised in Arabian countries (FAO, 2009). This Godgiven gift is praised for its strategic stockpile of meat, milk, culture and traditions in Arabian deserts especially under the predicted hazardous effects of climatic changes forthcoming within the next 3-5 decades (Trondalen, 2009). Camels are known to have peculiar physiological features that enable them to survive and reproduce under the harsh desert environmental conditions (El-hassanein, 1989). However, low reproductive performance is one of the most important factors affecting camel productivity. Compared to other productive farm animals, there are still many potential ways available to improve productivity and reproductive performance of Arabian camels. Many and complex hereditary and environmental factors are contributed to low fertility in camels (El-Hassanein, 2003). This low reproductive efficiency could be improved by a better understanding of the reproductive cycle and increased use of assisted reproduction techniques such as Artificial Insemination (AI) and Embryo Transfer (ET) (Skidmore, 2013). Applying AI, particularly with frozen semen, has been by far the most effective reproductive technology for selective genetic improvement in farm animal production. It has many advantages over natural breeding, such as reduction of injuries (during natural mating), facilitation of rapid dissemination of superior genetics, restriction of spreading infectious and transmitted venereal diseases, in addition to prolongation of the reproductive lifespan of males even beyond their death. For this reason, there is a considerable interest in the use of AI in Camel breeding programs. During the last two decades, several scientific efforts have been conducted to utilize AI and ET techniques in improving reproductive performance of elite camel breeds (race and milch breeds). Embryo transfer has been tremendously developed in racing camels since 1990 (Anouassi and Tibary, 2013). However, AI technique has not developed well as a routine method for breeding camels compared with its fast and universal application in other farm animals. This may be due to difficulty in collecting semen from aggressive males in rut, the viscous nature of camel semen (Bravo and Johnson, 1994; Bravo et al., 2000a) and the continued use of traditional natural breeding system in most breeding herds (Skidmore, 2013). It is aimed at articulation of different natural and managerial constraints limiting improvement of productivity and reproductive performance of camels. The need to put into practice a persistent and steady technique for semen collection and

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processing into valuable frozen doses has been discussed. Also, the urgency of establishing a sperm bank for semen of genetically superior camel breeds has been debated for conservation and efficient dissemination of superior genotypes purposes.

Breeding Management in Camel Herds

Under pastoral conditions, the breeding management is usually focused on the male selection at 3-4 years age (3 years in average) to become the new replacement breeding males (Ishag and Ahmed, 2011). Most of the herders select these males from within the herd. Typically, herders select these prospective breeding males according to specific criteria, the most important of which are the male' dam potency in milk production and fitness and the fitness of the male's sire, in addition to their body confirmation, fitness, racing ability, disease resistance and drought tolerance (Farah et al., 2004). As the selected males reach maturity and start to exhibit rutting behavior, herders should isolate them from the herd as the leader breeding male becomes very aggressive towards other competitor males and even humans. The age of sexual maturity of breeding males is averaged seven years while the average age of keeping breeding males in herds is 15 years (14-18 years age) (Ishag and Ahmed, 2011). A rutting male is normally able to mate with about 50 females in a breeding season depending on his *libido*, fecundity and the fertility level of the served females (Elmi, 1989). The male is typically proficient to locate the vulval opening of the female during mating, however, it is common that herders aid the entrance of the male's penis into the female genitalia. Practically, herders forbid the breeding male in herds from mating with his mother; however, they did not prevent the male from mating with his sisters and daughters (Kuria et al., 2011). The practice of selection of breeding males from within the herd and mating the male with his related females may expose the herd to the risks of inbreeding depression and weakness or malformation of calves at birth.

In race camels, herders are usually eager to mate their camels with racing legend studs. Owners of famous males are usually obliged to share breeding with herders with no studding charges. Before the beginning of the autumn breeding season, herders with their females often start to travel hundreds of kilometers to reserve close to a breeding farm having some famous champion males. They may camp there for months to give their females the chance of being mated with these legendary males. Actually, there is a deficiency in formal pedigree records for most of the racing camel herds however; herders can be able to recite the pedigree of their camels for several generations. Missing formal pedigree records makes it difficult to analyze the herd reproductive performance. In addition, mixing of herds from different regions during the breeding season may increase the possibility of transmission of *Brucella* infection which is likely the cause of abortion and reproduction loss in camels.

A conventional system of reproductive management in camel breeding is considered one of the major limits of improving reproductive performance of dromedary camels. These age-old methods make it difficult to ensure an optimum number of females are pregnant at the end of the season and can also lead to widespread of venereal infections with a consequent lowering of fertility (Skidmore, 2013). In a personal experience extended for 15 months (Dec. 2011 - Feb. 2013) working on a royal flock of race camels in Qatar, several constraints have led to submission of my resignation and insistence to accepting it. The main reason for this decision was the inability to accord with the persistent traditional natural breeding of elite males during two successive breeding seasons and the powerlessness to realize an established semen collection and preservation system for the priceless royal male camels. Few days after joining the work in Leawaina Hejen Hospital (LHH) (Dec. 6, 2011), a field visit to the royal breeding farm (about 15 km to the northwest of LHH) was executed. The main impression of this visit was the shock from the emulative congregations of several hundreds of female camels around the farm to have the best chance of being fertilized by any of the royal male camels having a champion reputation. This breeding festival extends from September to April every year for females coming from different regions of the state and from several neighboring states without any care or precautions against infectious and venereal diseases. About 40 famous champion males were subjected to intensive breeding sessions with the possibility of becoming infected with sexually transmitted diseases. Throughout two consecutive breeding seasons, the responders of the breeding farm insistently refused to assign a group of the breeding males to be subjected to a persistent training for collection and preservation of semen in LHH during the period from November to April (the optimal period for collection and processing of camel semen). Their infinite priority was the employment of all the race legend studs in natural breeding rather than their attendance to the newly established AI and ET unit in LHH. The responders start to bring males to LHH near the end of April. Most of the incoming males were often suffering from extremely sexual exhaustion and weakness and many of them had clinically proven to be infected with brucellosis. It is well known that the fertility of males is obviously reduced with increasing number of their consecutive use in mating and the infection of breeding studs with venereal diseases will help in wide spreading of these diseases within the served camel herds.

In the first breeding season (2011-2012), farm responders have been hardly satisfied to bring about 6 of the newly retired males from racing (7-8 years age) to LHH (Feb. 2012) rather than their inclusion to the breeding farm. These males have shortly mastered to mount with the camel dummy and gradually produced semen of a very good quality for

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few weeks before their pulling to the breeding farm in a response to the forceful demand for natural breeding with them. These males were not brought to LHH again.

The persistence on annual appending of the newly retired famous champion males to the natural breeding mission is certainly in opposition to the establishment a sufficient and enduring semen collection and preservation system. Considering the customary principles in any AI-center for productive farm animals (semen cryobank), male camels of desired genetic merits should be fully dedicated to semen collection and preservation program all through their breeding potency rather than to be employed in natural mating. These males will have the greatest contribution to the genetic progress of elite camel breeds if they can be accurately evaluated and selected for applying AI-program in accordance with their sound progeny testing and proper genetic evaluation (Hermas, 1998).

Nature of Copulation in Camels

Coitus in camels differs completely from that process which is carried out in farm animals. In cattle, for example, copulation occurs in the standing posture. The sexually excited male in a full penile erection aggressively jumps on the rear back of a standing female. Just after resting his chest on the female back, he starts to intercourse the erected penis into inside the vagina and suddenly expels semen in one shot. This process is terminated within few seconds and is called "an ejaculation". On the contrary, copulation in camels takes place in the sitting position (sternal recombency). The male in rut leads up from behind over the pelvis of the kneeling female until his front legs are on either side of the female shoulders. Some males start to flex all joints of their hind legs to squat behind the female and then emplace their chests on the female hump, while the others firstly drop their chests on the hump before sitting behind the female. After mounting the kneeling female, the male starts to protrude the forepart of his penis in a verminous movement searching for the vulval cleft of the female. Then, the male starts to erect and direct his penis into inside the vagina and penetrates the cervix with his penis (intromission), followed by pushing himself forward to embrace the female pelvis between his thigh of hindlegs. After full intromission, the male starts to carry out continual rhythmic mild urethral pulses intermediated with several consecutive clusters of a strong whole body strain with urethral pulses (Lichtenwalner et. al., 1996a). These rhythmic urethral pulses and penile strokes can be followed by watching contractions of pelvic, hind legs and abdominal muscles of the male during copulation. Furthermore, if we are using a camel dummy for semen collection, we can observe from underneath the dummy the repeatedly entrance of the penis and influx of semen into the bulbous neck of the semen collection tube (Figure 1) with the corresponding penile strokes. This means that each cluster is considered as a distinct ejaculation, i. e. during the copulation of a male camel, several ejaculations are carried out and the amount of semen and sperm concentration well increase as copulation period is prolonged (Lichtenwalner et. al., 1996b). Naturally, copulation duration in camels may extend for more than 40 minutes depending on the male libido, sexual potency, breed, age and frequency of use. Practically, the male himself will dictate the duration of copulation. Regarding the pattern of semen flood during copulation, it is better to indentify the delivered semen after collection from a male camel as "a collection" rather than "an ejaculation".

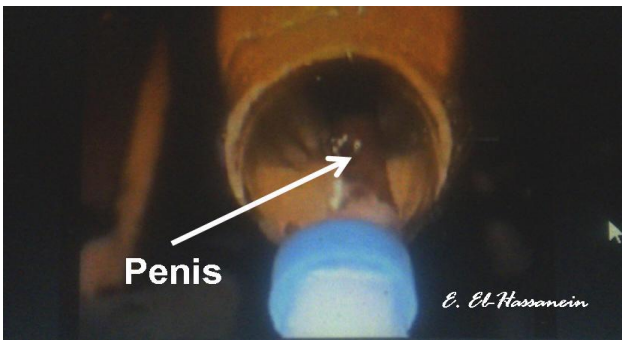

Figure 1a. Entrance of penis into inside the collection tube during consecutive penile strokes.

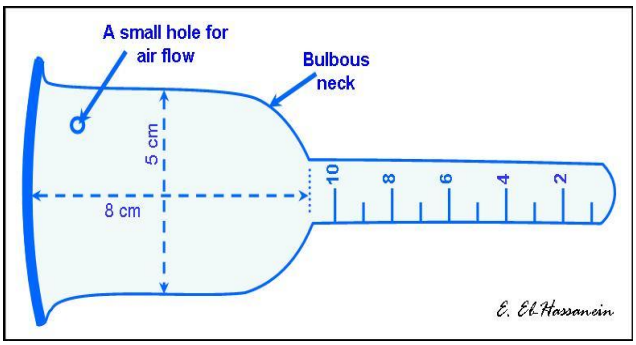

Figure 1b. Modified tube for semen collection using a camel dummy.

Semen Collection from Male Camels

Semen collection from genetically superior males is considered the main component in establishing an AI program. Consistent collection and evaluation of the male semen helps in allowing selection of suitable ejaculates of a reasonable quality to be processed and preserved for AI programs. Only males with excellent reproductive performance that produce high quality semen and have good production potential should be selected for AI. Consistent semen collection from breeding males in AI-centers helps in accurate evaluation of males, testing and formulation of standard extenders protocols and achieving optimal dilution, freezing and thawing rates for processing delivered semen into preserved semen doses of good quality post-thaw.

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By the onset of the breeding (rutting) season, the sexually mature male camel exhibits several peculiar morphological, behavioral and endocrinological traits (Yagil and Etzion, 1980; El-Hassanein et al., 2004; Bhakat et al., 2005; Deen, 2008; El-Bahrawy and El-Hassanein, 2011; Padalino et al., 2015). Additionally, males in rut are almost restless, aggressive, difficult to handle and often tend to bite other animals and humans (Abu-Zidan et al., 2012). The behavior of males in rut is mostly unpredictable and experience in handling them is important to avoid injuries. Therefore, semen collection in dromedary camels is often relatively long, unsafe, and tedious (Ziapour et al., 2014). Inability to consistently collect semen has been one of the most serious impediments to the application of AI programs in camels. Semen collection in camels is complicated by their recumbent mating posture, long duration of copulation and intrauterine deposition of semen (Arthur and Tigani, 1990; Bravo et. al., 2000a) in addition to the dripping manner of ejaculation and the challenge of handling the violently males in rut (El-Hassanein, 2003). Nowadays, attempts to collect semen from dromedary camels are carried out by using either electroejaculation or using a modified artificial vagina (AV) either in combination with a receptive (teaser) female or formerly fixed inside the newly invented device "*EL-Hassanein Camel Dummy*" (El-Hassanein, 2003).

Collecting semen by electroejaculation (EE)

Semen collection by using EE technique requires to kneel down the male, binding his front legs to shoulder and neck, forcing him to turn on his side and binding his hindlegs from the shin region together, followed by his sedation or general anesthesia (Hemeida et al., 2001; El-Hassanein, 2003; Mostafa et al., 2014).

Several studies have obtained less semen volume by using EE than that obtained by using an AV and a teaser female (Abdel-Raouf and El-Naggar, 1978; Tingari et al., 1986; Tibary and Memon, 1999; Bravo et al., 2000a; El-Hassanein, 2003; Marai and Zeidan, 2007), but other semen parameters were nearly similar in both techniques (Tibary and Memon, 1999; Marai and Zeidan 2007) or negatively affected by using EE (El-Hassanein, 2003). Due to the short duration of ejaculation using EE technique, the semen obtained is often of poor quality (Bravo et al., 2000a). On the contrary, other studies found an increase in ejaculate volume after using EE technique as compared to using an AV and a teaser female in Llama (Giuliano et al., 2008) and in dromedary camels (Mostafa et al., 2014). These studies have elucidated this increase in ejaculate volume by EE as an increase in volume of accessory fluids associated with electric stimulation of the accessory sex glands.

It is clear that the sedated or anesthetized male is forcibly ejaculated by using EE technique without any sexual stimuli for his gonads as it normally occurs in natural mating process. This may reflect that semen delivered by this technique does not resemble the semen that can be naturally produced. In general, EE technique is advices to be used only when semen collection by AV is not possible, as the practice of this technique has demonstrated many disadvantages (El-Hassanein, 2003), the most important of which are: consuming a lot of time and effort, restraining males by sedation or general anesthesia, needs a lot of laborers for tightening the males and removing ties, exposing males to bleeding injuries and even fractures, causing males to be fearful and unable to mount normally again, in addition to the possibility of contamination of the delivered semen with urine and cellular debris (Tibary and Memom, 1999).

Collection by an AV in combination with a receptive female

The pronounced and accustomed method of semen collection in camels is the use of an AV and a receptive female. A bull AV of 30 cm long and 5 cm internal diameter has been modified by adding a foam imitation cervix (8 cm in length) inside it in the part at which the rubber cone and the collection tube well be attached (Bravo et al., 2000a). For collection using an AV, a teaser female should be kneeled and her fore legs are tied with a rope to the neck while her hind legs are tied with a rope around the lumber region (El-Hassanein, 2003). Proper tying of the teaser before collection prevents her sudden movements during copulation that can cause injuries or even fractures for the male. As the male is fully squatted down behind the teaser and starts to protrude and erect his penis, the technician instantly sits beside the hind legs of the teaser, grasps the male's sheath and deviates the penis into inside the AV. The technician should hold the erected penis inside the AV throughout the copulation period (Figure 2).

Under the conditions of semen collection using an AV and a teaser female, the male camel is often excited and stressed by the intervention of the technician in mating process throughout the copulation period and also by the presence of several persons in the collection area. Therefore, males mostly have the tendency to dismount and slip sideways off the teaser without completion of the copulation. As long as the male has been used for natural mating for several consecutive breeding seasons, the male will dismount faster refusing to complete the copulation process. Elder breeding males can almost dismount just after the intervention of the technician for diverting the male's penis towards the AV. This mating behavior during semen collection using an AV and a teaser may reflect the reason for the reported wide ranges of the copulation durations and the volumes of semen delivered by this technique. Ejaculate volume can vary from 2 to 10 ml as there is great variation between males and even between ejaculates from the same male (El-

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Hassanein, 2003; Wani et al., 2008; Morton et al., 2013; Skidmore et al., 2013; Mostafa et al., 2014). Good quality semen can be collected by using AV at weekly intervals from dromedary camels, but the overall efficiency of semen collection by AV is low and needs to be further improved to adopt camel males for artificial insemination programs (Al-Bulushi et al., 2014). The larger volume samples of dromedary camel semen (16 ml) collected by Kutty and Koroth (2012) using AV were white, homogeneous, viscous and sperm rich. These values of semen volume were recorded for males after their copulation for durations ranged from 5 to 15 minutes, however, under natural mating, copulation duration may extend to more than 40 minutes. It is obvious that the elongation of male camel copulation duration is almost attained with donation of more semen volume and sperm concentration.

Figure 2. Semen collection by an artificial vagina and a teaser female.

Although collection of semen from male camels by AV and teaser females is considered to be the most standard and repeatable procedure, practicing the technique has however revealed several disadvantages, the most important of which are:- occurrence of incised bleeding injuries on the back of the teaser female, disturbance and excitation of the male by intervention of the technician in the mating process, exhaustion of the technician during the long copulation period, the risk of injuries to the operators, frequent failure of ejaculation in the AV, dismounting of males without completion of copulation (Tibary and Anouassi, 1997; El-Hassanein, 2003) in addition to refusal to serve the AV and sand contamination (Deen et al., 2003).

Collection by using an AV fitted inside the camel dummy

Several attempts to overcome the difficulties of semen collection from camelids were noticed early (Garnica et al., 1993). The most striking of these attempts was the construction of an alpaca or llama dummy of the same shape and size of a recumbent female for semen collection (Garnica et al., 1993; Lichtenwalner et al., 1996b; Bravo et al., 1997). The practical use of an AV mounted inside a dummy for semen collection from alpaca and llama has proven to yield copulation periods, mating behaviors and semen output mostly representative to that obtained in natural mating. Similar attempt to overcome different constraints of semen collection from dromedary camels was also conducted later in Egypt. El-Hassanein (2003) has successfully devised and tested a camel-dummy that resembles the shape and size of a natural female dromedary camel in the recumbent posture. The design and the structural formation of this devise has been discussed earlier in details (El-Hassanein, 2003). The dummy was designed to become visible as a recumbent natural female camel on the ground of the collection area when firmly fixed on a definite aperture in the ceiling of a small lab underneath it. It was also designed to mount an AV inside it such that the front edge of which is fitted in the same position of the vulva of the natural female. Fixation of AVs and exchanging them between different males are promptly carried out from the small lab underneath the dummy (Figure 3). Practically, using the dummy for semen collection from well trained males has proven that it is the most natural and reliable technique for semen collection from dromedary camels.

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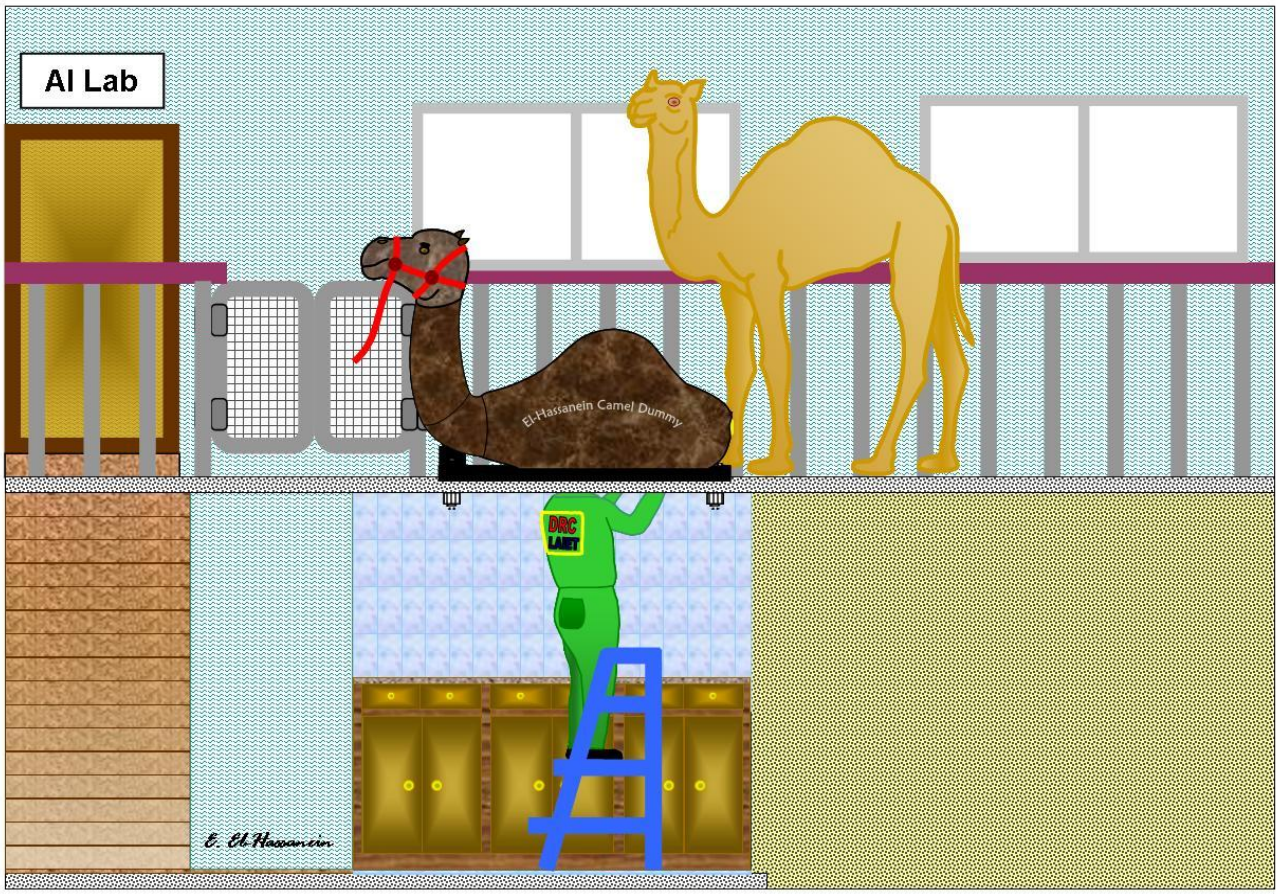

Figure 3. The technician promptly fixes and exchanges artificial vaginae for different males from the lab underneath the dummy.

Similar reports were also recorded in the South American camelids by Adams et al. (2009). Males that were trained well and accustomed to mating with the dummy have entirely reacted well throughout their copulation periods which exceeded up to 45 minutes and produced a collection semen volume of up to 35 ml. (Figure 4). These obtained results may reflect to how much the semen collection from dromedary camels can be advanced by applying the camel dummy technique. Replication of the male's copulation duration by this technique and hence the multiplication of the delivered semen quantity may imitate to a great extent the natural mating process in dromedary camels. The duration of copulation (15-45 min) and the volume of delivered semen in a collection varied between males (12-35 ml) depending on their sexual stamina and to how much they were trained well to savour mating with the dummy.

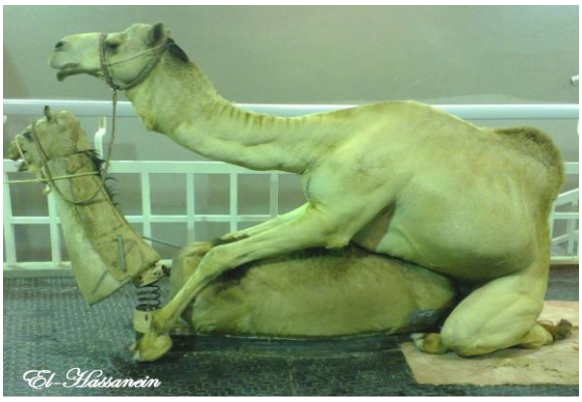

Figure 4a. A newly retired racing-male (8 years age) acting exclusively well with the camel dummy.

Figure 4b. A partially diluted collection of semen (1 semen : 1 extender) of a total volume of about 70 ml.

Management and training of males for semen collection using AVs

Under semi extensive herding production, pastoralists either release the desired breeding male to graze together with the breeding females during the breeding season (free mating) or confine the breeding male lonely in a captivity yard for managing the breeding activity of their herds (controlled breeding). Under intensive breeding management in

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AI-centers, where males are selected and intended for standard semen collection and preservation, each desired male camel is often kept in a well ventilated and half-shaded pen of about 75 m2 space.

Regarding the confinement-related problems in intensive management systems, several reports have proven that animals in captivity can develop abnormal stereotypical behaviors which reflect the impact of captivity on the animal welfare and hence on their performance (Broom, 1991; Mason, 1991; Inglis and Langton, 2006; Cooper and McGreevy, 2007). A recent striking study on the negative effect of captivity on the housed dromedary male camels in rut has been reported by Padalino et al. (2014). This study has asserted that male camels in full captivity housing systems have obviously revealed several oral and locomotor stereotypical behaviors. In our practice, we have also recorded two abnormal sexual behaviors. Some males in captivity were occasionally exhibited self-ejaculation. Other males were rhythmically protrude their penis when they were recumbent on the sandy ground and then retract it back into inside the scrotum after being contaminated with sand grains (Figure 5). These abnormal sexual behaviors were negatively reflected on the delivered semen either as a reduction in its volume or a contamination with sand particles.

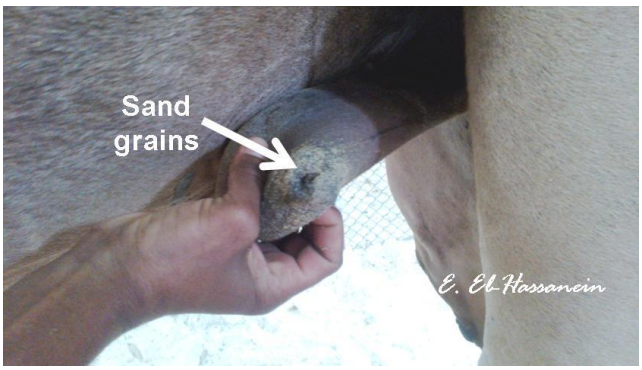

Figure 5a. Scrotal orifice filled with sand granules.

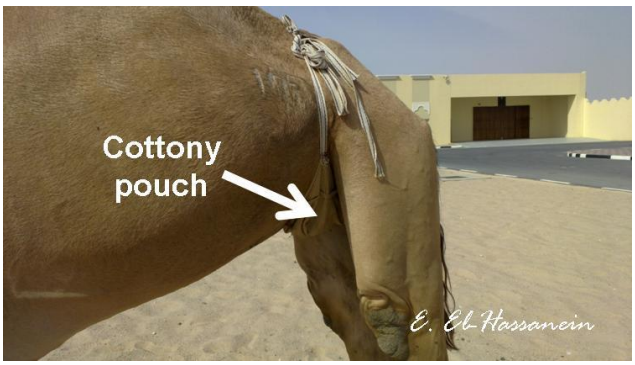

Figure 5b. Scrotum enclosed with a cottony pouch.

It is generally considered that the major effects of confinement on the male camels in rut are the limited opportunity for locomotion, the social isolation and the inability to forage on pasture. These captivity stressors may negatively affect their welfare status and their reproductive performance. It has been noticed that male camels in herds mostly show earlier sexual behaviors and longer breeding season compared to housed males. Recent reports have asserted that the allowance of a daily free movement for 1 hour and the social interaction with frequent female parade could improve the welfare and reproductive performance of male camels housed for semen collection (Vyas et al., 2001; Bhakat et al., 2005; Fatnassi et al., 2014a,b; Padalino et al., 2014).

By the onset of the breeding season, male camels housed for semen collection programs must be allowed to have the opportunity to daily regular movement for about 1 hour to improve the captive animal's physical fitness and muscle tone. This exercise regime should be carried out daily or bi-daily throughout the breeding season. On the scheduled days of training or actual semen collection, and just before the time of starting the training or collection process, a parade of receptive females (5-10 animals) should be carried out for about 1 hour in front of the confined males to enhance their olfactory and ocular stimuli before training or collection of semen.

Males adopted for semen collection by AV techniques (either in combination with a natural female or mounted inside a camel dummy) must be habituated to the collection area at the beginning of the natural breeding season (Sep.-Oct.). Each male should enter the collection area and spend about 20-30 minutes in the presence of the expected operators of the collection process and of either a restrained kneeling natural female or a fixed camel dummy. This accustoming program must be carried out bi-daily after a female parade and nearly at the expected time of actual semen collection.

Training males to accept mating with the AVs must be start during the course of acclimatization to the collection environment. The modified AVs must be prepared and filled with warm water and incubated for 2-3 hours inside an AVs-Incubator pre-switched on and adjusted at 50-55^{o}C. Attention must be given to the adjustment of temperature inside the AV at 40-45^{o}C throughout the training and collection periods.

For semen collection using an AV and a teaser female, intervention of the technician should be carried out only after squatting down of the male behind the teaser and as he begins to erect his penis towards the female's vulva. Operators of the collection process should be careful of sudden biting and their movement inside the collection area must be limited during male copulation.

In the case of using the camel dummy in training and for semen collection, the males can get excited and attracted to the dummy by trembling its head and neck, through pulling a long robe looped around its head, in combination with emission of a recorded female bleat inside the collection area as an audiovisual stimulus. Furthermore, sprinkling a little bit of urine of a receptive female around the tip of the AV at the rear end of the dummy is also practiced as an olfactory

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stimulus to arouse males for mating with the dummy. As in natural breeding practice of herders, some males may need assistance in directing their penis into inside the AV of the dummy.

In general, to optimize semen collection from dromedary camels using AV techniques (either in combination with a teaser female or fitted inside a camel dummy), males can be practically categorized into three brands:

1. Males which have recently reached sexual maturity (7-8 years age) and have not practiced natural mating yet: These males are usually acceptable to serve AVs after a short period of training and give excellent results in semen collection, i. e. semen volume and quality. These trained males will be the main source of semen in AI-program throughout their breeding lifespan.
2. Males which have practiced natural mating for 4-5 breeding seasons (8-12 years age): These males take a relatively longer time and more effort for training to serve AVs. Most males of this type often accept mating with AVs after a training course of about 2-3 weeks at the beginning of the breeding season.
3. Elder males which have professionally practiced natural mating for 5-10 breeding seasons (12-20 years age): These kinds of males are most likely to refuse mating with AVs. However, a small fraction of these males can accept training and collection with AVs after a long period of training (4-6 weeks of a condensed training course).

It is very essential to emphasize that alternation of the males between practicing natural mating and their employment in semen collection by the AVs is usually attended with an obvious mutiny of males to mate with the AVs. Consequently, to establish a persistent AI program, males which have been selected and trained well for semen collection by AVs (either in combination with a teaser female or formerly fitted inside a camel dummy) must be completely devoted for this mission in AI-centers and preventing their reuse in natural mating.

Processing and Preservation of Camel Semen

Successful in-vitro preservation of superior genetic materials from elite male camels will obviously facilitate its dissemination over a wide range of female herds through AI-programs. A collection from a male camel will be diluted by 3-5 folds (according to the collection concentration) and then processed into multiple standard semen doses to be used for fertilizing many of receptive females rather than the deposition of this collection in one female.

There are many challenges should be taken into consideration in processing and preserving camelid semen. The prominent viscous nature of seminal plasma and the detrimental effects of cold-shock during short-term preservation (chilling form) and the cryo-damage during long-term preservation (frozen form) are the major constraints in processing and preservation of camelid semen.

Elimination of viscosity in camelid semen

Viscosity of camelid semen causes difficulty in handling and evaluation of the delivered semen immediately after collection. It obviously hinders the interaction of the components of the used diluents with the sperm membranes, and consequently, exposes sperm to cold shock and cryodamage during processing and preservation. In camelids, the whole collection is mainly composed of highly viscous seminal plasma in which spermatozoa are totally embedded. Conversely, in stallions and boars as examples, the delivered semen is mostly composed of two distinct fractions: a sperm-rich fraction (75-90% of sperm) and a sperm-poor fraction (the bulbourethral gel fraction). The poor fraction is about 5-10% and 20-25% of the total ejaculate volume in stallions and boars, respectively. This gel fraction is practically filtered and excluded from the delivered semen and AI doses have been prepared by diluting and processing the sperm-rich fraction.

Freshly collected dromedary camel semen undergoes spontaneous liquefaction (Skidmore, 2005). Dilution of dromedary semen (1:1) in Tris-lactose-yolk extender revealed best liquefaction within 60-90 minutes of incubation at 37^{o}C and highest progressive sperm motility after storage for 24 hours either refrigerated or at room temperature (23^{o}C) (Wani et al., 2008). Several enzymes have been used to improve the rheological characteristics of camelid semen however; all the enzymes used have proven to have detrimental effects on sperm plasma membrane and acrosomal integrity. Bravo et al. (2000b) used trypsin, fibrinolysin, hyaluronidase and collagenase enzymes to reduce viscosity of llama and alpaca seminal plasma and found that collagenase was superior in reducing viscosity but all enzyme treatments had detrimental effects on sperm function and integrity, less of which was recorded in collagenase treatment. Similar findings were also reported by Giuliano et al. (2010) that when they used collagenase at 0.1% in H-TALP-BSA medium to eliminate viscosity in llama semen. Deen et al. (2003) found that adding 0.2 mM of caffaeine to a Tris buffer and dilution of dromedary camel semen at 1:1 ratio it significantly improved motility of individual spermatozoa while the addition of 1% α-chymotrypsin did not reduce semen viscosity. El-Bahrawy and El-Hassanein (2009) tested five different mucolytic agents to remove viscosity in dromedary camel semen after dilution in one step at rate of 1:3 with a Tris-lactose based extender. They found that α-amylase superiorly eliminated seminal viscosity and improved individual sperm motility post-dilution (46.0%) compared to control (27.5%), however, all mucolytic agents had deleterious effects on acrosomal integrity (11.8% detached) after 4 hours of incubation at 37^{o}C. Whilst, addition of α-amylase (10-15 µl/ml)

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to a Tris buffer for diluting dromedary camel semen significantly eliminates viscosity and improves sperm motility and acrosomal integrity post-thaw (El-Bahrawy, 2010), while lower concentrations of α-amylase (5 µl/ml) had no effect on viscosity (El-Bahrawy, 2010; Monaco et al., 2016).

In a proteomic analytical study on several mammalian seminal plasmas, dromedary camel and alpaca had proven to display the least complex seminal plasma proteome of the species studied (Druart et al., 2013). The authors found that the highly abundant camelid seminal plasma proteins (recently identified as beta nerve growth factors, ß-NGF) were rarely shared with other species and if so, were observed in relatively lower abundance. During liquefaction of camelid seminal plasma, abundant proteins with high-molecular mass in fresh semen start to degrade and gradually disappear in seminal plasma in conjunction with production of two low-molecular mass proteins in liquefied seminal plasma (Mal et al., 2016). It has been confirmed that ß-NGF is an ovulation-inducing factor for alpacas and causes 80% ovulation associated with a marked elevation in plasma progesterone concentration after alpaca treatment (Kershaw-Young et al., 2012a).

Earlier reports have attributed the viscous consistency in camelid seminal plasma to the abundance of mucopolysaccharides, recently named glycosaminoglycans (GAGs), which in camels can only come from secretions of the bulbourethral and the prostate glands (Garnica et al., 1993; Hassan et al., 1995). Also, Kershaw-Young et al. (2012b) suggested that GAGs (mainly keratan sulphate), that are secreted from the bulbourethral gland of alpaca, may be the cause of seminal plasma's prominent viscosity. The authors found also that keratan sulphate was correlated with viscosity. However, Kershaw-Young and Maxwell (2012) have proven that proteins, mainly mucin 5B which is secreted from the bulbourethral gland, are mainly responsible for the viscosity in camelid seminal plasma rather than GAGs. Mucin5B protein was found to be about five times more abundant in highly viscous seminal plasma and its concentration decreased considerably by liquefaction of semen. The authors found that keratanase enzyme that specifically degrades keratan sulphate, which constitutes about 85% of the total GAGs in camelid seminal plasma, did not completely reduce viscosity within 2 hours of incubation. Conversely, protease papain was found to be most effective in the complete elimination of viscosity within 30 minutes and in preservation of sperm viability and acrosomal integrity compared to GAGs enzymes and even to the proteinase k (Kershaw-Young et al., 2013). However, Morton et al. (2012) found that papain alone or supplemented with Tris-buffer efficiently reduced alpaca semen viscosity but detrimental effects on sperm integrity were observed. Also, Monaco et al. (2016) found that dilution of dromedary camel semen in Tris based buffer containing the protease papain underwent complete liquefaction within 90 minutes of incubation at 37^{o}C, however, higher percentage of head-to-head sperm agglutination was recorded and is supposed to be due to papain-induced protein degradation. In order to eliminate seminal plasma viscosity in camelids, semen can be diluted immediately following collection with a suitable extender at 1:1 ratio, then treated with 0.1 mg/ml papain for about 20 min at 37^{o}C (or until complete removal of viscosity) followed by addition of 10 uM E-64 (papain inhibitor) to halt the digestion of proteins by papain to conserve sperm cell-membrane integrity and viability (Kershaw-Yong and Maxwell, 2011).

Consequently, it is obviously needed to identify a reliable enzymatic protocol (type, concentration, incubation temperature and exposure time) that can be used to eliminate the viscosity of camel semen whereas sufficiently preserving sperm viability and integrity, i. e. to protect sperm function.

Short-term preservation (Liquid or Chilled form)

Camelid semen can be preserved in a liquid (chilled) form at 4-5^{o}C for use within 1-2 days without a significant decrease in its quality. Accordingly, a reliable daily ovarian scanning of naturally receptive or hormonally induced females should be conducted to assure the existence of the dominant follicle(s) at the optimal size (1.5-2.0 cm in diameter) for ovulation and timed-AI within the limited period of preservation. Diluents used for chilling-storage of semen must be containing a sugar as an energy source (glucose, lactose, sucrose or fructose), a non-permeating protein for protecting sperm cell membrane against cold-shock and damage (lipoprotein from egg yolk or casein from milk) in addition to the buffer medium (to maintain pH and tonicity) and the antibiotics.

Over the years, several extenders have been tested for chilling-storage of camelid semen including conventional (sugar-based, citrate-based, Tris- and Tris-tes-based buffers in addition to skimmed-milk) and commercial extenders (Green buffer, Biladyl, Androhep, Triladyl, Laiciphos, Biocephos, OptiXcell, EquiPlus and INRA-96) and have revealed obvious conflicting results.

In alpacas, dilution of epididymal sperm cells with lactose-yolk extender superiorly maintained sperm progressive motility and longevity (62%) for up to 24 hours at 4^{o}C as compared with dilution in citrate- and Tris- based extenders (Morton et al., 2007). However, dilution of collected alpaca semen with citrate-glucose-yolk (reviewed in Bravo et al., 2013) or with Biladyl (Morton et al., 2009) had improved sperm viability for up to 48 hours at 4^{o}C as compared with dilution in citrate, Tris-based, lactose, Androhep, Triladyl and skim-milk extenders. In Bactrian camel, Niasari-Naslaji et al. (2006) found that dilution of semen with SHOTOR (a Tris-based) extender or with Green buffer had revealed higher

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progressive forward motility (65.5 and 60.5%, respectively) after dilution as compared with dilution in lactose (31%) and sucrose (28%). In another study, Niasari-Naslaji et al. (2007) had recorded 72.3% progressive forward motility, 63% membrane integrity and 87.5% live sperm after dilution with SHOTOR, however, fast cooling after dilution to 4°C and storage for 24 hours revealed a progressive motility of 47% compared with slow cooling (31%).

The marked reduction in sperm progressive motility after cooling of diluted Bactrian semen either at slow (57%) or fast (35%) rate, compared with the post-dilution related value (72.3%), may reflect the need to optimize the cooling rate. It is noteworthy to mention that too fast-cooling of sperm will susceptible them to damage by intracellular ice formation, while too slow-cooling may lead to sperm damage by long exposure to concentrated solutions resulting from progressive conversion of extracellular water to ice. Optimization of pH value and osmotic pressure in diluted semen and the rate of cooling of it from 35 to 5°C is considered crucial for successful retaining of sperm viability and longevity post-chilling.

In dromedary camels, earlier reports had stated that dilution of semen with a Tris-based buffer superiorly maintained sperm viability for up to 48 hours at 5°C compared with dilution in lactose buffer (Vyas et al., 1998), Biociphos (Deen et al., 2004) or in citrate or sucrose based extenders (Wani et al., 2008). However, Zeidan et al. (2008) found that dilution of camel semen with fructose-yolk-citrate, lactose-yolk-citrate, sucrose-yolk-citrate or Tris-yolk-fructose extender revealed better sperm motility and longevity after storage at 5°C for 3 days compared with dilution in glucose-yolk-citrate, skim-cow-milk and skim-camel-milk extenders. Other studies showed that Green buffer was found to be superior to Tris-buffer in preserving sperm motility, integrity and viability in dromedary semen preserved at 5°C for up to 48 hours (Ghoneim et al., 2010; Waheed et al., 2010). Recently, Al-Bulushi et al. (2016) recommended dilution of dromedary semen with OptiXcell, Green buffer or Triladyl for storing at 4°C for up to 48 hours.

An attempt was conducted to compare the effect of diluting dromedary camel semen with freshly prepared (4°C) or stored frozen (-20°C) Green buffer extender on sperm parameters after dilution and on the *in-vivo* fertility after AI (Morton et al., 2011). Dilution in fresh or frozen-thawed Green buffer revealed higher motility (70.7 and 68.8%, respectively) compared with sperm motility in neat semen (35%), pregnancy rates were higher for single and multiple ovulating camels inseminated with semen diluted in fresh buffer (72.7 and 83.3%, respectively) compared to camels inseminated with semen diluted in frozen-thawed buffer (27.3 and 11.1%, respectively). The reported AI results may reflect alteration in the buffer components by freezing and thawing that led to deprivation of its protecting ability for sperm membrane and DNA against cold-shock.

Recent studies have been focused on using commercial Green buffer and INRA-96 (IMV, France) extenders for dilution and chilling-storage of dromedary semen. It is reviewed that both extenders were found to be comparably able to maintain sperm motility and integrity after dilution (56%), but sperm viability was higher after chilling in INRA-96 (65%) compared with Green buffer (56%) and pregnancy rates were similar with the two extenders (34%) (Skidmore et al., 2013). Also Morton et al. (2013) reported that dilution of dromedary semen with Green buffer or with INRA-96 significantly improved sperm motility after dilution (67.4 and 59.1%, respectively) compared with motility in neat semen (45.5%) and both extenders comparably retained sperm motility (47.6 and 48.3%, respectively), sperm viability (58.9 and 62.2%, respectively), sperm membrane integrity (54.9 and 57.6%, respectively) and acrosomal integrity (84.8 and 84.6%, respectively) after being stored at 4°C for 24 hours. However, chilling-stored semen diluted with INRA revealed superior sperm fertilizability (23.5%) than that diluted with Green buffer (0.0%) which may reflect sperm DNA damage during chilling-storage of semen diluted with Green buffer. It has been reported that sperm with damaged DNA, when succeeded to fertilize oocytes, the resulting embryos may fail to develop or implant in the uterus or may be naturally aborted at a later stage (Alvarez, 2003).

The noticeable reduction in sperm progressive motility, after cooling and storage for 24 hours, by up to 30-35% of corresponding post-dilution values (Niasari-Naslaji et al., 2007; Morton et al., 2013) may reflect susceptibility of sperm cells to cold-shock and membrane damage during cooling of semen from body temperature to near the freezing point of water (from 35 to 5°C). The primary lesions associated with cold-shock damage are the morphological alterations in sperm plasma membrane and changes in membrane permeability (Barrios et al., 2000).

It is known that storing semen in a liquid form will be associated with sperm ageing and a decline in their incubation lifespan due to accumulation of toxic metabolic products mainly in the form of reactive oxygen species (ROS) resulting from the lipid peroxidation of sperm membranes (Salamon and Maxwell, 2000). During processing and preservation of semen, sperm membrane will be susceptible to lipid peroxidation due to oxidation of the membrane polyunsaturated fatty acids and thereby high production of hydrogen peroxide. ROS accumulation leads to oxidative stress which may cause damage of sperm membrane, reduction in motility, viability, DNA integrity and lowering fertility (Gavella et al., 1996; Aitken et al., 1998, 2010; Kumer et al., 2011).

Oxidative stress plays an important role in the decline of sperm quality during chilled storage through the production of hydrogen peroxide and supplementation with an antioxidant additive (such as: thioglycol, adenosine, prolactin, lycopene, catalaze, cysteine, ascorbic acid, Vitamin C, vitamin E) is often used to provide protection against oxidative stress in liquid-stored semen. Moreover, it is suggested that lowering the final concentration of egg yolk in the

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diluent, as a substrate for the dead sperm enzyme aromatic-L-amino acid oxidase, it will also reduce the production of hydrogen peroxide during storage of diluted semen. Medan et al. (2008) reported that the addition of catalaze enzyme at 500IU/ml significantly increased sperm motility (from 53.2 to 62.7%) and decreased dead sperm (from 22.4 to 16.5%), sperm abnormalities (from 13.4 to 7.9%) and acrosomal damage (from 7.5 to 4.5%) in dromedary camel semen diluted in Tris-fructose-yolk extender and stored at 5^{o}C for 5 days. The authors concluded that addition of catalaze enzyme to semen extender can be used to prolong camel sperm survival during storage at 5^{o}C.

Egg yolk is the most common non-permeable protein added to semen extender for protection of sperm against cold-shock. Phospholipids and low-density lipoproteins in egg yolk can lessen the chilling injuries on sperm by binding to sperm membrane and increasing its permeability whilst preserve its composition and physical properties (Holt, 2000). However, egg yolk had proven to contain progesterone (Bowden et al., 2001) which plays an important role in the sperm capacitation during processing and storage. Also, some components in egg yolk interfere with biochemical assays and metabolic investigations.

In general, egg yolk is composed of plasma and granules fraction. The yolk plasma is mainly constituted of 85% low-density lipoproteins and 15% livetin, whilst yolk granules by 70% high-density lipoproteins, 16% phosvitin and 12% low-density lipoproteins (McCully et al., 1962). Low-density lipoproteins are the main egg yolk constituent representing 2/3 of the yolk dry matter and 22% of the yolk proteins. It is considered the main contributor of yolk emulsifying properties (Martinet et al., 2002) and the most likely source of protection for sperm against effects of storage at 5^{o}C (Watson and Martin, 1975). It is also reported that extender containing purified low-density lipoproteins preserves the sperm membrane integrity after ejaculation and dilution by preventing seminal plasma proteins from binding with sperm surface and causing lipid efflux from sperm membrane (Bergeron et al., 2004). Low-density lipoproteins can be extracted from egg yolk at 97% purity according to the procedure of Moussa et al. (2002). It has also been proven that low-density lipoproteins contain a low progesterone level than egg yolk due to the filtering effect of the dialysis procedure during its extraction (Bencharif et al., 2008). Therefore, it is hypothesized that extender containing extracted low-density lipoproteins provides good protection for sperm membranes and acrosomes than the whole egg yolk.

Therefore, further research attempts are needed to test a wide range of antioxidants and substitution of egg yolk with extracted low-density lipoproteins in camel extenders in order to lessen oxidative stress produced during chilling-storage of dromedary semen and to protect sperm membranes and DNA integrity against cold-shock damage.

Long-term preservation (Frozen form)

The aim of freezing semen is the production of a cryopreserved stock of genetically elite sperm cells to be used throughout several decades for AI-programs and applying other reproductive technologies such as *in-vitro* and *in-vivo* embryo production and transfer. During semen processing for cryopreservation, sperm cells are susceptible to several stressful stages that can cause biochemical and anatomical alteration in their compartments (acrosome, DNA, mitochondria, axoneme and plasma membrane). A thriving sperm-freezing protocol should prevent lethal intracellular ice crystal formation and to reduce cell membrane damage during and after freezing of semen. The composition of the semen extender plays a critical role in protecting sperm against chilling-shock and cryoinjury which occur during cooling (from 15 to 5^{o}C), freezing (from -5 to -50^{o}C) and thawing (from -50 to -5^{o}C) critical temperature stages of processing.

Cryoprotectants are often included in the cryopreservation extender to reduce the physical and chemical stresses derived from cooling, freezing and thawing of sperm cells (Purdey, 2006). The efficacy of the utilized cryoprotectant agent is almost always based on comparing the pre-freezing and the immediate post-thawing sperm viability and its capability to protect sperm compartments against cryoinjury. Sperm cryopreservation extenders should contain a permeating cryoprotectant (glycerol, ethylene glycol, propylene glycol, dimethyl sulfoxide or amides) in addition to the non-permeating cold-shock protectants, the buffer medium, carbohydrates as energy source and antibiotics. The non-permeating cryoprotectors create an extracellular osmotic pressure that induces cell dehydration and lower the incidence of intracellular ice formation in addition to their interaction with phospholipids in the cell plasma membrane and increasing the sperm survival to cryopreservation. However, permeating cryoprotectors penetrate inside the sperm cells preventing the formation of ice crystals, stabilizing cell-membrane lipids and limiting shrinkage of the sperm cells at subzero temperatures. To this date, glycerol is the most common cryoprotectant in use for sperm cryopreservation for its osmotically permeating properties. It replaces intracellular water and maintains cellular volume, interacts with ions and macromolecules and depresses the freezing point of water so that less ice forms inside cells (Holt, 2000).

When alpaca and llama semen was liquefied after collection with collagenase (1 mg/ ml) and diluted in two-steps with citrate-yolk-glycerol extender and cryopreserved in liquid nitrogen, the percent of sperm motility reported after liquefaction, after final dilution (pre-freezing) and after thawing at 35^{o}C for 8 seconds were: 80% oscillatory motility, 60% progressive motility and 30-40% oscillatory movement, respectively (reviewed in Adams et al., 2009) and pregnancy rate recorded after insemination was 26% (5/19). In a previous study, alpaca semen was diluted in two-steps with either Tris-citrate-yolk or skimmilk-fructose-yolk extender, each of which was split to be supplemented either with

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glycerol or ethylene glycol as a cryoprotectant agent (Santiani et al., 2005). The authors reported that skimmilk-based extender containing either glycerol or ethylene glycol seems to promote the survival of more viable sperm with intact acrosomes than the Tris-based extender. Morton et al. (2007) reported higher pre-freezing and post-thawing motility and acrosomal integrity in epididymal alpaca semen diluted with lactose-glycerol based extender and frozen in pellet form than that diluted and frozen with citrate-glycerol and Tris-glycerol based extenders. Also, a study on the effect of glycerol concentration and EquexSTM® supplementation in diluent on the post-thaw viability of epididymal alpaca sperm was conducted by Morton et al. (2010). They reported that epididymal alpaca sperm can be cryopreserved after dilution in lactose (11%)-egg yolk (20%) extender containing 3-4% final glycerol and 1% final EquexSTM® paste concentration. EquexSTM® interacts with egg yolk in the extender, modifies the protein structure of lipoproteins and improves stability of plasma membrane lipids by adding cholesterol (Pena and Linde-Forsberg, 2000).

In Bactrian camels, semen is diluted in two-steps with lactose (11%)-egg yolk (20%) + glycerol (6%) and OEP-Exquex (1.5%) to reach a final glycerol concentration of 2% after dilution (Seime et al., 1990) and achieved post-thaw motility rates of 70%, but morphological abnormalities reached 48%. Orvus ES Paste (OEP) is a detergent that causes the emulsion and dispersion of yolk-lipids in the extender facilitating their interaction with the sperm membrane surface to protect sperm cells against cryoinjury (Pursel et al., 1978). In Chinese studies, dilution of Bactrian semen in two-steps with sucrose (12%)-egg yolk (20%) and glycerol (7%) revealed higher post-thaw motility, viability and acrosomal integrity compared with different extenders (Zao et al., 1996) and realized a pregnancy rate of 93% (29/31) after insemination (Chen et al., 1990). However, Niasari-Naslaji et al. (2007) found that dilution of Bactrian semen in two-steps with SHOTOR (a Tris-based extender) containing 6% glycerol was superior in preserving progressive motility (35%), membrane integrity (49.5%) and sperm longevity (61.5%) post-thaw compared with 4 and 8% glycerol concentrations. The authors also compared SHOTOR containing 6% glycerol and Green/Clear buffers extenders on post-thaw viability and found that SHOTOR efficiently preserved progressive motility (29.9%) post-thaw compared with Green/Clear buffer (4.2%), however, the two extenders comparably preserved sperm membrane integrity (44.4 and 37.4%, respectively) and longevity (56.0 and 46.8%, respectively) post-thaw.

In dromedary camels, El-Bahrawy et al. (2006) found that the dilution of dromedary semen with Tris-citrate-yolk-glycerol, Tris-sucrose-yolk-glycerol, Tris-lactose-yolk-glycerol, lactose-yolk-glycerol and skimmilk-yolk-glycerol extenders revealed comparable pre-freezing sperm motility (63.3-68.7%), but dilution in Tris-lactose-yolk-glycerol extender superiorly maintained the highest post-thaw motility (62.3%) and sperm survival (93.2%) compared with dilution in the other extenders. Also, El-Hassanein (2006) found that Tris-sucrose-yolk-glycerol extender significantly reduced the dilution effect on dromedary sperm viability and had improved their freezability after fast freezing as compared with dilution in sucrose-yolk-glycerol and Tris-yolk-glycerol extenders. In a more recent study, El-Bahrawy et al. (2012) found that supplementation of Tris-citrate-yolk-glycerol camel extender with 15 µl/ml α-amylaze enzyme significantly improved sperm post-thaw sperm motility (61.6%) and decreased acrosomal damage (10.4%) and sperm primary and secondary abnormalities (5.0 and 7.0%, respectively). On the other hand, when dromedary semen was diluted and frozen with Triladyl, a post thaw sperm motility of >40% was recorded in 34% of freezing attempts and only 4 samples yielded a post-thaw sperm motility of >50%, however, a drastic reduction in post-thaw motility was recorded after 3 months of storage (Kutty and Koroth, 2012).

Regarding the type and concentration of the cryoprotectant used, El-Bahrawy et al. (2006) found that addition of 2% glycerol to Tris-lactose-yolk camel extender superiorly preserved post-thaw sperm motility (45.8%) and survival (73.3%) compared with using 3% glycerol or 2% and 3% dimethyl sulfoxide (DMSO). However, inclusion of 6% DMSO in Tris-fructose-yolk buffer for dilution and cryopreservation of dromedary camel semen had significantly improved post-thaw motility (66.7%), freezability (95.2%) and acrosomal integrity (84.7%) compared with addition of 2, 4 and 6% glycerol or 2 and 4% DMSO (Abdel-Salaam, 2013).

Dromedary epididymal sperm have been harvested and cryopreserved for using in in-vitro fertilization of dromedary oocytes. Abdoon et al. (2013) found that dilution of epididymal sperm with Ovixcell® (a soybean lecithin-based extender) or with Tris-fructose- yolk-glycerol extender significantly improved post-thaw sperm motility (47.5 and 45.0%, respectively) and viability (73.1 and 71.7%, respectively) and improved cleavage (37.3 and 83.8%, respectively) and Morulae and blastocyst (58.1 and 52.2%, respectively) rates. Also, El-Badry et al. (2015) diluted camel cauda epididymides sperm with a Tris- yolk-glycerol extender and reported post-thaw sperm motility, liveability and acrosomal integrity of 32.8, 67.6 and 71.2%, respectively. Frozen-thawed cauda epididymides sperm revealed higher fertilization, cleavage, morula and blastocyst rates (38.6, 28.4, 12.4 and 8.1%, respectively) when used for *in-vitro* fertilization of camel oocytes.

Earlier reports revealed that glycerol has deleterious effects on sperm cells due to its osmotic stress, changes in membrane organization, fluidity and permeability as well as changes in the membrane lipid composition (Watson, 1995). Adequate permeating cryoprotectant should be able to rapidly penetrate the sperm cells at freezing and also to rapidly exit cells at thawing. Amides (such as: acetamide, methylacetamide, formamide, methylformamide and

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dimethylformamide) are permeable cryoprotectants with lower molecular weight than glycerol and can cross sperm cell membrane faster than glycerol, thus exert less osmotic pressure than that can be exerted by glycerol (Carretero et al., 2015; Squires et al., 2004). Besides the crucial role of permeating cryoprotectants in preserving sperm integrity and viability during cryopreservation, substitution of egg yolk in extender by purified low-density lipoproteins has reported to improve the freezability and fertility in many species such as: cattle (Hu et al., 2011), buffalo (Akhter et al., 2011), dogs (Prapaiwan et al., 2016), boar (Jiang et al., 2007) and sheep (Tonieto et al., 2010).

To this date, results of semen freezing protocol and *in-vivo* pregnancy rates by using frozen-thawed semen are not yet satisfactory in the dromedary camels (Deen et al., 2003; Skidmore, 2003; Monaco et al., 2015). It is noteworthy that even when inseminations were carried out using equivalent amounts of motile and viable cryopreserved or raw spermatozoa, pregnancy rates were lower after AI with cryopreserved semen (0-26%) than with raw semen (50-80%) (Bravo et al., 2000a). Poor fertilizability of frozen semen may reflect a cryodamage of sperm DNA in frozen-thawed sperm even that revealing acceptable post-thaw motility and viability. Earlier report of Griveau and LeLannou (1997) demonstrated that sperm plasma membrane and DNA integrity are susceptible to damage during freezing-thawing process due to lipid peroxidation. Also, Bilodeau et al. (2000) reported that excessive generation of ROS molecules is evident during cryopreservation of mammalian semen that reduces the viability and fertilization capacity of sperm.

Therefore, further studies are needed to develop the cryodiluents for dromedary semen and the freezing and thawing protocols. Substitution of egg yolk with purified low-density lipoproteins in addition to utilizing amides as cryoprotectors may lessen the oxidative stress on the processed sperm and protects sperm membrane and DNA against cryodamage.

CONCLUSION

To establish a persistent AI-strategy for germblasm preservation from elite dromedary camel breeds, selected males must be dedicated to semen collection programs in AI-centers rather than permanently practicing natural mating. The dependence on the ease and sufficient semen collection with the camel dummy, as a technique resembling natural mating the most, is the optimal possibility to create a semen collection and storage strategy for preservation of semen from elite dromedary male camels. Persistent abundance of semen under controlled conditions in AI-centers provides excellent opportunities to develop extenders with optimal additives needed for elimination of viscosity and for proper protection of sperm against cold-shock and cryodamage during processing of semen.

Spermiogram assays after different stages of processing (neat semen, dilution, equilibration, post-thawing and incubation post-thaw) should include a set of both compensable and non-compensable traits (Saake, 2008). Including assays of non-compensable factors (such as chromatin or DNA consistency), which is important for predicting the male fertilizability and conception rate, helps to select suitable collections for processing and discarding processed collections of predicted low-fertilizing ability before using in AI (Oliveira et al., 2013; Saake, 2008).

Persistent preservation of successfully processed camel insemination-doses helps to initiate developing of other assisted reproductive technologies such as *in-vivo* and *in-vitro* embryo production and transfer as well as intra-cytoplasmic sperm injection regardless the limited male breeding season.

Competing interests

The author has no competing interest to declare regarding the publication of this article.

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Sero-Prevalence of Contagious Bovine Pleuropneumonia in Three Senatorial District of Kaduna State, Nigeria Using Latex Agglutination Test

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ABSTRACT

A study was conducted to determine the sero-prevalence of Contagious Bovine Pleuropneumonia (CBPP) in cattle in 3 senatorial districts of Kaduna State, using Latex Agglutination Test (BoviLAT PA 6223). One Local Government Area (LGA) from each senatorial district was randomly selected for this study thus; Ikara (Northern), Chikun (Central) and Kauru (Southern). A total of 300 blood samples were each collected from the selected LGAs in each senatorial district using simple random sampling without replacement technique. An overall sero-prevalence of 26.0% (234/900) was recorded, with Kauru having a higher sero-prevalence of 46.0% (138/300), followed by Ikara (17.0%) (51/300) and Chikun (15.0%) (45/300) LGAs. The sero-prevalence was highest (30%) in the age group of >6 years old and lowest (19.3%) in the age group <1-3 years old (P=0.0027). Based on sex of the cattle, there was no statistical significant association (P=0.1424) in the sero-prevalence of CBPP between the female (27.1%) and the male (21.9%) cattle. Though the sero-prevalence showed some degree of variation, there was no statistical significant difference (P=0.0572) in infection among Sokoto Gudali (57.1%), Red Bororo (50%) and White Fulani (25.3%) breeds of cattle. The study revealed the sero-prevalence of CBPP in cattle in the study area. Although BoviLAT Latex Agglutination is not capable of differentiating between antibodies from vaccinated animals and those of natural infection, it however provides, a fast and easy to perform diagnostic technique in the field, and therefore, good for early detection of cattle with CBPP.

Key words: Chikun, Ikara, Kauru, Latex Agglutination, *Mycoplasma mycoides*, Sero-prevalence.

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INTRODUCTION

Contagious Bovine Pleuro Pneumonia (CBPP) is a highly contagious disease of cattle that is caused by *Mycoplasma mycoides* subsp. *mycoides* small colony (*Mmm*SC) (21, 39). Cattles infected with CBPP (caused by *Mmm*SC) has a tendency of becoming "carriers" which may consequently lead to the spread of the disease (Egwu et al., 1996, Musa et al., 2016). The disease is usually spread by movement of animals across international boundaries with devastating consequences on cattle, particularly in severe outbreaks (Santini et al., 1992; Egwu et al., 1996; Huebschle et al., 2004; Thiaucourt et al., 2011; Tambuwal et al., 2011). Transmission occurs mainly by aerosol through close contact with infected animals within herds and from herd to herd through direct contact and repeated contact between sick and healthy animals, and occasionally from latent carriers intermittently shedding Mycoplasma organisms from sequestrasted lung lesions (Radostits et al., 2006).

Contagious Bovine Pleuro Pneumonia is among the OIE list of 118 animal diseases, infections and infestations (OIE, 2016). This is due to its potential for rapid transboundary spread and associated economic impacts. For similar reasons, CBPP is included in the list of 6 priority diseases for FAO's EMPRES–Livestock programme (FAO/OIE, 1995). In addition, the second priority vaccine for the Pan African Veterinary Vaccine Centre (PANVAC) is that of CBPP (PANVAC, 1991; OIE, 1996). Since the first incidence of CBPP in Nigeria in 1924 (Foluso, 2003), the disease has become endemic with pockets of outbreaks occurring particularly in the Northern part of the country, where most of the cattle populations are located (Osiyemi, 1981; Fayomi and Aliyu 1992; FAO/OIE 1995). The disease has been reported in Kano, Katsina, Borno, Sokoto and Kaduna States of Nigeria (Nawathe, 1992).

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Nigeria has the highest cattle population of about 16.3 million in West Africa (Ikhatua, 2011), but is constantly threatened with CBPP (Ajuwape et al., 2004), and "a live with the disease" attitude has always prevailed in the last few years among pastoralist and farmers who hardly report cases of CBPP but rather resort to treatment with antibiotics like any other bacterial disease (Chima et al., 1999). Animal Diseases Information System (NADIS) classified Nigeria as an endangered zone based on her CBPP status. CBPP is considered to be a disease of economic significance due to its ability to compromise food security through loss of protein, increased production costs due to costs of disease control and inhibition of sustained investment in livestock production (Ikede and Taiwo 1988).

Latex agglutination test is a rapid agglutination test that is easier to interpret and can be performed in the field when compared to Slide Agglutination Test (SAT), which lack sensitivity, and detects only animals in the early (acute phase) stage of the disease and can only be used on herd basis (Ayling et al., 1999b; Turner and Etheridge, 1963). Although Latex agglutination is not capable of differentiating between antibodies from vaccinated animals and those of natural infection, it however provides, a fast and easy to perform diagnostic technique in the field, and therefore, it is good for early detection of cattle with CBPP. This test uses a "specific" polysaccharide antigen extracted from the *Mmm*SC capsule, which is then bound to latex beads. "specific" means found to be specific by empirical means, testing it against different antigens until the one was found that did not cross-react or gave false-positive results (John et al., 2003). This test has been evaluated using CBPP negative sera from England and CBPP positive sera from Africa, Portugal and Italy (Ayling et al., 1999a). Sensitivity was comparable to the internationally recognized complement fixation test, but is far simpler and more rapid to perform. This test may have great potential in parts of Africa where there are great distances between the outbreaks, usually in nomadic herds, and diagnostic laboratories enabling control measures to be implemented rapidly (March et al., 2003). This study was aimed at detecting the presence of CBPP antibodies in the field using BoviLAT Latex Agglutination Test as an alternative to other tests in the three senatorial districts of Kaduna State, Nigeria.

MATERIALS AND METHODS

Study Area

The research was carried out in 3 senatorial districts of Kaduna State, which is located in the North-West geo-political zone of Nigeria. Kaduna state lies between Longitude 30'' and 0900 East of the Greenwich Meridian and has a Latitude of 0910 and 11°30'' North of the Equator (KADP) (Figure1). Kaduna State has 23 Local Government Areas (LGAs) and a human population of 6.07 million (NPC, 2006). The state is an agrarian state and also has potentials for livestock industry, with about 70-75% of the population engaging in farming activities (NLR, 1992).

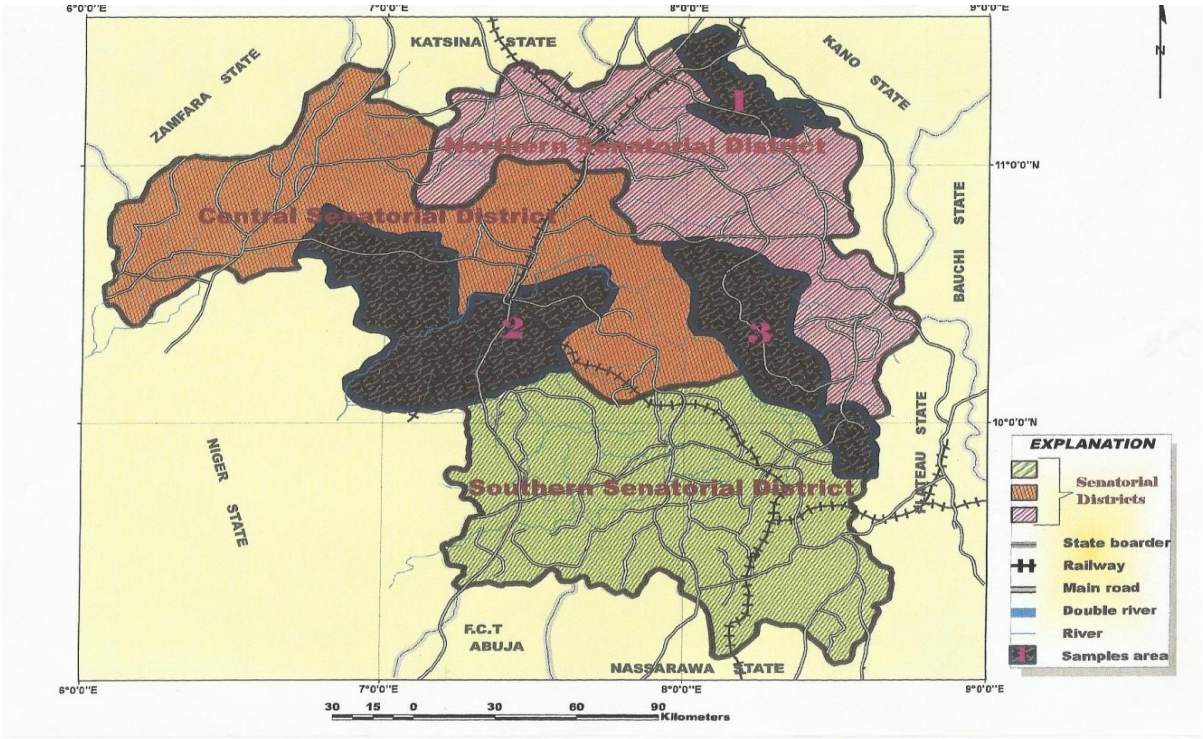

Figure 1. Map of Kaduna State showing the three Senatorial Districts with Sampled areas (**1:** Ikara Local Government Area -representing the Northern Senatorial District, **2**: Chikun Local Government Area -representing the Central Senatorial District and **3**: Kauru Local Government Area -representing the Southern Senatorial District).
Source: Department of Catography; National Geoscience, Kaduna Zonal Office, Barnawa, Kaduna, Kaduna State, Nigeria (Production map from KADP, 2012).

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Sample Size

Sample size was determined as described by the method of Mugo (2011).

$$n = \frac{Z^2 Pq}{d^2}$$

Where:
n= sample size
q = 1-p
P = expected prevalence of 47% (Danbirni, 2010)
d = desired absolute precision = 5% or 0.05
Z = appropriate value for the standard normal deviation for the desired confidence = 1.96
Therefore,

$$n = \frac{1.96^2 \times 0.47(1-0.47)}{0.05^2} = 382.7$$

This sample size shows a minimum of 382.7 cattle heads that can be investigated. However, to increase precision, 900 heads of cattle were bled and sera obtained.

Collection and processing of blood samples

One local government area (LGA) in each of the senatorial district (Northern, Central and Southern) was randomly selected to represent the district. The LGAs are; Ikara for (Northern), Chikun (Central) and Kauru for Southern senatorial districts. Each of the 3 selected LGAs has 11 political wards and 3 political wards were randomly selected from each of the LGAs. A cattle population of a minimum of 20 heads was considered as a herd in this study (Tambuwal et al., 2011), and 10 herds were randomly identified in each of the selected ward. Ten heads of cattle from 10 herds were randomly sampled and properly restrained in each of the 3 selected wards in a LGA (making a total of 300 heads of cattle in each LGA). Ten millilitres of blood was aseptically collected from the jugular vein of each cattle using an 18G needle mounted on a 20ml syringe and their respective ages, sexes and breeds labelled and recorded on each sample bottle. The blood samples collected were kept in a slanting position at ambient temperature for about 6 hours and the sera separated from the cellular component of the blood were collected and transferred into labelled plain sterile sample bottles and stored at 4^{O}C until use.

Sera Analysis (BoviLAT Test Procedures)

The test was carried out according to the manufacturer's procedure for BoviLAT test Kit (BoviLAT PA6223). To perform the test, the sera was brought out from the cold box and allowed to attained ambient temperature. Twenty micro litre of serum was dropped onto a black reaction card using plastic dropper. This was carefully dispensed to avoid air bubbles. The BoviLAT Latex reagent was well shaken and a drop of the reagent was added close to the spot where the serum was dropped. The BoviLAT Latex reagent and the serum were mixed together using a wooden stick (sterile tooth pick) and the mixture spread out inside the reaction cell. The reaction card was rocked from left to right for three minutes and any agglutination or otherwise was recorded. A maximum of six reactions were done at a time.

Reaction Process

The latex cards are coated with Capsular Polysaccharide (CPS) purified from *Mmm*SC cells. Antibodies recognising the CPS that banded and cross-linked the latex particles causing agglutination at different degrees in the positive cases are as follow; (i) Positive (+++), Strong clumping of latex beads, and agglutination beginning within one minute, (ii) Positive (++), Clear agglutination of latex beads, agglutination beginning between one and two minutes and (iii) Positive (+), Fine agglutination of latex beads, agglutination formed between two and three minutes. The sera from animals that might not be suffering from CBPP will not show any reaction, and therefore, it is recorded as negative (-), No agglutination formed within three minutes (Figure 2 A, B, C, D, E).

Data Analysis

The data obtained were presented in tables using Microsoft Excel 2007 and analyzed with Graphpad Prism version 4.0 for Windows and Chi-square (X^2). Values of $P \leq 0.05$ were considered significant. The prevalence of CBPP was determined using the formula:

$$\text{Prevalence} = \frac{\text{Positive sample} \times 100}{\text{Total samples analyzed}}$$

Ethical approval

This work was jointly approved by the Ahmadu Bello University Zaria ethics committee on the use of animals for research purposes and the department of livestock resources, Kaduna State ministry of Agriculture, Nigeria.

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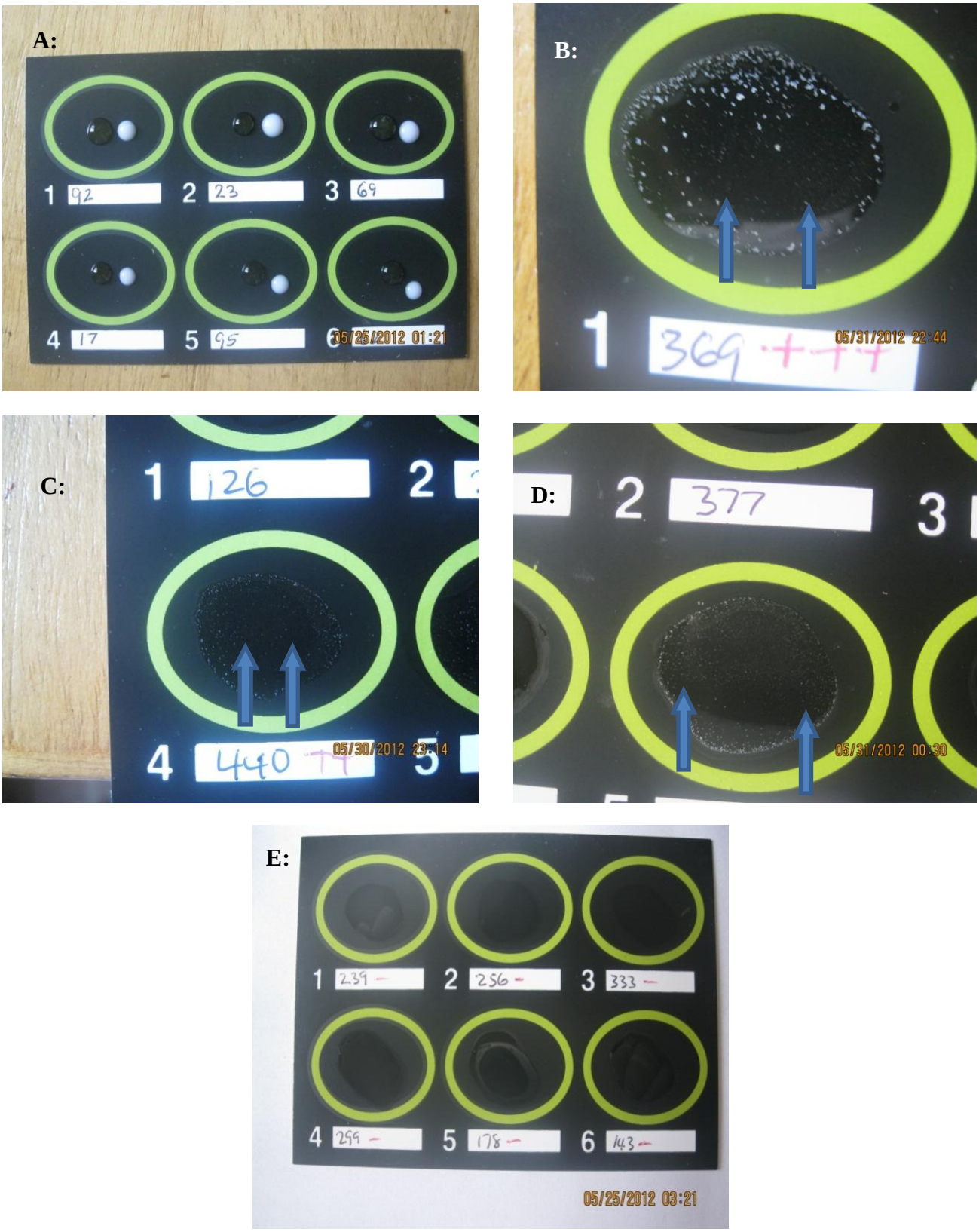

Figure 2.

A: Reaction beads card coated with capsular polysaccharides purified *Mmm*SC made up of 6 reacting cells containing spot of serum and reagent next to each other in the cells (arrows), for the latex agglutination test to diagnosise CBPP in cattle.

B: Strong clumping of latex beads (arrows). Agglutination of CBPP antibodies began within one minute, 3 positives (+++) indicates a positive case.

C: Clear agglutination of latex beads (arrows).

D: Fine aggluttination of the latex beads (arrows). agglutination of CBPP antibodies formed between two and three minutes, 1 positive (+) indicates a positive case. Agglutination of CBPP antibodies began between one and two minutes, 2 positives indicates a positive case.

E: Negative (-). No agglutination formed in all the reaction cells of *Mmm*Sc after three minutes (homogeneuos solution), indicating negative cases.

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RESULTS

An overall sero-prevalence of 26.0% (234/900) was recorded. Forty six percent (138/300) of the 300 cattle sampled in Kauru LGA were sero-positive, while, 17.0% (51/300) and 15.0% (45/300) of the 300 cattle sampled from Ikara and Chikun LGAs respectively, were similarly sero-positive for CBPP. Sero-prevalence of CBPP was significantly associated with LGAs sampled (P=0.0001) (Table 1).

From the 275 heads of cattle in the 3 senatorial districts with an age range of <1-3 years, 17.1% (47/275) were sero-positive for CBPP. This consist of 31.0% (27/87) sero-positivity for cattle in Kauru, 11.9% (12/101) for Ikara and 9.2% (8/87) for Chikun LGA. Similarly out of a total of 375 heads of cattle within the age range of 4-6 years old from the 3 districts, 24% (90/375) were sero-positive for CBPP out of which, Kauru, Ikara, Chikun LGAs 47.8% (56/117), 11.1% (19/171) and 10.2% (15/147) sero-positivity respectively.

Two hundred and fifty heads of cattle with ages >6 years old from the 3 districts were sampled and 38.8% (97/250) of the cattle were sero-positive for CBPP. Among the cattle with ages >6 years old in the 3 selected LGAs from the 3 district, Kauru LGA had a higher 57.3% (55/96) sero-prevalence rate of CBPP than Chikun 33.3% (22/66) and Ikara 22.7% (20/88) LGAs. There is a significant association (P=0.0027) in the sero-prevalence for CBPP between the age groups with the sero-prevalence being higher 38.8% (97/250) in ages >6 years old, and lowest 17.1% (47/275) in the younger cattle aged<1-3 years old (Table 2).

Table 3 shows that 196 male cattle were sampled for sero-prevalence for CBPP from the 3 senatorial district, and 21.9% (43/196) were found to be sero-positive for CBPP. While out of the 704 females sampled, 27.6% (191/704) were sero-positive for CBPP. Male cattle from Kauru LGA had higher 31.4% (17/54) sero-prevalence for CBPP than Chikun 18.2% (10/55) and Ikara 18.4% (16/87) LGAs. Similarly, female cattle sampled from Kauru had a higher 49.2% (121/246) sero-prevalence rate for CBPP than Ikara 16.4% (35/213) and Chikun 14.3% (35/245). There is no significant association (P=0.1424) between sexes in the CBPP sero-prevalence in the cattle sampled and equally no association between sexes in the CBPP sero-prevalence in the cattle sampled in the 3 LGAs.

Among the cattle sampled for this study in the 3 senatorial district, 97% (879/900) were of the White Fulani (Bunaji) breed, while, 1.6% (14/900) and 0.8% (7/900) were Red Bororo (Rahaji) and Sokoto Gudali (Bokoloji) breeds respectively. The White Fulani breed of cattle in the 3 district had a sero-prevalence rate of 25.4% (223/879), out of which the white Fulani breed of cattle from Chikun LGA had the lowest sero-prevalence rate 13.9% (41/295) while those from Kauru LGA had the highest 45.9% (134/292) sero-prevalence rate. Of the Red Bororo breed of cattle sampled in the 3 senatorial district, 50% (7/14) were sero-positive. None of the Red Bororo breed of cattle from Ikara LGA but 40% (2/5) were positive for CBPP. There was no significant association in the sero-prevalence between the breeds of cattle sampled (P=0.0572) (Table 4).

Table 1. Sero-prevalence of CBPP in Cattle by Local Government Area in the three Senatorial Districts of Kaduna State, Nigeria

LGA*	No. Sampled	No. Positive	% Positive
Ikara	300	51	17.0
Chikun	300	45	15.0
Kauru	300	138	46.0
Total	900	23.4	26.0

P-value=0.0001, X^2= 93.87 df=2; *LGA= Local government area

Table 2. CBPP Sero–prevalence by age in the cattle of three senatorial districts of Kaduna state, Nigeria, during 2016

Age (years)	Local government areas						Total sampled	Total positive (%)
	Ikara		Chikun		Kauru			
	Sampled	Positive (%)	Sampled	Positive (%)	Sampled	Positive (%)		
<1-3	101	12(11.9)	87	8(9.2)	87	27(31.0)	275	47(17.1)
4–6	171	19(11.1)	147	15(10.2)	117	56(47.9)	375	90(24.0)
>6	88	20(22.7)	66	22(33.3)	96	55(57.3)	250	97(38.8)
Total	300	41(13.7)	300	45(15.0)	300	138(46.0)	900	234(26.0)

P value = 0.0027, X^2 = 9.010, df = 1, OR = 0.5299

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Table 3. Sero-prevalence of CBPP by sex in the cattle of three senatorial districts of Kaduna State, Nigeria during 2016

Sex / LGA*	Male		Female	
	Sampled	Positive (%)	Sampled	Positive (%)
Ikara	87	16(18.4)	213	35(16.4)
Chikun	55	10(18.2)	245	35(14.3)
Kauru	54	17(31.5)	246	121(49.2)
Total	196	43(21.9)	704	191(27.1)

P value=0.1428, X^2=2.148, df=1, OR=0.7549; *LGA= Local government area

Table 4. Sero–prevalence of contagious bovine pleura pneumonia in cattle by breed in three senatorial districts of Kaduna state, Nigeria during 2016

Breed / LGA*	White Fulani (Bunaji)		Red Bororo (Rahaji)		Sokoto Gudali (Bokoloji)	
	Sampled	Positive	Sampled	Positive	Sampled	Positive
Ikara	292	48(16.4%)	3	-	5	2(40%)
Chikun	295	41(13.9%)	4	3(75.0%)	1	1(100%)
Kauru	292	134(45.9%)	7	4(57.1%)	1	1(100%)
Total	879	223(25.3%)	14	7(50.0%)	7	4(57.0%)

P value = 0.00573, X^2 = 5.718, df = 2; *LGA= Local government area

DISCUSSION

Contagious Bovine Pleuropneumonia (CBPP) has been an endemic disease in Nigeria since it was first reported in 1924 (Foluso, 2003). This may be due to the transhumance and nomadic nature of cattle rearing and inadequate control measures for CBPP in Nigeria (Egwu et al., 1996). Latex Agglutination Test (BoviLAT PA6223) for CBPP is a fast and easy diagnostic technique to perform in the field (Ayling et al., 1999b).

The 26.0% sero-prevalence of CBPP found in this study is, lower than the 47% sero-prevalence reported by Danbirni et al. (2010), in a herd of cattle with concurrent infection of CBPP and bovine Tuberculosis in Igabi LGA of Kaduna State and that of Suleiman et al. (2015) who reported 30.2% of seropositivity to CBPP within agro-pastoral areas of Nigeria. However, the result of this study was higher than that of Okaiyeto et al. (2011) who reported a sero-prevalence of 16.7% and 17.5% for adults and young cattle respectively, in a herd of cattle with CBPP outbreak in Kafur LGA Katsina State, using LAT and Musa et al. (2016) who also reported 3.33% prevalence of CBPP from lung samples sampled in Maiduguri and Yola abattoir both in north eastern Nigeria. The result of this study also differ from the work of Nawathe (1992) and Adamu and Aliyu (2006) who in their separate studies recorded a lower sero-prevalence of 0.52% and 0.33% respectively in Borno State and Aliyu et al. (2000) who recorded a sero-prevalence of 0.29% in 5 other States in Northern part of Nigeria. The higher sero-prevalence rate recorded in this study could be as a result of the inadequate prevention and control measures that resulted in absence or irregular vaccination programmes for cattle over the years, as well as the introduction of infected cattle into the areas (particularly through transhumance and nomadism) that were initially thought to be free of the disease (Aliyu et al., 2000). It could also be as a result of epidemiological trend of the disease with the presence of carriers in some herds which might not have been detected clinically and hence the maintenance and gradual spread of the disease (Egwu et al., 1996).

Southern senatorial district (Kauru) has a higher sero-prevalence of 46.0% when compared to northern (Ikara) and central (Chikun) senatorial district with 17.0% and 15.0% sero-prevalence respectively. The higher sero-prevalence of CBPP in cattle in the southern district may not be unconnected to the strategic location of Kauru LGA, where a major cattle route, from the North- western part of Nigeria passes through to the southern part of the country. Kauru LGA shares boundary with Kajuru and Zangon kataf LGAs where two grazing reserves, (Libere and Laduga) are located, and harbours large population of cattle. The movement of these cattle in and out of Kauru might be responsible for the introduction and easy spread of CBPP. Furthermore, a previously reported outbreak of CBPP by Danbirni et al. (2010) in a herd of cattle in Igabi LGA, which shares boundary with Kauru LGA, might also have contributed to the higher sero-prevalence of the disease in southern district due to uncontrolled movement of cattle between the 2 LGAs. The presence of the CBPP in older than younger animals agrees with the findings of Boelert et al. (2005), who reported that age is a measure of exposure and chances of being infected. Thus, younger animals have a lower risk of being infected. The result of the study shows that sex and breed is not a factor in the epidemiology of CBPP, agrees with Santini et al.

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(1992), who reported that ruminants of *Bos* genus are generally susceptible to CBPP indicating uniform susceptibility among breed and sex.

CONCLUSION

Sero-prevalence of CBPP in cattle was found to be high in the study area. Since there was no history of recent vaccination against CBPP in the area prior to this study, and the sero-prevalence of CBPP in cattle was found to be high; there is therefore, a need for the authorities concerned to intensify vaccination of cattle against the disease. BoviLAT should be encouraged due to its simple, fast and easy to perform technique for the diagnosis of CBPP in the field.

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Conflict of interest

There is no conflict of interest

Author's contribution

Billy Ishaya La'ah and Arhyel Gana Balami conducted the research and wrote the manuscript, A.K.B Sackey, S.N.A Sa'idu and L.B Tekdek supervised the research and corrected the manuscript while S.O Okaiyeto provided the test kits.

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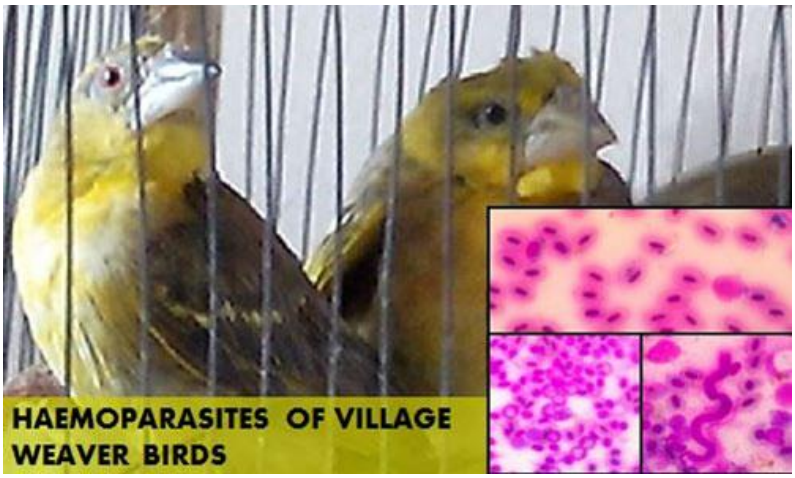

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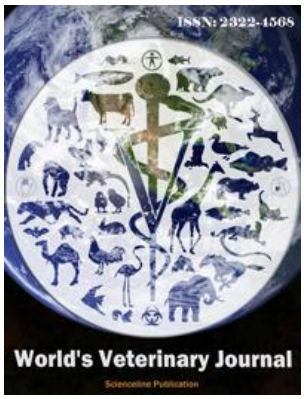

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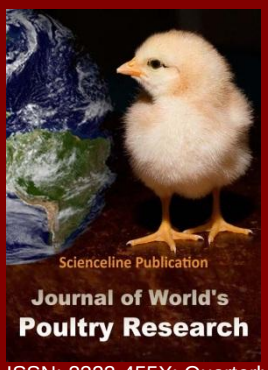

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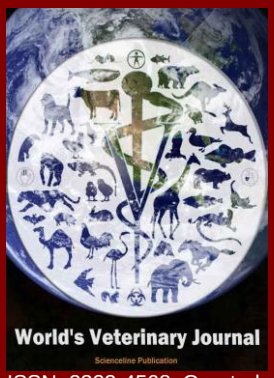

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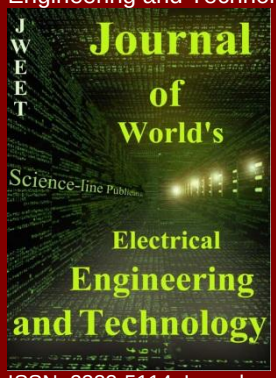

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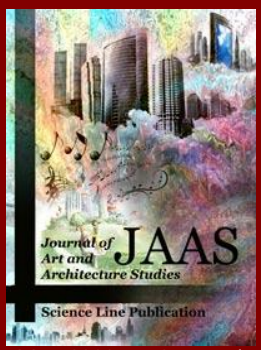

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