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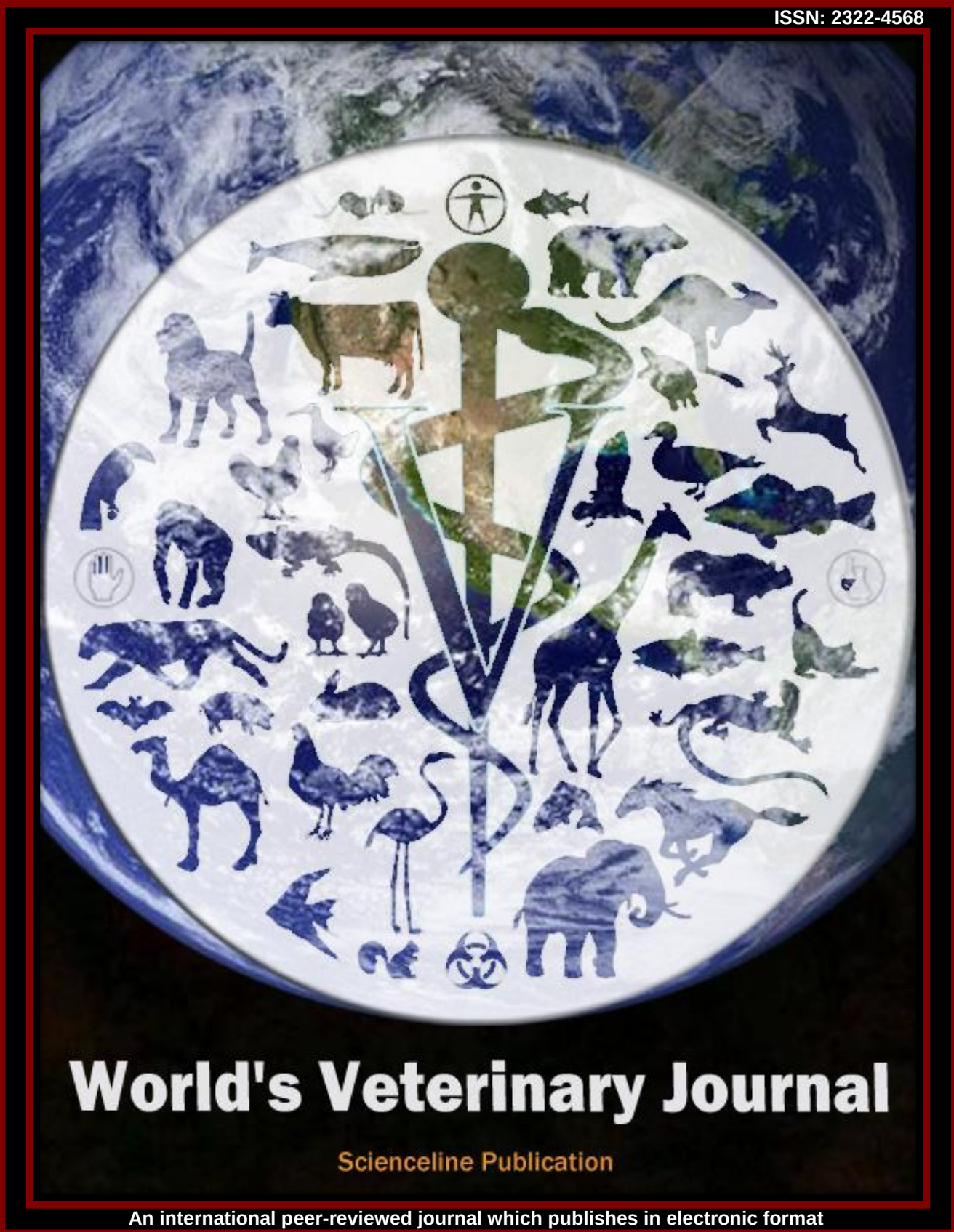

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Research Paper

The Epidemiology of Canine Parvovirus Enteritis in Dogs of Makurdi, Benue State, Nigeria.

Tion MT, Apaa TT, Saganuwan AS, Nwankwo HC, Tughgba T, Anumtyo TM, Amine AA, Nguetyo SA, Igoh, AF and Akpehe-Ishor W.

World Vet. J. 8(3): 48-54, 2018; pii:S232245681800005-8

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ABSTRACT

Since its emergence, canine parvovirus enteritis has remained the most significant and important cause of enteritis in puppies between six weeks and six months of age. The aim of this study was to carry out a retrospective study in order to assess the effect of certain factors on the prevalence of the disease in diagnosed cases presented to the veterinary teaching hospital Annex, university of agriculture Makurdi from 2010 to 2016. The overall prevalence of the diagnosed cases of canine parvoviral enteritis has been at 5.7% for the past seven years. Age, sex, breed, vaccination status was showed to have been associated with the infection. Puppies of up to five months of age, males, breeds such as the Nigerian local breed, Alsatian, Caucasian, Rottweiler and Russian shepherd dogs and unvaccinated dogs are prone to this disease. Furthermore, this study revealed that early presentation and an aggressive support management of these cases had brought about a high recovery outcome of 85.4%. The distribution of the disease according to the season showed the highest incidence of cases occurred in 2012 (25.8%) and had descended down to (9%) by 2016 with most cases witnessed around January (22.5%) in dry season and June (15.7%) in wet season. The need for educating both veterinarians and dog owners about core vaccinations in dogs is paramount.

Keywords: Canine parvoviral enteritis, Dogs, Makurdi, Prevalence

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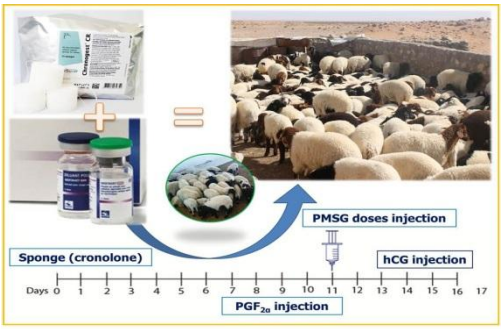
Research Paper

Application of Different Hormonal Protocols for Improving Reproductive Performance of Barki Ewes.

Ashour G, El-Bassiony MF, Dessouki ShM and El-Wakeel MA.

World Vet. J. 8(3): 55-64, 2018; pii:S232245681800006-8



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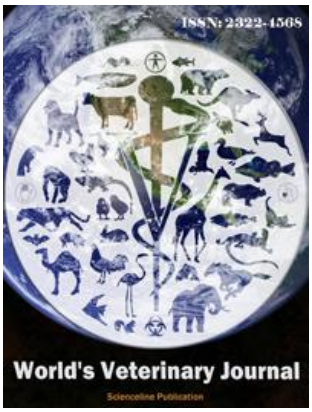
ABSTRACT

This investigation was designed to assess reproductive improvement in Barki ewes using different hormonal protocols. Seventy-five non-pregnant and non-lactating Barki ewes were randomly assigned into three equal groups (25 ewes each), namely G1, G2 and G3. (G1) served as control, while (G2) was treated with an intravaginal progestagen impregnated sponge for 12 days then was removed. On the ninth day, all treated ewes received an intramuscular injection of prostaglandin F2α. (G3) was also synchronized as G2, in addition to on the 10th day ewes were received an intramuscular injection of 750 IU PMSG in descending doses for three days so that the last dose was injected at the 12th day concurrently with sponges' removal. Meanwhile, on the 14th day, all ewes in G3 were received an intramuscular injection of hCG hormone (500 IU/ewe). The results showed an increase in plasma progesterone level from the first day of pregnancy and rises up to the last day before parturition. Progesterone was found to be higher in G3 (that had higher twining rate) than in G2 than in G1. Insignificant increase in estrus response was observed in groups (G2, G3) compared to G1 (100, 100 and 92%, respectively). Conception rate was significantly higher in G3 (100%) than G2 (92%) and G1 (88%). While, abortion rate was higher in G1 and surpassing G2 and G3 (9.0, 0.0 and 0.0 %, respectively). Lambing rate

was significantly higher in G3 as compared to G2 and G1 groups (100, 92 and 80 %, respectively). While, weaning rate was recorded to be insignificantly higher in G2 than in G1 and G3 (100.0, 95.45 and 94.29%, respectively). It could be concluded that, hormonal manipulation using intravaginal progestagen impregnated sponge and PMSG in the presence of hCG; would be a proper way for enhancing the reproductive efficiency of Barki ewes.

Keywords: Barki ewes, Synchronization, Superovulation, Twining, Reproduction



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The Epidemiology of Canine Parvovirus Enteritis in Dogs of Makurdi, Benue State, Nigeria

Tion Matthew Terzungwe[1*], Apaa Ternenge Thaddaeus[1], Saganuwan Alhaji Saganuwan[2], Nwankwo Henry Chukwuebuka[2], Tughgba Terzungwe[3], Anumtyo Theresa Mwuese[4], Amine Aondowase Andrew[3], Nguetyo Samuel Aondonenge[4], Igoh Ann Faith[4], Akpehe-Ishor Washima[4]

[1]*Department of Veterinary Medicine, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria.*
[2]*Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria.*
[3]*Department of Veterinary Surgery and Theriogenology, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria.*
[4]*College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria.*

[*]Corresponding author's Email: tions_doc@yahoo.co.uk

ABSTRACT

Since its emergence, canine parvovirus enteritis has remained the most significant and important cause of enteritis in puppies between six weeks and six months of age. The aim of this study was to carry out a retrospective study in order to assess the effect of certain factors on the prevalence of the disease in diagnosed cases presented to the veterinary teaching hospital Annex, university of agriculture Makurdi from 2010 to 2016. The overall prevalence of the diagnosed cases of canine parvoviral enteritis has been at 5.7% for the past seven years. Age, sex, breed, vaccination status was showed to have been associated with the infection. Puppies of up to five months of age, males, breeds such as the Nigerian local breed, Alsatian, Caucasian, Rottweiler and Russian shepherd dogs and unvaccinated dogs are prone to this disease. Furthermore, this study revealed that early presentation and an aggressive support management of these cases had brought about a high recovery outcome of 85.4%. The distribution of the disease according to the season showed the highest incidence of cases occurred in 2012 (25.8%) and had descended down to (9%) by 2016 with most cases witnessed around January (22.5%) in dry season and June (15.7%) in wet season. The need for educating both veterinarians and dog owners about core vaccinations in dogs is paramount.

Key words: Canine parvoviral enteritis, Dogs, Makurdi, Prevalence

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INTRODUCTION

The use of dogs as companion to humans cannot be overemphasized. Dogs are used as trackers, instrument of war, and healers of both the physical and emotional problems of humans (Carmichael, 2003). The population of dogs in Nigeria is estimated to be around 4.5million (Bourn et al., 1994) and infectious diseases such as parvovirosis could reduce their population. The affection humans develop towards pets continues to deepen in this twenty-first century (Daodu et al., 2017).

Canine parvovirus type 2 (CPV-2) is a highly contagious and often fatal viral disease that infects the gastrointestinal tract of dogs (Touihri et al., 2009). CPV is a small, non-enveloped with spherical capsid, consisting of three proteins and a linear, single-strand DNA virus of the family Parvoviridae (Maclachan and Dubovi, 2010). CPV-2 infection does not have predilection for sex, age or breeds of dogs (Castro et al., 2007; Gombac et al., 2008). CPV-2 affects dogs between the ages of six weeks and six months. It seldom affects older dogs, because of their natural immunity (McCaw and Hoskins, 2006; Prittie, 2004). But some breeds such as Rottweiler, Doberman pinscher, American Pit-bull terrier, Labrador retriever and German shepherd dog are at an increased risk of infection (Smith-Carr et al., 1997; Houston et al., 1996).

Certain factors that predispose puppies to CPV-2 infection are lack of protective immunity, intestinal parasites and overcrowding, poor sanitary, and stressful environmental conditions (Smith-Carr et al., 1997; Hoskins, 1997). CPV-2 can be transmitted from infected to susceptible dogs mainly through direct transmission (faecal-oral route), or indirect transmission where dogs can also become infected through exposure to fomites such as shoes, clothing, the hands of humans, food bowls and other utensils (Carmichael, 1994; Smith-Carr et al., 1997; Hoskins, 1997; Decaro et al., 2005b, Nivy et al., 2011). CPV-2 can also be transmitted via house flies, flesh flies and blow/bottle flies (Bagshaw et al., 2014).

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CPV-2 has mutated twice in the early 1970s to mid 1980s given rise to two antigenic variants namely; CPV-2a and CPV-2b (Parker et al., 2001). In 2000, a third antigenic mutant named CPV-2c emerged from Italy (Buonavoglia et al., 2001). All the three antigenic variants have been distributed in different parts of the world (Bingga et al., 2014; Touihri et al., 2009; Wilson et al., 2014).

The emergence of CPV-2 infection in Nigeria can be traced back to the mid 1980s (Ezeokoli et al., 1985). Chollom et al. (2013) discovered CPV-2 with the use of conventional polymerase chain reaction (PCR). Case reports, seroprevalence, treatment regimen, immunity following CPV vaccination and risk factors affecting the disease in Nigeria have been reported (Eghafona et al., 2007; Ezeibe Maduike et al., 2010; Nwoha, 2011; Shima et al., 2015). CPV-2a strain has been reported in Nigeria (Dogonyaro et al., 2013; Apaa et al., 2016). The virus is often shed in the faeces of infected dogs within 4-5 days of exposure before clinical signs develop and then throughout the period of illness, and for 10days after clinical recovery (Cynthia and Scott, 2010).

CPV-2 infection manifests as an acute haemorrhagic enteritis and myocarditis. Dogs with enteritis show depression, loss of appetite, lethargy, vomiting, high fever and severe mucoid or bloody and foul smelly diarrhea (Lamm and Rezabek, 2008; Prittie, 2004). Presently, myocarditis though seen can develop in puppies less than eight weeks old dog born to unvaccinated bitches (Hoskins, 1997).

Definitive diagnosis is done by the detection of CPV-2 in the faeces of affected dogs, serology, and necropsy and histopathology (Pollock and Carmichael, 1988). Other methods of detection include electron microscopy, viral isolation, fecal hemagglutination, latex agglutination, counter-immunoelectrophoresis, immunochromatography, PCR (Macintire and Smith-Carr, 1997; Pollock and Carmichael, 1988; Desario et al., 2005; JinSik et al., 2006) which is more sensitive and reliable than traditional techniques (Desario et al., 2005).

CPV infection can be managed by aggressive symptomatic and supportive therapy (Prittie, 2004; Brown and Otto, 2008) involving fluid therapy, antibiotic, antiemetic, nutritional support, antiviral treatments and pain management (Mylonakis et al., 2016). Prevention is by vaccination of dogs with either attenuated or modified live vaccines (Martella et al., 2005). But maternally derived antibodies protect neonates as well (10 days) and it interferes with vaccines. Thus, causing vaccine failures (Pollock and Carmichael, 1982).

In Makurdi metropolis, there have been several reported cases of morbidity and mortality of CPV in dogs but there is paucity of information on prevalence, morbidity and mortality rates of the disease. Hence epidemiology of canine parvovirus infection was studied at Veterinary Teaching Hospital (VTH) of the University of Agriculture Makurdi (UAM).

MATERIALS AND METHODS

Study area

This study was conducted in Makurdi metropolis, Benue State, Nigeria. Makurdi, the capital of Benue state lies between latitude 70 15'- 70 45'N and longitude 80 15'- 8 0 40'E. It has a population of about 500,797 (The World Gazetter, 2007), it lies in the Guinea savannah vegetative belt and on the bank of the river Benue which is the second largest river in Nigeria. The river divides the town into North and South banks and the town covers an area of 16 km^2 (Omudu and Amuta, 2007). It has a tropical climate with a temperature ranging between 21.70 -24.70^{0}C (minimum) and 29.70-33.70^{0}C (maximum). The climate of Makurdi town is the tropical wet and dry type, Koppen's Aw classification, with double maxima (Ayoade, 1983). The rainy season lasts from April to October, with five months of dry season (November to March). Annual rainfall in Makurdi town is consistently high, with an average annual total of approximately 1173 mm (Abah, 2012).

Data collection

Data of 1571 treated cases of CPVE in dogs presented at the VTH Annex, UAM from 2010 to 2016 were assessed. History, clinical signs and therapeutic regimens of CPV were reviewed. The presented signs were foul smelly haemorrhagic diarrhoea, emaciation, vomiting and lethargy. The vaccination history, treatment outcome, sex, breeds and age of dogs were recorded. The status of the vaccination history was categorized as vaccinated, unvaccinated and unknown. Similarly, the treatment outcomes of the cases following medical intervention were classified as either "recovered or dead". The suspected cases were confirmed using SensPERT® canine parvovirus antigen test kits (VetALL, Korea).

Statistical analysis

The data collected were analysed using Microsoft Office Excel (2007) for descriptive analysis of variables such as age, sex, and vaccination history and treatment outcome.

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Ethical approval

This study was certified and approved and was performed according to the ethics of committee of the college of veterinary medicine, University of Agriculture, Makurdi, Nigeria.

RESULTS

Of the 1571 dogs that were brought to the VTH Annex, UAM, Makurdi, for over a period of seven years (2010 - 2016), 89 (5.7%) were diagnosed of CPV enteritis. Table 1 shows the analysis of diagnosed CPVE cases according to age, sex, vaccination status, treatment outcome and season where as the analysis of diagnosed CPVE cases by breed of dogs, month and year have been presented in diagrams 1-3. The local Nigerian breed had the highest frequency of cases 40 (44.9%), followed by Alsatian 18 (20.2%), Caucasian 7 (7.9%), Rottweiler, Russian shepherd 6 (6.7%), Mixed 4 (4.5%), Bull mastiff, Neapolitan mastiff 3 (3.4) and Pit-bull and Chihuahua 1(1.1%) (Diagram 1). The highest incidence of cases was recorded more in the month of January 22 (22.5%), June 14 (15.7%), March and July 11 (12.4%), February 8 (9%), May 7 (7.9%), April, October 4 (4.5%), December 3 (3.4%), September 2 (2.3%) and November 1 (1.1%) respectively (diagram 2) while the peak incidence of cases were recorded in the year 2012, 23 (25.8%), 2013, 16 (18%), 2014, 15 (16.9%), 2011, 11 (12.4%), 2015, 9 (10.1%), 2016, 8 (9%) and 2010, 7 (7.9%) respectively (Diagram 3).

Table 1. Analysis of diagnosed canine parvoviral enteritis cases base on age, sex, vaccination status treatment outcome and season, presented to the veterinary teaching hospital, university of agriculture, Makurdi from 2010 to 2016

Groups	Number of diagnosed cases of canine parvovirus enteritis	
	Frequency	Percentage (%)
Age (Months)		
0-5	71	79.8
6-12	12	13.5
>1 year	6	6.7
Sex		
Male	54	60.7
Female	35	39.3
Vaccination status		
Vaccinated	25	28.1
Unvaccinated	42	47.2
Unknown	22	24.7
Treatment outcome		
Recovered	76	85.4
Dead	13	14.6
Season		
Dry (Nov- March)	43	48.3
Wet (April- October)	46	51.7

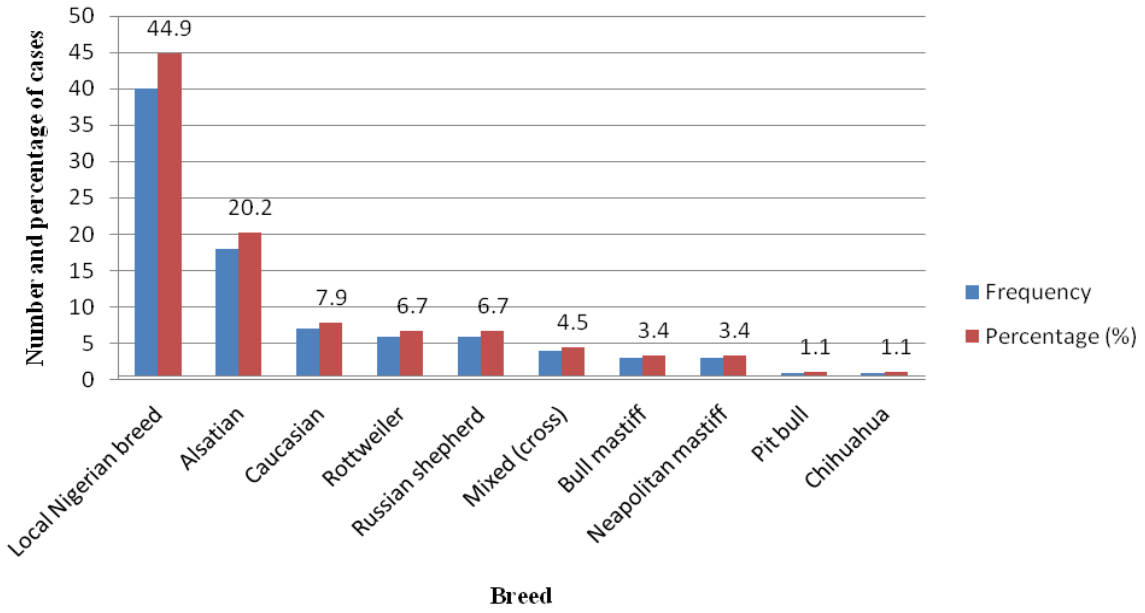

Diagram 1. The distribution of diagnosed canine parvoviral enteritis cases base on breed in university of agriculture Makurdi, Nigeria during 2010-2016.

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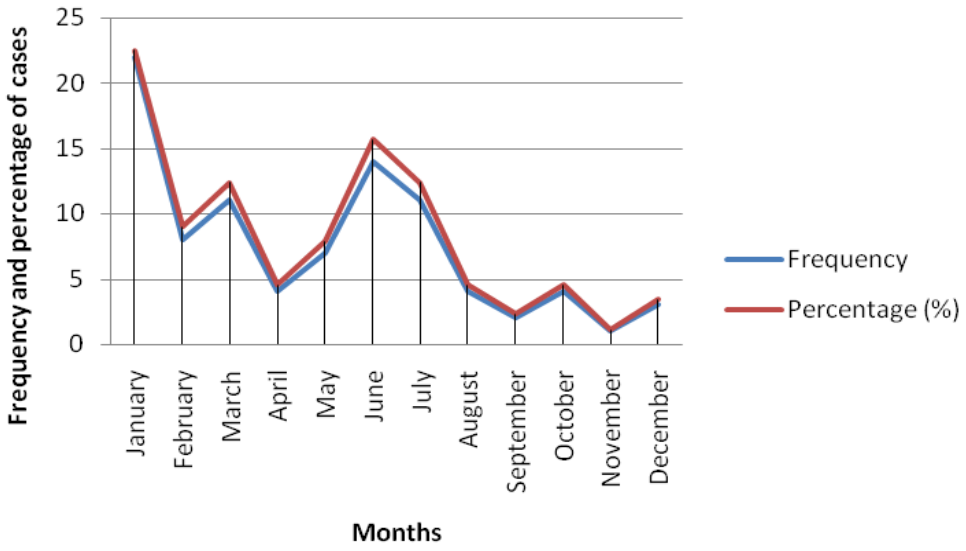

Diagram 2. The distribution of diagnosed canine parvoviral enteritis cases base on months in university of agriculture Makurdi, Nigeria during 2010-2016.

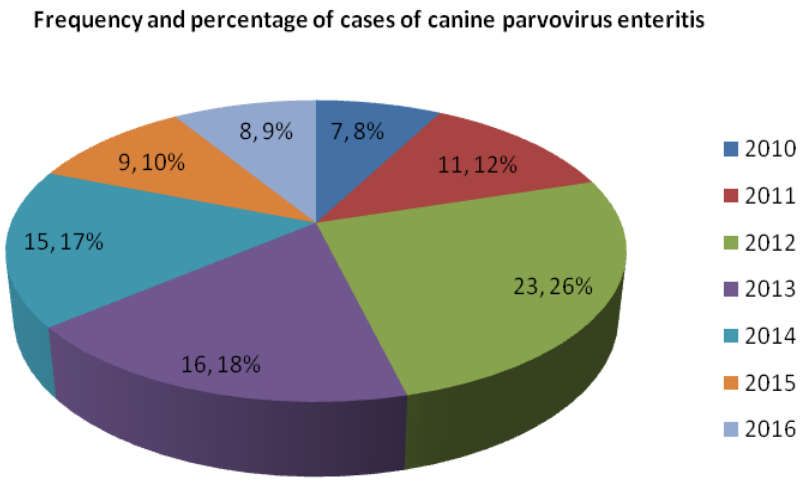

Diagram 3. The distribution of diagnosed canine parvoviral enteritis cases in Nigeria by years at the veterinary teaching hospital (2010-2016).

DISCUSSION

The study has shown that CPVE has been endemic in Makurdi metropolis for years with a 5.7 % prevalence rate (89/1571). Although, the prevalence rate in this study appears to be much lower compared to the values reported in Ilorin 6.4% (105/1645) (Daodu and Ajiboye, 2018), Effurun/Warri 13.4% (204/1527) (Shima et al., 2015), Jos North/South 17.4% (12/70) (Ogbu et al., 2016), Tunisia 32.1 % (54/168) (Tagorti, 2018), Nigeria and south Africa 96.7% (30/31), 98.2% (106/108) (Dogonyaro et al., 2013) and Zimbabwe 84.9% (191/225) (Mcree et al., 2014) but it was higher than the report from Vom 2.8% (87/3075) (Mohammed et al., 2005). The low prevalence may be due to low patronage of the VTH by dog owners, poor record keeping of medical cases and/or lack of proper diagnostic techniques/equipment then. Therefore, this report is a well representation of the cases of CPVE in Makurdi since the VTH is the major veterinary hospital where cases are handled in Makurdi metropolis.

The fact that the group (0-5 months) had a higher incidence rate of 79.8% (71/89), 6-12 months, 13.5% (12/89) and > 12 months, 6.7% (6/89) agrees with the reports from Effurun/Warri 60.3% (0-5 month), 27% (6-11 months) and 12.7% (12 months and above) (Shima et al., 2015), Argentina 86% (1-5 months) and 14% (6 months and above) (Calderon et al., 2011), United States of America 59% (less than 6 months) and 41% (more than 6 months) (Glickman et al., 1985), Slovenia 67.6% (1-5 months), 25.7% (6-12 months) and 6.8% (more than 12 months) (Gombac et al., 2008), India with 40.75% for (0-6 months), 24.19% for (7-12 months) and 10% for (12 months and above) (Basava, 2012). It has already

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been reported that CPVE primarily affects puppies that are between the age of 6 weeks to 6 months (Marcovich et al., 2012; Mccaw and Hoskins, 2006; Prittie, 2004).

Local Nigerian breeds 44.9%, Alsatians 20.2%, Caucasians 7.9%, Russian shepherd and Rottweiler 6.7%, mixed breed 4.5%, Neapolitan mastiff and Bull mastiff 3.4% each and the least Pit-bull and Chihuahua 1.1% each. The reasons for breeds of dog being susceptible to this dreaded disease remain unknown. Although, there have been earlier reports that Doberman Pinscher, Rottweiler and German Shepherd puppies are under greater risk of developing CPV enteritis than other breeds (Glickman et al., 1985; Houston et al., 1996; Castro et al., 2007; Shima et al., 2015). Our findings disagree with the report that Alsatians, Rottweilers and Doberman pinschers appears to be more at risk (Gombac et al., 2008; Castro et al., 2007). Basava (2012) reported that, the Spitz breed had the highest prevalence (43.75%), followed by Doberman (42.85%), Pomeranian (37.5%), Alsatian (31.03%), Mongrel (28.71%) and Pug, Mastiff, Golden retriever, Labrador (27.77%). Dogs with highest incidence of cases of CPVE were the males (60.7%) as against females with (39.3%). This result agrees with the reported values in males (83.33%) and females (16.7%) respectively (Castro et. al., 2007; Basava, 2012) but disagrees with the finding of Umar et al. (2015) who reported that female (58.5%) were more at risk than the males (41.5%). In Nigeria, male dogs are preferred to females by owners as security with exception of the few that breed them.

The prognosis of CPVE is as low as 9.1% in the absence of treatment, and 64% or higher with treatment (Otto et. al., 1997). In the present study, the prognosis after treatment is 85.4%. This finding corroborates with the reports of Prittie (2004) and Macintire and Smith-Carr (1997) indicating that CPVE could be treated symptomatically. But unvaccinated dogs (47.2%) had the highest prevalence, followed by vaccinated dogs (28.1%) and dogs with unknown vaccination status (24.7%). This also agrees with the result of Basava (2012) who reported (35.4%) for unvaccinated and vaccinated dogs (16%) respectively. The lower prevalence rate of CPVE in vaccinated dogs indicated that current vaccine offer protection (Cavalli et al., 2001).

Monthly prevalence of CPVE in January (22.5%), June (15.7%), March and July (12.4%), February (9%), May (7.9%), April and October (4.5%), December (3.4%), September (2.3%) and November (1.1%) show that cases are seen in the dry season (48.36) and more in the wet season (51.7%), respectively. Shima et al. (2015) reported highest prevalence in January (17.2%) and lowest in April (2.0%). In contrast, Basava (2012) reported highest prevalence in July (48.97%) and the lowest in March (20%) and Houston et al. (1996) also reported that dogs are most likely to be admitted between July to September in Canada. Meanwhile, in the last seven years, the disease had its highest prevalence in 2012 (25.8%) followed by 2013 (18%), 2014 (16.1%), 2011 (12.4%), 2015 (10.1%), 2016 (8%) and 2010 (7.9%) respectively. This was due to lack of awareness on the preventive measures of the disease and poor regimen management.

CONCLUSION

CPVE is endemic in Makurdi metropolis and could be affected by age, sex, breed, vaccination status, treatment (management) and seasonal variations. Vaccination and therapy of the affected dogs could improve the well-being and longevity of the dogs. This result will serve as recorded information to veterinarians, dog owners and breeders in and around Makurdi in giving adequate care and taking preventive measures generally but particularly during the high prevalence period of CPVE.

DECLARATIONS

Author's contribution

ATM, AAA, NSA, IAF, AIW collated the data. NHC and TT analysed the data. TMT, ATT and SAS designed the work while TMT and SAS reviewed the manuscript.

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Competing interests

The authors have declared that no competing interest exists.

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Application of Different Hormonal Protocols for Improving Reproductive Performance of Barki Ewes

Gamal Ashour[1], Moharram Fouad El-Bassiony[2*], Sherif Mohamed Dessouki[1] and **Mohamed Awad El-Wakeel[2]**

[1] *Department of Animal Production, Faculty of Agriculture, Cairo University, 12613, Giza, Egypt*
[2]*Animal and Poultry Physiology Department, Desert Research Center, 11753, Cairo, Egypt*

*Corresponding author`s Email: moharramf@yahoo.com

ABSTRACT

This investigation was designed to assess reproductive improvement in Barki ewes using different hormonal protocols. Seventy-five non-pregnant and non-lactating Barki ewes were randomly assigned into three equal groups (25 ewes each), namely G1, G2 and G3. (G1) served as control, while (G2) was treated with an intravaginal progestagen impregnated sponge for 12 days then was removed. On the ninth day, all treated ewes received an intramuscular injection of prostaglandin $F_{2\alpha}$. (G3) was also synchronized as G2, in addition to on the 10th day ewes were received an intramuscular injection of 750 IU PMSG in descending doses for three days so that the last dose was injected at the 12th day concurrently with sponges' removal. Meanwhile, on the 14th day, all ewes in G3 were received an intramuscular injection of hCG hormone (500 IU/ewe). The results showed an increase in plasma progesterone level from the first day of pregnancy and rises up to the last day before parturition. Progesterone was found to be higher in G3 (that had higher twining rate) than in G2 than in G1. Insignificant increase in estrus response was observed in groups (G2, G3) compared to G1 (100, 100 and 92%, respectively). Conception rate was significantly higher in G3 (100%) than G2 (92%) and G1 (88%). While, abortion rate was higher in G1 and surpassing G2 and G3 (9.0, 0.0 and 0.0 %, respectively). Lambing rate was significantly higher in G3 as compared to G2 and G1 groups (100, 92 and 80 %, respectively). While, weaning rate was recorded to be insignificantly higher in G2 than in G1 and G3 (100.0, 95.45 and 94.29%, respectively). It could be concluded that, hormonal manipulation using intravaginal progestagen impregnated sponge and PMSG in the presence of hCG; would be a proper way for enhancing the reproductive efficiency of Barki ewes.

Key words: Barki ewes, Synchronization, Superovulation, Twining, Reproduction

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INTRODUCTION

Low reproductive performance has been observed in sheep flocks raised traditionally in arid regions. This poor performance may be attributed to several factors including fertility, selection, nutrition and disease (Webb et al., 2004; Yavuzer, 2005). Increasing Barki sheep productivity by increasing lambing frequency and fecundity is considered to be the most important factor in the development of Barki sheep production. In this regard, increasing the rate of fecundity in sheep offers the best opportunity to increase the efficiency of lamb meat production (Koyuncu and Alticekic, 2010). Natural lambing in Barki sheep occurs throughout the year in a scattered manner negatively affecting survival and growth rates of the lambs born during the unfavorable season of the year. Consequently, one of the most common ways to alleviate problems related to disorderly lambing is the using of exogenous source of hormones for artificially controlling the time of mating (Mekuriaw et al., 2016). In addition, the use of hormonal manipulation for estrus synchronization creates the opportunity for timed breeding and lambing. This in turn results in taking the advantages of seasonal variation of forage availability, photoperiod, labor resources and market demands. Estrus synchronization is a valuable management tool that has been employed successfully in enhancing reproductive efficiency, particularly in cows, ewes and does as early reported by Kusina et al. (2000). The success of a hormonal treatment for estrus synchronization depends on the productive status of ewes, season of the year, the type of hormonal protocol and the method of administration. Multiple ovulation programs are also possible with the use of estrus synchronization for improving herd productivity.

Estrus synchronization in small ruminants is achieved either by reducing the length of the luteal phase of the estrous cycle with prostaglandin $F_{2\alpha}$ or by artificially extending it with exogenous progesterone or potent progestagens (Jainudeen et al., 2000 and Kusina et al., 2000). Synchronization of estrus has been practiced for the last few decades when many protocols varying in degrees of success which based on the use of intravaginal progestagen devises inserted for 7 to 14 days followed by gonadotropin injection at the time of devices' removal and introduction of teaser ram

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(Zarkawi, 2001). Also, hormonal treatments to control ovulation and reproduction are a perquisite for successful breeding and increasing the number of pregnant females (Motlomelo et al., 2002 and Husein et al., 2005). In small ruminants, several programs have been used for improving fertility by controlling the ovarian activity. Acceptable results of estrus responses have been achieved by using progestagen and equine Chorionic Gonadotropin (eCG) together at the time of progestagen withdrawal (Fonseca et al., 2005; Husein et al., 2005).

Application of assisted reproductive technologies is a prerequisite for improving reproductivity and productivity of livestock, especially in arid and semi-arid environments species which are usually characterized by low fertility. Most recently, Kaya et al. (2018) noted that hormonal protocols are one of the main items to improve, herds productivity including superovulation as proposed by Souza et al. (2014). In practice, generality of the superovulation protocols used for embryo production in ewes, both *in vivo* and *in vitro*, consists of the administration of intravaginal progestagen impregnated sponges; both for synchronization of estrus and ovulation or for avoidance of spontaneous ovulation (Cognie et al., 2003). The objectives of superovulation included inducing a high number of ovulations and subsequent high fertilization rate, while at the same time ensuring a normal physiological environment in the reproductive tract for embryo development. The application of superovulation technology to improve endogenous secretions of pregnant hormones increases offspring productivity and gross revenue in the small scale farm (Andriyanto and Manalu, 2011).

The present study was conducted to place on record the lambing rates of ewes following and to investigate the possibility of improving fertility and increasing twinning rate in Barki ewes under arid conditions of the North Western Coast of Egypt using different hormonal protocols for estrus synchronization and superovulation.

MATERIALS AND METHODS

Experimental region

The present study was carried out during the period from June 2015 to January 2016 at Animal Production Unit, Sustainable Development Center for Matrouh Resources, Matrouh Governorate, which belongs to the Desert Research Center (DRC), Ministry of Agriculture and Land Reclamation. The station is located at 240 Km West of Alexandria and 222 Km Egyptian western boarders.

Ethical approval

This experiment was performed according to all ethics and animal rights (Desert Research Center). As much as this work had considering all rules and regulations in conformity with the European union directive for the protection of experimental animals (2010/63/EU).

Experimental animals, feeding and management

Seventy-five, non-pregnant and non-lactating, Barki ewes averaged 48.10±0.72 kg Live Body Weight (LBW) and ranged between 2.5 and 3.5 years old were used in this study. All ewes were clinically examined for any reproductive disorders as well as general health status. Vaccination against the major prevailing epidemic diseases, internal and external parasites were controlled in proper time. Ewes were weighed before starting the experiment, and kept under an intensive production system and housed in semi-open yards throughout the experimental period. Lambs were left all the day time with their dams for suckling up to weaning age at three months of age. All groups were daily fed on a concentrate (0.75 kg) and berseem hay (0.5kg) per head during the experimental period to cover their nutrient requirements during different physiological status according to Kearl (1982). Lambs were daily fed only on their dams' milk from birth to weaning age at three months of age. The daily ration was offered in a certain mode of feeding starting with concentrate mixture at 08:30 h followed by chopped rice straw at 12:00 h and continuing to the next morning feeding. All animals drank fresh water three times a day (08:00, 14:00 and 20:00 h).

Experimental design

Animals were randomly divided into three equal groups (25 ewes per group (Figure 1). The first group (G1) was served as a control without any hormonal treatment and was left without synchronization or superovulation. While, the second group (G2) was treated using an intravaginal progestagen impregnated sponge (20 mg cronolone, Chronogest® CR, product of intervet international B. V. and manufactured in the European Union, (EU) was inserted. Sponges remained in situ for 12 days and were removed on the 12th day. On the ninth day, all ewes were received an intramuscular injection of prostaglandin $F_{2\alpha}$ (1 ml Synchromate each 1 ml solution containing 0.250 mg Cloprostenol, Bremer Pharma GMBH, Germany). The third group (G3) was also synchronized using an intravaginal progestagen impregnated sponge for 12 days and received an intramuscular injection of prostaglandin $F_{2\alpha}$, on the ninth day, while on the 10th day ewes of the third group (G3) were received an intramuscular injection of 750 IU PMSG (Laboratorios Hipra, S.A. Avda. Ia Selva, 135, 17170 Amer (Girona) Spain). PMSG injections were applied in gradual decreasing doses for

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three days that each ewe received 275 IU at the 10th day, the second dose (250 IU) was injected at the 11th day, while the last dose (225 IU) was injected at the 12th day at the time of sponges' removal. Meanwhile, on the 14th day, all ewes in G3 group received an intramuscular injection of 500 IU/ewe of hCG hormone (Epifasi lyophilized ampoule contains 5000 I.U. of Human Chorionic Gonadotrophin, hCG) and 10 mg of lactose, manufactured by Egyptian int. pharmaceutical industries Co. (EIPICO, Egypt). During the synchronization period, all synchronized animals were subjected to a twice daily check (morning and evening) to ensure that sponges remained in their position during the treatment period.

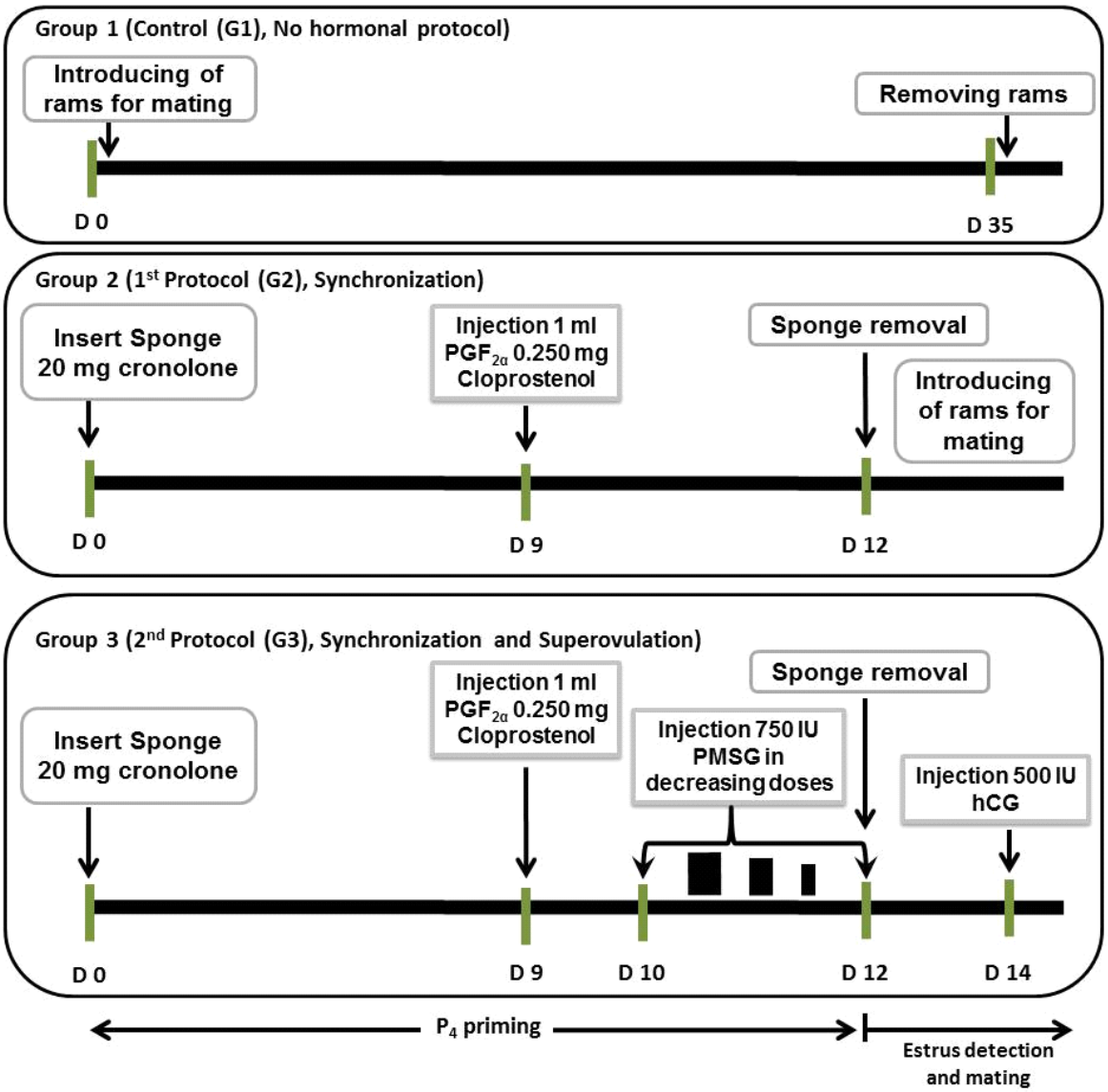

Figure 1. Experimental design and timeline for the treatment administration for the control (G1), synchronization (G2) and synchronization + superovulation (G3) groups.

Blood sampling and progesterone assay

Blood samples (5 ml) were collected from the jugular vein into EDTA (ethylenediamine tetra-acetic acid) containing tubes. Blood samples were collected immediately before sponge insertion on day 0 from ewes. The rest of the samples were withdrawn on days 1, 3, 5, 7, 9, 10, 12, 13, 15, 17, 19, 30, 60, 90, 120, 150, 180 from all groups. Blood plasma was pipetted into 1.5 mL Eppendorf tubes using sterilized plastic disposable Pasteur pipettes and then stored at -20 ºC until assayed for (P4) concentration. Progesterone hormone was quantified by ELISA method using BIOS kit provided by Chemux Bioscience Corporation, 385 Oyster Point Blvd Suite 5-6., South San Francisco, CA 94080, USA. The standard curve ranged between 0-50 ng/ml. The sensitivity of the curve was 0.2 ng/ml.

Estrus detection and mating

Nine fertile Barki rams were introduced to the ewes of all experimental groups, three rams for each group for estrus detection and mating; started 24 hours after removing the sponges (G2 and G3) and left to one week. While the control group (G1), rams left with ewes from the zero day throughout two estrus cycles (35 days total). Rams were allowed to

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interchange among different ewe groups to avoid sire/group confounding effect. The fertile rams' chest was colored in a different color for each group.

Rams were introduced to ewes for seven days and left with them for estrus detection and natural mating. Estrus was checked continuously by observation of the color marks on the ewe's rumps. Ewes were considered in estrus and mating had occurred when the paint mark was heavy and evenly distributed or copulation was observed.

Reproductive parameters

The following traits were estimated for each of the treated groups:

- Estrus response = number of ewes showing signs of estrus / total ewes treated in each group ×100.
- Conception rate = number of ewes conceived / number of ewes showing estrus and mated in each group ×100.
- Lambing rate = number of ewes lambed / number of ewes mated in each group ×100.
- Weaning rate = number of lambs weaned / number of lambs born in each group ×100.
- Abortion rate = number of ewes aborted / number of ewes conceived in each group ×100.
- Litter size at birth = number of lambs born / number of ewe lambed.
- Litter size at weaning = number of lambs weaned / number of ewe lambed.
- Average litter weight at birth = the kilograms lambs born / number of ewe lambed.
- Fecundity rate = number of lambs born / number of ewe mated.
- Weaning weight = the kilograms lambs weaned / number of lambs weaned.
- Mortality rate up to weaning (%) =live lambs born - lambs weaned / live lambs born x100.

Statistical analysis

A General Linear Model procedure (SAS, 2004) was used for the statistical analyses of progesterone concentration, milk yield and composition during the experimental period using the following model:

$Y_{ijk} = \mu + P_i + T_j + (P*T)_{ij} + e_{ijk}$

Where,

Y_{ijk} = Any observation of k^{th} animal within j^{th} treatment within i^{th} period
μ = Overall mean
P_i = Effect of i^{th} period
T_j = Effect of j^{th} treatment (j = 1-3, 1= Tr1, 2= Tr2, 3= Tr3)
$(P*T)_{ij}$ = The interaction between period and treatment
e_{ijk} = Experimental error

Significant differences among means were detected using Duncan's multiple range test (Duncan, 1955). While, reproductive traits (estrus response, conception, lambing, weaning, fecundity, abortion, single, twining, triple, mortality rates) and litter size at birth, litter size at weaning, weaning weight, sex ratio were analyzed using Chi-square test.

RESULTS AND DISCUSSION

Estrus response, and conception and abortion rates

The data presented in table (1**)** revealed that estrus response was non-significantly high in G2 (FGA+PGF2α) and G3 (FGA+PGF2α+PMSG and hCG) than the G1 (control group). The recorded data were (100, 100 and 92%, respectively). The data also indicated that conception rate was significantly higher (P<0.05) in G3 than in G2 and G1 (100, 92 and 88%). On the other hand, abortion rate was higher in control group (G1) surpassing G2 and G3, as both groups G2 and G3 showed no abortion (9.0, 0.0 and 0.0%, respectively) (Table 1).

Table 1. Estrus response and conception and abortion rates % of Barki ewes as affected by different hormonal protocols (LSM±SE)

Traits	Treatment (T)			Overall mean	± SEM	Significance
	G1	G2	G3			
Estrus response	92.00 (23/25)	100.0 (25/25)	100.0 (25/25)	97.33 (73/75)	5.30	NS
Conception rate	88.00 (22/25)	92.00 (23/25)	100.00 (25/25)	93.33 (70/75)	4.87	*
Abortion rate	09.00 (2/22)	00.00	00.00			

G1 = control group; G2 = FGA and PGF2α; G3 = FGA, PGF2α, PMSG and hCG; NS = non-significant

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The results of this study confirm that using the intravaginal progestagen impregnated sponge is an effective tool for the synchronization of the estrus. These results were previously confirmed in goats at the beginning of the breeding season in semi-desert conditions (Riaz et al., 2012). In our study, progesterone treatment lasted for 12 days showed high efficiency. Hosseinipanah et al. (2014) concluded that the occurrence of estrus using CIDR in non- reproductive season for 10, 12 and 14 days was associated with the onset of good estrus and use of GnRH had positive effect on fertility in treatment groups, with the highest significant value for the 12 days P4 treatment. In the same year, Zoharaa et al. (2014) recorded higher pregnancy rates as observed in the 30 mg FGA treatment (100%) compared with 100 µg Cloprostenol (88.2 %), 175 µg Cloprostenol (75%) and 45 mg FGA (93.8 %) treatments. It was concluded that though FGA sponge protocol presented superior results.

In comparison with our results, Abdul Muin et al. (2013) suggested that CIDR treatment for 14 days with 400 IU PMSG and 0.05 mg cloprostenol prior to CIDR removal gave better result in estrus synchronisation than in CIDR treatment for 9 days with 0.05 mg cloprostenol given 24 hours before CIDR removal. In the present study both treatments were high to estrous response with no significant except to the control group. But the results of this study agree with recent report of Waheeb et al. (2017) who concluded that intravaginal progesterone sponge for 7 days+500IU eCG at sponge removal is convenient for estrus synchronization of ewes raised in field conditions during breeding season specially that the reproductive and fertility parameters recorded in his study were acceptable and within values reported previously in farm conditions. Also, Dendena (2017) stated a fertility rate of 85.1% after the administration of 500 IU of eCG, which was considered lower than PMSG treatment in this study, this may be referred to using higher dose (750 IU) in our treated group. Thus, our results were comparable to what has been most recently reported by Fornazari et al. (2018) as these stated that short-term progestagens (FGA e MAP) + eCG were 100.0% efficient in Assaf breed in cycle control worth to mention that in the same study the administration of 750 IU of eCG determined a fertility rate of 76.5%, which was controversially to our results. From our point of view, less prolific and less seasonal breeds tend to be more responsive to eCG administration, specially that different follicular populations present on the ovaries before progestagen treatments + eCG may also condition fertility rate (Omontese et al., 2016). In general, the present results meet these last investigators' observations that exogenous gonadotropin administration advanced ovulation and higher estrus synchronization precision (Letelier et al., 2011; Valentim et al., 2016), as they support ovarian mechanisms affecting follicular growth and maturation and promoting the proper luteinization of the CL (Valentim et al., 2016). In most studies, it is mentioned that in adult animals the expected estrus rate is greater than 90%, while nulliparous goats can reach up to 97.2% and lactating animals up to 85.7% (Abecia et al., 2011; Navanukraw et al., 2014; Alvarado-Espino et al., 2016).

Lambing rate, abortion rate, weaning rate, fecundity rate, litter size at birth, litter size at weaning and mortality rates (%)

Lambing rate is expressed as the percentage of ewes lambed out of ewes mated. As shown in **table 2** lambing rate was significantly higher ($P<0.05$) in G3 as compared to G2 or G1 (100, 92 and 80%, respectively). While, weaning rate was recorded to be higher in G2 than in G1 and G3 and showed non-significantly rates of 100.0, 95.45 and 94.29%, respectively.

Table 2. Lambing rate, weaning rate, fecundity rate, litter size at birth, weaning rate, litter size at weaning and weaning weight of Barki ewes as affected by different hormonal treatment (LSM±SE)

Traits	Treatment (T)			Overall mean	± SEM	Sig.
	G1	G2	G3			
Lambing rate (%)	80.00 (20/25)	92.00 (23/25)	100.00 (25/25)	90.67 (68/75)	4.63	**
Weaning rate (%)	95.45 (21/22)	100.0 (26/26)	94.29 (33/35)	96.39	5.95	NS
Fecundity rate (%)	0.88^{b} (22/25)	1.04^{b} (26/25)	1.40^{a} (35/25)	1.10	0.10	**
Mortality rate (%)	4.55 (1/22)	0.00 (0/26)	5.71 (2/35)	3.61	--	NS
Litter size at birth/ewe	1.10^{b} (22/20)	1.13^{b} (26/23)	1.40^{a} (35/25)	1.22	0.05	*
Litter size at weaning/ewe	1.05 (21/20)	1.13 (26/23)	1.32 (33/25)	1.17	0.05	NS
Day 90 (Weaning weight)(kg)	16.71	17.53	17.45	17.28	--	NS

G1 = control group; G2 = FGA and PGF2α; G3 = FGA, PGF2α, PMSG and hCG; Sig. = significance; NS = non-significant
Means within each row with different superscripts are significantly different at 5% level.

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As reported in our study, fecundity rate was significant higher in G3 and G2 than in G1 (1.40, 1.04 and 0.88), respectively (**Table 2**). Moreover, litter size at birth as expressed as number of born lambs per ewe lambed is considered to be an important parameter of fertility. Data illustrated in table 2 indicated that litter size at birth was significantly the highest in G3 followed by G2 as compared to G1 (1.40, 1.13 and 1.10, respectively). Litter size at weaning was significantly higher in G3 then G2 than in G1 (1.32, 1.13 and 1.05 respectively). The data also revealed that weaning weight showed insignificant increase in G2 as compared to G3 and G1 (17.53, 17.45 and 16.71, respectively). Non-significant higher values were recorded for mortality rate in G3 and G1 than in G2 (5.71, 4.55 and 0.00%, respectively).

In the tropics, biological and economic efficiency of animal production are predominantly determined by reproductive performance. Fertility is categorized as important parameters for determination the productivity of sheep; fertility may be estimated by several indicators as litter size per lambing for example which is more important than weight gain of lambs. Profit gain from increasing lamb survival is the major concern of sheep producers (Elliott et al., 2011). According to Elliott et al. (2011), sheep producers believed that using teasers for estrus synchronization, minimizing handling and interrupting ewes during the peri-partum period, and limiting duration of the breeding period would be beneficial to lamb survival. Contrarily to our results, it has been confirmed that high dose of eCG increases the ovulation rate but also reduces the embryonic survival rate (Diskin and Morris, 2008).

Formerly Gongnet (1996) reported malnutrition as the main cause of lamb mortality before weaning in lambs, for that reason, in recent study by Allou et al. (2013) advised with proper diet to enhance the adequate health that improve the survivability and lambs growth during the first three months of age. While, Gbangboche et al. (2005) referred the lamb survival rate before weaning to the age of the sheep at the first lambing influences, this was confirmed by Allou et al. (2013), who had stated that lambs survival is positively and beneficially correlated with this influence . Early reports indicated that the number of lambs surviving until weaning has been found to be influenced by ewes' body condition score at mating (Carson et al., 2001) rather than other surrounding factors.

Lambs with intermediate birth weights have a lower mortality risk than small and very large lambs (Piwczynski et al., 2012). However, the association between lamb birth weight and mortality was not detected in a study by Brien et al. (2009). In this regard, due to negative effects of large litters on lamb survival, close supervision should be provided to non-singleton lambs (Chniter et al., 2011). Litter size is influenced by several factors, and is low in ewes younger than 2 years of age and ewes having low weight at mating (Atashi et al., 2013). Weight gain in lambs is described by two subsequent phases, the first lasts from birth up to 90 days with a moderate weight change, this phase is followed by very fast changes in weight form 90 days until 200 days (7 months) of age (Allou et al., 2013).

Progesterone concentration

The results in Figure 2 to elucidate that plasma progesterone levels (ng/ml) as affected by FGA and $PGF_{2\alpha}$ (G2) and FGA, $PGF_{2\alpha}$, PMSG and hCG (G3) as compared to the control group (G1). It could be observed that P4 levels attended to increase from the first day of pregnancy and rises to the end of the day before parturition. Also, progesterone was higher in G3 which contains the highest values of litter size at birth of newborn lambs as a result of increasing twinning rates in response to hormonal treatment for the superovulation. Mean concentration of P4 level was found to be 8.47 ng/ml in G3 followed by G2 (8.05 ng/ml) while the lowest value was observed in G1 (7.40 ng/ml).

Early pregnancy is a critical period for maternal recognition, and the lack of nutrients that stimulate an increase in circulating progesterone can increase early embryonic losses (Viñoles et al., 2012). According to Mann et al. (2006), most embryonic losses occur during the first few days after fertilization and during the implantation process, inadequate luteal function being one of the main causes. The maintenance of pregnancy in ruminants depends on the continued secretion of progesterone by the corpus luteum, which inhibits luteolysis. Progesterone deficiency due to primary luteal insufficiency has been reported as a cause of embryonic death (Mann and Lamming, 2001; Diskin and Morris, 2008). Increase concentrations of progesterone (P4) during meta-oestrus and early di-oestrus improves embryonic growth and the production of interferon-τ (IFN-τ) (Spencer, 2013 and 2016; Arosh et al., 2016), which in turn improves the relationship between embryo and uterus and increases embryonic survival rates (Mann et al., 2006).

The higher values of progesterone levels, found in G3 followed by G2 than that in the control group, may be due to the number of corpora lutea present as it is the source of progesterone in sheep. El-Tarabany (2012) found that progesterone concentration decreased significantly in ewes conceived single than in ewes conceived twin's fetuses by 34.3%. Also, Khan and Ludri (2002) found that in twin bearing goats' plasma progesterone level was significantly higher than in single bearing goats during all days of experiment. Manalu et al. (1998) reported that ewes with a high litter size had heavier placental tissue. Moreover, ewes with a high litter size had high ovulation rates that resulted in high number of corpora lutea as sources of progesterone during the embryonic phase of pregnancy and, probably, throughout the pregnancy period

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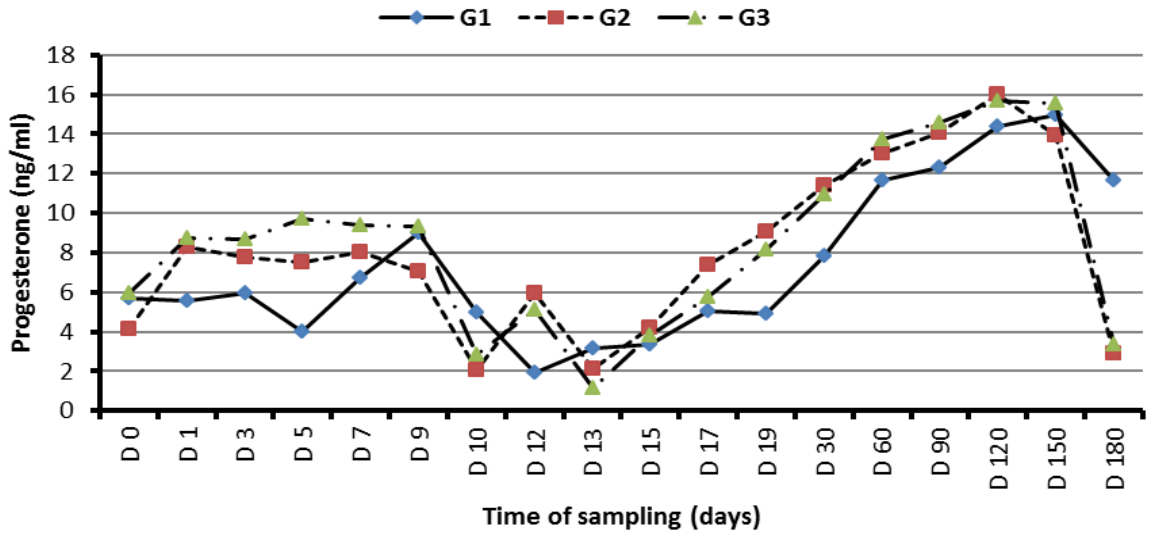

Figure 2. Plasma progesterone levels (ng/ml) as affected by FGA and PGF2α (G2) and FGA, PGF2α, PMSG and hCG (G3) compared to the control group (G1). G1 = control group; G2 = FGA and PGF2α; G3 = FGA, PGF2α, PMSG and hCG

Type of lambing and sex ratio

The data presented in **table 3** demonstrate that single rate was significantly higher in the control non treated group (G1) as compared to other treated groups, being 90.00, 86.96 and 64.00%, respectively. Results of present study indicated that twining rate significantly increased in the hormonal manipulated group (G3) than (G2) and (G1) being 32.00, 13.04 and 10.00%, respectively, **(Table 3**). The influence of the superovulation treatment was observed for G3, as triple rate increased significantly in G3 as compared to other groups, with values of 4.0, 0.0 and 0.0%, respectively. Sex ratio (female %) exhibited significantly the highest values for G3 (60.00%) followed by G2 (59.09%) and finally G1 (46.15%). Conversely**,** sex ratio (male %) was significantly higher in G2 than in G1 and G3, recording 53.85, 40.91 and 40.00%, respectively.

A like to the Barki breed in our study herein, Awassi breed is a monotoccus with an ovulation rate of 1 and very low incidence of twinning (Jaber et al., 2004). For that reason, the twins and triplets born lambs weighed less at birth as compared with singles in control groups, this might be due to the competition between fetuses for nutrient supplied by the placenta from maternal circulation for growth due to the low capacity of uteri to provide for twins (Baneh and Hafezian, 2009). In Barki, twining in general and especially triple births is not common. Moreover, Clement et al. (1997) in his study assumed that the longer of lambing interval is accompanied with the increase in the range of sizes with the rank of lambing. Herein this study triples birth in group 3 (G3) is probably accompanied with the effect of PMSG injection. Sheep male lambs' are known to be born heavier than females, (Gbangboché et al., 2005), who also reported that this trend in weight significant different may last up to 12 months or more, this observation is reported under same rearing conditions for birth weight for males and females, but these variations tends to disappear at 120 days of age (Poivey et al., 1982; Fall et al., 1983). Binns et al. (2002) and Brien et al. (2009) found higher mortality in male than female lambs. On the other hand, another study reported a higher survival risk in male lambs (Atashi et al., 2013), while mortality risks for gender were not statistically different in a study in Quebec (Arsenault et al., 2003).

Table 3. Types of lambing and sex ratio (%) of Barki ewes as affected by different hormonal protocols (LSM±SE)

Traits	Treatment (T)			Overall mean	± SEM	Significance
	G1	G2	G3			
Single rate	90.00 (18/20)	86.96 (20/23)	64.00 (16/25)	--	--	--
Twining rate	10.00^{b} (2/20)	13.04^{b} (3/23)	32.00^{a} (8/25)	18.34	2.74	**
Triple rate	0^{b}	0^{b}	4.00^{a} (1/25)	--	--	--
Sex ratio (female)	59.09 (13/22)	46.15 (12/26)	60.00 (21/35)	55.42 (46/83)	1.62	*
Sex ratio (male)	40.91 (9/22)	53.85 (14/26)	40.00 (14/35)	44.58 (37/83)	1.62	*

G1 = control group; G2 = FGA and PGF2α; G3 = FGA, PGF2α, PMSG and hCG. Means within each row with different superscripts are significantly different at 5% level.

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CONCLUSION

It could be concluded that, hormonal manipulation using intravaginal progestagen impregnated sponge and PMSG in the presence of hCG; for estrous synchronization and superovulation; would be a proper way for enhancing the reproductive efficiency of Barki ewes.

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Competing interests

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

Author's contribution

Dr. Gamal Ashour designed the experiment, article writing and revision, Dr. Moharram Fouad El-Bassiony designed the experiment, laboratory analyses, statistical analysis, tabulation of experimental data, manuscript writing, commenting and approval, Dr. Sherif Mohamed Dessouki helped in statistical analysis, tabulation of experimental data and article writing; while, Mr. Mohamed Awad El-Wakeel helped in field study, collected data, laboratory analyses, manuscript writing. All authors have read and approved the final manuscript.

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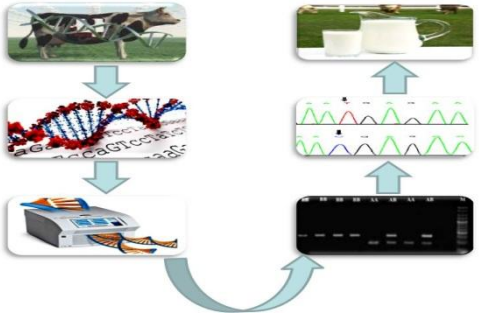

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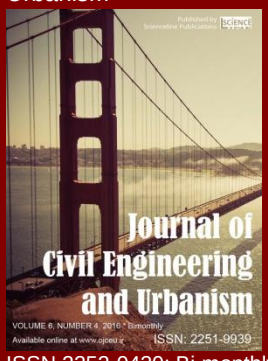

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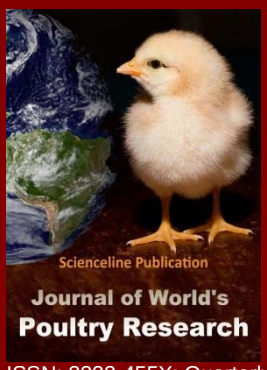

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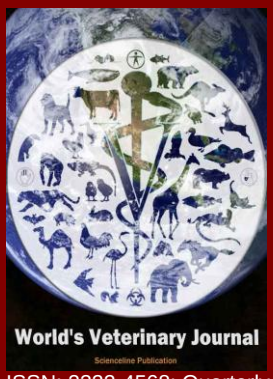

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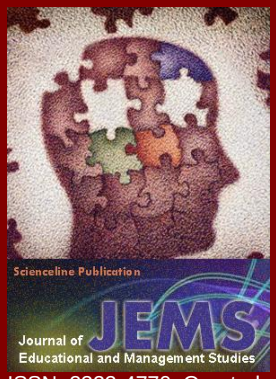

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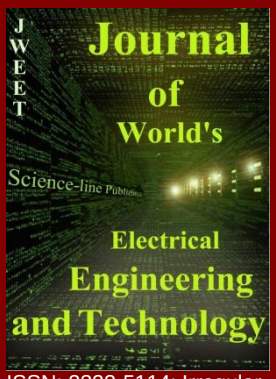

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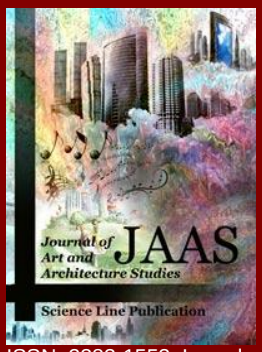

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