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Full length Research paper

Estrus synchronization of Desert ewes under dryland farming in North Kordofan, Sudan

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Short title: Estrus management in free ranging Desert ewes

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Estrus synchronization, as a climate change adaptation, was imposed on two hundred Sudan Desert ewes (9-10 months old, 28 kg av. wt.), under dryland farming in North Kordofan, Sudan, during January-August 2019. The ewes were supplemented with a concentrate mixture (200 g/head/day) one-month prior to trials, divided into four similar groups and randomly assigned to four treatments. A control receiving no treatment (A), synchronized with FGA sponges (40 mg) for 14 days plus 400 IU PMSG (B), or plus 600 IU PMSG (C), or by the ram effect (D). Ten rams were introduced for natural mating. Highest (P<0.01) fertility (82.0%) and fecundity (78.0%) rates were for ewes in C and B, respectively. Prolificacy rate was slightly highest (P>0.05) in C (85.4%) while comparatively highest (P>0.05) litter size resulted in B (1.1). Highest lamb weights (P<0.01) at birth and at 30 days of age were for B (2.95 and 10.16 kg, respectively). Plasma progesterone concentrations were higher (P<0.01) for A, B and C compared with D. Estrus synchronization had improved desert ewe fertility and twining rates. Combined ram effect and estrus synchronization protocols for improving desert ewe productivity under dryland conditions in Sudan and similar ecological zones need to be studied.

Keywords: desert ewes, drylands, climate change adaptation, estrus synchronization, progesterone, reproductive parameters

INTRODUCTION

Sheep in Sudan is estimated at 40.21 million head, accounting for 37.7% of the total livestock population in the country (Wilson, 2018). It is classified into four main types: Desert, Baggara, Garag and Jebelli, with Desert sheep accounting for 65% of sheep in Sudan (Gornas *et al*., 2011). Sudan Desert sheep plays an important role in the domestic meat supply and in foreign exchange earnings (Wilson, 2018). In recent years, the use of Sudan Desert sheep as an export commodity has increased, with the majority being from North Kordofan region, contributing between 600 thousand to one million heads annually (el Dirani *et al.*, 2009).

Desert sheep is a continuous breeder and could mate and reproduce throughout the year (Gornas *et al*., 2011). However, sheep farmers, particularly under rangeland dryland farming conditions, have adopted breeding control strategies to cope with the changing climatic conditions and production constraints in order to match lambing with the rainy season nutritious grazing resources (El-Hag *et al.*, 2001). Such practices lead to low reproductive performance and have negative effects on sheep productivity (El-Hag *et al.* 2007). It is therefore imperative to seek means that could improve reproductive management of Desert sheep flocks for sustainable production (el Dirani *et al.*, 2009) as an adaptation option to climate change.

Estrus synchronization is the most acceptable worldwide

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technique for reproduction management (Moakhar *et al.*, 2012). Progesterone/progestogen-based protocols are widely used to synchronize estrus and ovulation in several farm animal species (Fierro *et al.*, 2016). Ovarian response of sheep and goat to oestrus synchronization varies with the type of intravaginal sponge, kind of progestogens, breed of sheep (Mahboub *et al.*, 2013), nutritional status (Khotijah *et al.*, 2015), environmental stress (Dobson *et al.*, 2012) and male effect (Alavez-Ramirez *et al.*, 2016). The overall objective was to improve free ranging Desert ewes' productivity under dryland farming conditions in North Kordofan, Sudan, and as adaptation to climate change.

MATERIALS AND METHODS

Ethical approval: Animals Use in Research was according to the committee of the University of Khartoum regulations. The guidelines and regulations set out by the Sudan Veterinary Council were strictly followed during animal handling and sampling.

All experimental protocols used are approved by the University of Khartoum and in accordance with the University of Khartoum Laboratory Authority guidelines.

Study Area

North Kordofan lies between Lat. 11°15´-16°30´N and Longit. 27-32°E, Sudan. Soils in the area include sandy soils (more than 60%), clay soils (6%), clay-loamy soils (18%), and other important soils include loamy soils locally known as "Gardud" soils (2%) (El Tahir *et al.*, 2009). The important land use categories are rangelands comprising about 50.6% of the region area, traditional rainfed agriculture 22.1%, forest area 1.82%, and bare areas 25.2%. The region suffers from frequent drought spells due to extreme fluctuations in rainfall which generally varies from 150-450 mm/year and extends from July-October. Severe climatic conditions and land mismanagement (overgrazing, over-cropping, deforestation) further augment the situation through causing vegetation cover to become very poor and the loss of endemic woody and range plant species that were once dominant (El Tahir *et al.*, 2009).

Animals, estrus synchronization treatments and experimental layout

Two hundred ewes (9-10 months old, average body weight of 28 kg) of Sudan Desert sheep of the Hamari subtype were monitored for reproductive performance during the period January-August 2019. The whole flock was first injected with Ivomec (Ivomectin^R) at 0.5cc/sc/head against internal and external parasites and vaccinated against sheep pox, hemorrhagic septicemia, and anthrax. All ewes were ear tagged and initially weighed. The flock was maintained on open rangelands. A concentrate mixture (60% sorghum+29% groundnut seed cake+10% wheat bran+1% common salt) was given at 200g/ewe/day, for one month prior to the commencement of estrus synchronization trial. Mineral salt lick and water were offered free choice.

The ewes were divided into four similar groups of 50 heads each, based on weight and age, then randomly assigned to four estrus synchronization treatments. The first Group (A) was kept as a control while the other three groups were assigned to three different protocols for estrus synchronization. In ewes of Groups B and C, intravaginal sponges impregnated with 40mg fluorogestone acetate (FGA) synthetic progesterone were inserted using an applicator (Algan *et al.*, 2017) for 14 days. At the time of sponge removal, Group B ewes were injected with 400IU of pregnant mare serum gonadotrophin (PMSG), while Group (C) ewes were injected with 600IU of PMSG. Then, ten rams of the same Hamari subtype of Desert sheep, with great sexual activity (4-5 years old), were introduced for mating. Some ewes were hand mated by taking estrus ewes to rams. Rams were rotated among ewe groups to remove ram effect. Group (D) was synchronized through isolating rams from the flock for a month. After the period of separation, rams were suddenly introduced to the group of ewes and mated. The ewe flock was monitored for productive and reproductive performance.

Productive and reproductive parameters

Conception rates were determined by non-return to estrus. Pregnancy rates were determined by abdominal palpation 90-110 days post insemination. Lambing, dates of birth and number of lambs born per ewe were recorded.

Other productive and reproductive parameters calculated for each group (Zarkawi and Al-Daker, 2018) were fertility rate ([lambed ewes/ewes inseminated]x100), fecundity rate ([Lambs born alive/ewes inseminated]x100), prolificacy rate ([Lambs born alive/Lambed ewes]x100), lambing rate ([Lambed ewes/pregnant ewes]x100), and litter size (number of lambs per ewe that lambed).

Progesterone determination

Blood samples were taken from the jugular vein using vacationer tube with no additive from a representative sample of 29 ewes (8 from Group A, 8 from Group B, 7 from Group C, 6 from Group D) for six successive months, one month before the commencement of the trial and every month for five months thereafter. Harvested samples were stored at 4°C (in an ice bath) immediately after collection. Then, centrifuged at 3000 rpm for 15 minutes.

Collected samples were stored at -20°C until analyzed with Radio-Immuno Assay (RIA) for plasma progesterone determination (Algan *et al.*, 2017).

Statistical analyses

The chi-square test was used for analysis of reproductive performance rates, mortality, and type of birth (singleton, twinning, triplet). Fertility parameters, lamb weights at birth and 30 days of age, and plasma progesterone concentrations were compared by independent sample t-test. Effects of estrus synchronization treatment, lamb type of birth, sex of lamb on lamb weight at birth and 30 days of age were analyzed as a factorial experiment using least square mixed model procedures (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of breeding management on reproductive performance of Sudanese desert ewes

Successful estrus synchronization programs have a key role in lambing rate efficiency and profitability of sheep production systems (Almadaly *et al.*, 2016). Ewes treated with FGA + PMSG (Groups B and C) exhibited overt signs of estrus during the 24-36hrs and recorded higher (P<0.01) conception rates compared with the control (Group A) (Table 1). Respective conception rates for ewes in Groups B, C and under ram effect (D) were 82.0, 86.0 and 88.0% in comparison with a lower (P<0.01) conception rate of 66.0% for ewes in Group A (control).

These results were in agreement with the findings of Zeleke *et al.* (2005) who used progesterone with PMSG. They found that synchronization of estrus in sheep had resulted in greater than 90% of ewes on heat in a 24-36 hrs period and conception rate of 70-80%.

Lambing rates were highest (P<0.01) for ewes synchronized with FGA + PMSG (Groups B and C) compared with the control and ram effect groups (Table 1).

Abortion rate was highest (P<0.01) in Group D (ram effect) compared with the other three ones (Table 1). Furthermore, estrus synchronization treatment exerted no effect (P>0.5) on lamb sex, lamb type of birth and lamb mortality (Table 1).

However, comparatively the highest numbers of male lambs were for ewes in Group B while the lowest numbers were for Group C.

All lambs born to Groups A and D were singles, whereas ewes in Groups B and C gave birth to twin lambs. Algan *et al.* (2017) studying the effect of intravaginal sponges impregnated with different doses of PMSG on some reproductive parameters in Pırlak ewes, in Turkey, found that conception and lambing rates, and overall singleton and twinning rates did not differ between groups.

The highest (P<0.01) ewe fertility rates obtained (Table 1) were (82.0%) in Group C whereas the lowest rates were in the control and ram effect groups (Groups A and D; 62.0%).

Table 1: Effect of breeding management on reproductive performance of Sudan Desert Ewes under dryland conditions in North Kordofan, Sudan

Parameter	Group A	Group B	Group C	Group D	χ^2 (+df)
No. of ewes	50	50	50	50	50
Ewe reproductive performance:					
Conception rate	33(66.0%)**a**	41(82%)**b**	43(86%)**b**	44(88%)**b**	0.776**(df=3)
Abortion rate	2(4.0%)**a**	2(4%)**a**	2(4%)**a**	13(26%)**b**	2.221**(df=3)
Lambing rate	31(62.0%)**a**	39(78%)**b**	41(82%)**b**	31(62%)**a**	10.638**(df=3)
Mortality rate	7(14%)	6(12%)	6(12%)	6(12%)	0.137ns(df=3)
Lamb sex:					
Males	17(54.8%)	30(73%)	21(51.2%)	20(64.5%)	0.228ns (df=9)
Females	14(45.2%)	11(27%)	20(48.8%)	11(35.5%)	
Lamb Type of Birth:					
Singles	31(100.0%)	36(87.8%)	35(85.4%)	31(100%)	0.231ns (df=9)
Twins	0	5(12.2%)	6(14.6%)	0	
Lamb mortality:					
Alive	26(83.9%)	32(78%)	35(83%)	24(77.4%)	0.853ns (df=9)
Died	5(16.1%)	9(22.0%)	6(14.6%)	7(22.6%)	
Fertility parameters (%) of Desert ewes:					**SE±**
First inseminated	31	39	44	26	
Second inseminated	3	2	6	6	
Total ewes inseminated	34	41	50	32	
Fertility rate %[1]	62.0**a**	78.0**a**	82.0**a**	62.0**b**	4.27**
Fecundity rate %[2]	76.5**a**	78.0**a**	70.0**b**	75.0**a**	1.50**
Prolificacy rate %[3]	83.9	82.1	85.4	77.4	4.38**ns**
Litter size[4]	1.0	1.1	1.0	1.0	0.05**ns**

Estrus synchronization treatments: Group A = control (no treatment), Group B: FGA+ 400 IU PMSG, Group C: FGA+ 600 IU PMSG, Group D: synchronized with the ram effect. Numbers between parenthesis are percentages.
a,b,c, Means in the same row with no letter in common are significantly different (**P<0.01, **ns**= not signifi1cant (P>0.05)
[1]Fertility rate = (lambed ewes/ ewes inseminated) x 100, [2]Fecundity rate (%) = (Lambs born alive/ ewes inseminated) x 100.
[3]Prolificacy rate (%) = (Lambs born alive/ Lambed ewes) x100, [4]Litter size = Number of lambs per ewe that lambed

These findings were in line with Koyuncu and Alticekic, (2010) who concluded that the use of FGA+PMSG for estrus synchronization can stimulate follicular development and higher ovulation rate and fertility in ewes. The highest (P<0.01) fecundity rates were for ewes in Group B (78.0%) and lowest were for ewes under Group C (70.0%). In contrast, higher fecundity rate of 133.0% was attained with the use of FGA+PMSG in Karakul ewes (Safdarian *et al*., 2006). These differences may be due to age (Fornazari *et al*., 2018), natural mating synchronization protocols (Wani *et al*., 2017) and management conditions of ewes. Prolificacy rates ranged from a low value of 77.4% for ewes under the ram effect treatment (Group D) to a high value of 85.4% for those under Group C, but no significant differences (P>0.05) were detected (Table 1).Similarly, Viňoles *et al*. (2011) studying pregnancy rate and prolificacy after artificial insemination in ewes following synchronization with prostaglandin, sponges, or sponges with bactericide, concluded that synchronization with progestagen sponges plus equine chorionic gonadotrophin (eCG) or by three injections of prostaglandin (PG) had resulted in similar prolificacy.

Plasma progesterone level before and during pregnancy

Progesterone concentrations were higher (P<0.01) in Groups A, B and C compared with Group D throughout the pregnancy period (Table 2). Progesterone concentrations rose from basal levels and remained elevated in all ewe groups throughout pregnancy and reached their highest levels during the 3rd and 4th months of pregnancy (Zarkawi and Al-Daker, 2018).

Table 2: Plasma progesterone concentrations (ng/ml) of desert ewes before and during pregnancy

Sampling time	Group A	Group B	Group C	Group D	SE±
Number of ewes	8	8	7	6	
1st month prior to treatment	2.95±0.36	3.11±0.36	2.25±0.39	1.55±0.43	0.196**ns**
Immediately After treatment	7.50±2.1**a**	10.14±2.1**b**	5.95±2.3**a**	4.93±2.53**a**	1.147*****
1st month after treatment	9.70±2.9	13.98±2.9	11.89±3.2	7.93±3.5	1.593**ns**
2nd month after treatment	12.02±3.19**a**	18.70±2.87**b**	13.59±3.19**a**	4.70±3.53**c**	1.641******
3rd month after treatment	14.78±4.08**a**	25.41±4.08**b**	17.04±4.5**a**	5.06±4.95**c**	2.243******
4th month after treatment	20.90±5.70**a**	37.31±5.7**b**	23.85±6.27**a**	6.36±6.90**c**	3.125******
5th month after treatment	32.69±7.18**a**	39.46±7.18**a**	33.02±7.9**a**	13.48±8.70**b**	4.374*****

Estrus synchronization treatments: Group A = control (no treatment), Group B: FGA+ 400 IU PMSG, Group C: FGA+ 600 IU PMSG, Group D: synchronized with the ram effect.
a,b,c, Means in the same row with different letters are significantly different (*****P<0.05, ******P<0.01). **ns**=not significant (P>0.05)

Effects of estrus synchronization treatment, sex of lamb and lamb type of birth on Lamb weights

Estrus synchronization treatment had significant effects on lamb weights at birth and at 30 days of age (Table 3). Lambs born to ewes in Group B recorded higher (P<0.05) weights in comparison with other three groups, with Group A recoding the lowest (P<0.05) weights at birth and at 30 days of age (P<0.01). Sex of lamb had significant effects (P<0.01) on lamb weight at birth and on lamb weight at 30 days of age (Table 3). Male lambs had higher weights compared with female lambs (Yilmaz and Altin, 2011).
Single lambs had higher (P<0.01) weights at birth and at 30 days of age in comparison with twin lambs. Norouzian (2015) found that single lambs were 0.5 kg heavier (P<0.01) at birth than those born as twins. However, no significant estrus synchronization treatment x lamb type of birth interaction effect was found on lamb weights at birth or at 30 days of age (Table 3).

Table 3: The effects of estrus synchronization treatment, sex of lamb, and lamb type of birth on lamb weight at birth and at 30 days of age under dryland conditions in North Kordofan, Sudan

Factor	N	Wt. at Birth	N	Wt. at 30 days
Estrus synchronization treatment (EST):				
Group A (Control)	31	2.43±0.088**c**	26	8.25±0.340**c**
Group B	41	2.95±0.149**a**	32	10.16±0.273**a**
Group C	41	2.67±0.170**b**	35	10.13± 299**a**
Group D	31	2.67±0.089**b**	24	9.19±0.327**b**
SE±		0.012*		0.167**
Sex of Lamb:				
Male	88	2.87±0.081	67	10.04±0.25
Female	56	2.53±0.072	50	8.86±0.146
SE±		0.023**		0.056**
Lamb Type of Birth:				
Single	133	2.65±0.047	111	9.21±0.0986
Twin	11	2.47±0.016	6	7.915±0.048
SE±		0.003**		0.167**
Interaction effects (±SE):				
EST x Lamb Sex		0.157**ns**		0.149**ns**
EST x Lamb type of birth		0.055**ns**		0.100**ns**
Lamb sex x lamb type of birth		0.187**ns**		0.263**ns**
EST x Lamb sex x Lamb type of birth		0.442**ns**		0.641**ns**

Estrus synchronization treatments: Group A = control (no treatment), Group B: FGA+ 400 IU PMSG, Group C: FGA+ 600 IU PMSG, Group D: synchronized with the ram effect.
a,b,c, Means in the same column with different letters are significantly different (*P<0.05, **P<0.01, ns=Not significant P>0.05)

CONCLUSIONS

Estrus synchronization treatments were effective in improving desert ewe fertility and twining rates under dryland rangelands conditions. These protocols should be adopted since ram separation under free ranging conditions might be difficult.
Further research work is needed to study the combination of ram effect and other estrus synchronization protocols to improve reproductive performance in desert ewes under dryland rangeland conditions in North Kordofan, Sudan and similar ecological areas.

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Authors' Contributions

RMI laboratory work, data analysis and wrote the draft manuscript, AAK data tabulation and analysis,AM A-N coordination, MT supervision, YK design and review, IA A-B design and analysis, RMAO lab work and data analysis, HAS lab work and data analysis, M-K AA project management, FM El- design and data analysis. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

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