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Fertility Response of Desert Ewes to Hormonal Oestrous Synchronization and Artificial Insemination Using Fresh Diluted Semen

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Abstract: Forty Desert ewes were allocated for monitoring the effect of different hormonal treatments and Artificial Insemination (A.I) on their reproductive performance with an regard to oestrous response and fertility rates. The animals were randomly divided into four equal groups (10 ewes each) with average age of 2.00±0.80 years and body weight of 39±3.00 kg. The control group (A) was allowed to cycle naturally and handmated, while the other three groups were assigned to different hormonal treatments.These treatments consisted of a double intramuscular injection of Luprositol (3.75 mg^{-1}) 14 days part (group B); intravaginal sponges impregnated with 40 mg^{-1} Fourogestone Acetate (FGA) inserted for 13 days (group C) and treatment C plus an intramuscular injection of 500 iu Pregnant Mare Serum Gonatotrophin (PMSG) at the time of the sponge removal (group D). Oestrus was detected by the aid of a vasectomized ram, with an excellent libido, introduced to each group immediately after the end of each treatment. Time elapsed from end of treatment to onset of oestrus, oestrous signs and duration of oestrus were monitored. All the ewes in the three treatment groups were artificially inseminated with fresh semen, diluted with homogenized cow milk, 52 h after the end of treatments. Those returning to oestrus were artificially reinseminated. Conception rates were determined by non-return rates and later on by abdominal palpation (ballotement) on day 90-110 post insemination. The results obtained indicated that all the employed treatments can induce and synchronize oestrus in Sudanese Desert ewes. The percentage of ewes responding to treatment B (80%) by showing oestrous signs was significantly higher ($p<0.05$) than the other two treatments, followed by treatment D (55.50%) and C (44.40%). The duration of the induced oestrus was signiticantly longer ($p<0.05$) in treatment B (43.40±5.20 h) as compared to treatment C (27.30±2.80 h) and D (27.2±3.00 h). The pregnancy rate to first insemination was significantly higher ($p<0.05$) in treatment D (100%) as compared to B (40%), C (77.80%) and the control (40%). However, the overall pregnancy rates, based on abdominal ballotement between 90 and 110 days were significantly ($p<0.05$) higer in group C (100%) and D (100%) compared to group B (80%) and the control A (50%). Lambing confirmed the results obtained at abdominal ballotement which was 9.23% less than those obtained by the non - return rates. The twining rates were 20, 37.50, 33.30 and 33.30% for treatments A,B,C and D respectively. There were no significant differences ($p>0.05$) among the different hormonal treatments.

Key words: Oestrous synchronization, A.I, desert ewes, fertility rates

INTRODUCTION

Modern systems of sheep production are geared towards intensification of management, to accelerate the rhythm of lambing (Karagiannidis *et al*., 2001). Oestrous synchronization is the most acceptable, worldwide, technique for reproductive management (Gordon, 1983; Simonetti *et al*., 2000; Iida *et al*., 2004). Natural progesterone, synthetic progestagens and prostaglandins with or without combination with other hormones were used for oestrous synchronization. (Noakes *et al*., 2001). Intravaginal devices impregnated with progestagens are the most popular means for oestrous synchronization, in ewes, with fertility results ranging between 20 and 70% (Simonetti *et al*., 2000; Karagiannidis *et al*., 2001).

Variability in fertility response to progestagens treatment was attributed to variations in oestrous duration and timing of ovulation and these were found to be further synchronized by PMSG injection at the end of progestagens treatment (Robinson *et al*., 1987; Romano *et al*., 1996; Karagiannidis *et al*., 2001; Barrett *et al*., 2004).

Insemination at a predetermined time following synchronization with progestagens combined with PMSG achieved acceptable fertility rates in ewes (Dzuik *et al*., 1972; Smith, 1977; Karagiannidis *et al*., 2001). In the Sudan, Desert sheep constitute more than 65% of a sheep population of more than 47 million heads (Mcleroy, 1961; MAR, 2003). Many ecotypes, denoted by tribal names, exist in the area between latitude 13° and 17° North and

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longitude 25° and 37° East, raised mainly under the traditional pastural system (Medani, 1996). Desert sheep dominate the livestock exports of Sudan because of their potential for mutton production and marketing features merits (Hassan and Mukhtar, 1971; suleiman *et al*., 1978, 1990; Abdel Malik *et al*., 2002). Despite this substantial contribution in the national economy, little effort has been exerted to improve their reproductive performance (Makawi *et al*., 2005).

This study was designed to investigate the effect of controlled breeding and Artificial Insemination (AI) on reproductive performance of Desert sheep with respect to:

- Oestrous response to different hormonal protocols for oestrous synchronization.
- Fertility results following oestrous synchronization and A.I. using fresh diluted semen.

MATERIALS AND METHODS

Animals: Forty Sudanese Desert ewes with an average age of 2.00±0.80 years and body weight of 39.00±3.00 kg were selected from a flock of 80 ewes kept at Khartoum University farm. Selection was made on basis of body condition scoring and fertility as indicated by regularity of oestrous cycle and previous lambing.

Husbandry and management: The ewes were ear tagged and housed in well ventilated open shaded pens. The animals were drenched with 5% albendazole (Valbazen, Smith kline, Beecham, USA) at a dose of 7.5 mL 50 kg^{-1} for deworning. An adaptation period of three weeks was allowed during which the animals were taken outdoors to graze on green sorghum grass (Abu 70) daily between 7:00 a.m. and 3:00 p.m. A ready-made concentrate mixture, manufactured by Khartoum University Feed Mill, was given at a rate of 0.5kg/head/day. The concentrate diet consisted of maize (45%), groundnut cake (19%), molasses (13%), mineral mixture (3%) and salt (1%). Clean fresh water and mineral salt licks were offered *ad libitum* indoors.

Experimental design

Oestrous synchronization: The ewes were randomly divided into four equal groups of 10 animals with representation of the different ages and body weights in each group. The first group (A) was kept as a control while the other three groups were randomly assigned to three different hormonal treatments for oestrous synchronization as follows:

Treatment (B): Each ewe was given two intramuscular injections of 3.75mg Luprostiol, a synthetic $PGF_2\alpha$ analogue (Prosolvin, Intervet International, B.V., Boxmeer, Holland), 14 days apart.

Treatment (C): Intravaginal sponges impregnated with 40 mg fluorogestone acetate (F.G.A), a synthetic progeserone (Chronogest, Intervet International B.V.Boxmeer, Holland) were inserted using an applicator as described by Evans and Maxwell (1987) for 13 days.

Treatment (D): The ewes were treated with intravaginal sponges as in treatment (C). At the time of sponges removal the ewes were injected with 500 IU. of PMSG (folligon; Intervet International. B.V., Boxmeer Holland).

Oestrous detection: At the end of each treatment a vasectomized, sexually active, Desert ram was introduced to the control (group A) and treated groups (B,Cand D) for monitoring onset of oestrus and duration of induced oestrus. Detection of oestrus was carried out for 30 minutes, three times per day (morning, afternoon and evening) for five days starting immediately after the end of the treatments.

Insemination: The control group was handmated by taking oestrous ewes to male in a separate pen, while the synchronized ewes (regardless of being in oestrus or not) were artificially inseminated 52 h after the end of treatments. The semen was deposited intra-cervical as far as the cervix allowed the passage of the insemination gun. A volume of 0.5 mL of fresh semen diluted in homogenized cow milk containing 200×10^6 active spermatozoa was used. The ewe to be inseminated was restrained by an assistant in a vertical position with the hind quarters upwards and the forelimbs on the ground between the assistant's leg (Gordon, 1983). Semen was collected from three mature Desert ram through electroejaculation using a ram probe (Ruakura, mark IV, B. A. O'leary Waitomo, New Zealand). Inseminated ewes returning to oestrus were monitored by the vasectomized ram 17-60 days postinsemination.

Fertility measurement: Conception rates were determined at this period, based on non-return to oestrus following two inseminations. Pregnancy rates were determined by abdominal palpation (ballotement) 90-110 day post-insemination. Some other parameters were also investigated and were calculated according to Charring *et al.* (1992), these include:-

- Fertility rate: (Lambed ewes /ewes inseminated) × 100
- Fecondity rate: (Lambs born alive /ewes inseminated) ×100
- Prolificacy rate: (Lambs born alive /Lambed ewes) ×100

- Lambing rate: (Lambed ewes /Pregnant ewes) × 100
- Litter size: number of lambs per ewe

Statistical analysis: Analysis of variance for factorial randomized complete block design was used (Gomez and Gomez, 1984). Dunkan's New Multiple Range Test (DNMRT) was applied to determine degree of significance between treatment means.

RESULTS

Oestrous parameters: Eight (80%) ewes responded by showing oestrus after the first injection of Luprostiol (Treatment B). However, nine (90%) ewes responded to the second injection (Table 1). One of the two ewes which failed to respond to the first dose of Luprostiol f failed also to respond to the second treatment. Oestrus was observed in four ewes out of nine (44.4%) following removal of the intra vaginal sponges (Treatment C) on day 13 of insertion. In this group a nulliparous ewe lost its sponge befor removal. Likewise, five ewes out of nine (55.5%) showed oestrous signs following termination of FGA and PMSG treatment (Treatment D).

Table 1: Oestrous response in Desert ewes, mean interval from treatment to onset of oestrus and mean duration of oestrous period (h) following synchronization with Luprostiol (B), FGA sponges (C), FGA sponges + PMSG (D)

	Control		Treatments	
Parameters	A	B	C	D
No of ewes treated	10	10	9	9
Number responded (%)	-	9 (90%)^{a}	4 (44.4%) ^{b}	5 (55.5%)^{b}
Mean interval from treatment end to onset of oestrus (h)	-	54.0±5.2	53.5±6.4	49.4^{a}±1.90
Mean duration of oestrous period (h)	30.4±2.40	43.4±5.5^{a}	27.2±3.8 ^{b}	27.2 ^{b}±3.0

a.b Values in rows denoted by different superscipts differ significatly.

Table 2: Non-Return rates, pregnancy rates, Oestrus during pregnancy and Lambing rates in Desert ewes following synchronization with Luprostiol (B), FGA (C), FGA + PMSG (D)

	Control		Treatments	
Parameters	A	B	C	D
No of ewes inseminated	10	10	9	9
Non-return rate after first insemination (%)	7(70%)^{a}	5(50%)^{b}	7(77.8%)^{a}	9 (100%)^{a}
Non-return rate after	-	4(40%)	2(22.2%)	-
Overall non-return rate second insemination (%)	7(70%)^{c}	9(90%)^{b}	9(100%)^{a}	9(100%)^{a}
Pregnancy rates after 90-110 days, (ballotement) %	5(50%)^{c}	8(80%)^{b}	9(100%)^{a}	9(100%)
Fertilety rate (%)	50 ^{c}	80 ^{b}	100 ^{a}	100 ^{a}
Fecondity rate (%)	60^{c}	110^{b}	120^{a}	120^{a}
Prolificacy rate (%)	120 ^{b}	137.5 ^{a}	133.3 ^{a}	133.3 ^{a}
Lambing rate (%)	100	100	100	100
Litter size	1.2	1.1	1.33	1.33

a.b Values in rows denoted by different superscipts differ significatly

A vaginal sponge was also lost from a nulliparous ewe in this group. The response of ewes to treatment B was significantly ($p<0.05$) higer than to treatments C and D. All of the ewes (n:10) in the control group D showed spontaneous oestrus. The mean intervals from the end of treatments to first signs of oestrus were not statistically different between treatment groups. Nevertheless, there were significant ($p<0.05$) difference between treatment groups in the duration of the induced oestrous periods. The control (A) and treatment B showed longer durations than treatments C and D.

Fertility parameters: The non-return rates to oestrus following first A.I. in the treatment groups and handmating in the control (group A) were significantly ($p<0.05$) different. The highest value being secored by treatment D and the lowest by treatment B. These differences were bridged by the second A.I. and the overall non - return rates were not significantly different in the treatment groups. However, the value showed by the control group (A) remained unchanged (Table2).

The pregnancy rates from 90-110 days following A.I. Were not significantly ($p>0.05$) different in the treatment groups C and D, but lower values were shown by treatment group B followed by the control A (Table2). Fertility and fecundity rates (%) were highest in treatments C and D followed by treatment B, whereas the prolificacy rate (%) was highest in treatment B followed by treatments C and D.

The lambing rate was shown to be 100% in all treatment groups, while the Litter size was higher with treatment C and D (1.33) than treatment B (1.1) and the control group (1.2).

DISCUSSION

The three treatments used in this study were found to induce oestrus in Sudanese Desert ewes. The oestrous response obtained with treatment B (Luprostiol) was significantly higher than those recorded for FAG alone or with PMSG. This could be attributed to the rapid fall of plasma progesterone level (Acritopoulou *et al*., 1977) accompanied by an inerease in 17-β oestradiol (Maracek *et al*., 1989) which usually follow treatment with $PGF_2\alpha$ and its analogues. The oestrous response to treatment B was close to that reported by Fukui and Roberts (1977) and Wolf *et al*. (1990). The dose of Luprostiol used in this study was 3.75mg as suggested by Evans and Maxwell (1987).

The incidence of oestrus was not significantly ($p>0.05$) different when FGA with or without PMSG was used. The percentage of ewes responded to FGA alone

was lower than that reported by Kliniskii and Zhirkov (1977) and Ainsworth and Wolynetz (1982). However, FGA with PMSG resulted in oestrus response less than recorded by Robinson *et al.* (1987). These low results questioned the efficiency of the vasectomized ram in heat detection, since all ewes in treatment C and D showed swollen hyperaemic vulva clear copious mucous discharge and the os cervix was open at the time of insemination. Similar finding was observed by Maxwell (1986) where 34.4% of ewes synchronized as in treatment D was not detected in oestrus by a vasectomized ram. Absence of behavioural oestrus despite the presence of vaginal changes could be attributable to the high sensitivity of the female reproductive tract to rising oestrogen level compared to the neural tissues (Llewelyn *et al.*, 1993).

The mean intervals from termination of treatments to onset of oestrus were not significantly ($p>0.05$) different between the treatment groups. This agreed withfinding of Greyling *et al.* (1980) in ewes treated with progestagen sponges and cloprostenol and with Wolf *et al.* (1990) who used double injection of cloprostenol. However, a little longer period was observed by Mathur *et al.* (1987), while shortor periods were reported by Hanrahan and Quirke (1975) in Finnish sheep breeds (Lewis *et al.,* 1974; Boland *et al.,* 1983) in USA and Acritopoulou *et al.* (1977) in Britain. The relatively shorter interval in treatment D compared to Treatment C was due to the injection of PMSG which is known to shorten the interval from progestagen withdrawal to onset of oesrus. This results conforms well with those recorded by Kuksova and Stroumova (1972) and Robinson *et al.* (1987).

The prostaglandin $F_{2\alpha}$ analogue (Treatment B) resulted in longer mean duration of oestrus compared to the other treatments. Induced luteolysis produces increased amount of oestrogen in the blood (Smith *et al.*, 1977; Bindon *et al.*, 1979; Quirke *et al.* 1981) and this effect could explain the prolongation in the duration of oestrus.

Conception rates based on non-returning to oestrus was the lowest in ewes treated with $PGF_{2\alpha}$ following first insemination. This result could be explained in view of the interference of $PGF_{2\alpha}$ with efficiency of sperm transport through the cervix (Hawk and Conley, 1975). However an overall higher non-return rate was gained after the second insemination in this group. This confirms the finding of Mathur *et al.* (1987). Ewes treated with progesterone showed slightly low non-return rate after the first insemination. This effect could be attributed to an abnormal steroid balance on the transport and survival of spermatozoa (Quinlivan and Robinson, 1969, Langford *et al.*, 1982; Roberts, 1986). The overall non-return rate recorded for this group was close to that reported by Alsayed (1996) in Desert ewes. The highest non-return rate encountered in this study was scored by the ewes treated with progesterone plus PMSG following the first insemination.

This goes in line with many reports where PMSG was stated to synchronize ovulation (Boshott *et al.*, 1973, Whyman *et al.*, 1979; Romano *et al.*, 1996; Karagianni dis *et al.*, 2001; Barret *et al.*, 2004). Ewes in the control group, on the other hand, showed comparable results to those of Berg and Anderesen (1989).

Pregnancy rate obtained after 90-110 days post insemination, through abdominal ballottement was lower in ewes treated with $PGF_{2\alpha}$ than in those treated with progesterone alone or with PMSG. These findings agree with that of Greyling *et al.* (1980). The differences in pregnancy rates following the different hormonal treatments could be due to differences in the timing of the LH surges and the consequent ovulation (Cumming *et al.*, 1973). Ovulation in ewes treated with $PGF_{2\alpha}$ occurs 70 h after treatment (Acrito poulou and Haresign, 1980). Thus, insemination at a predetermined time (52 h after treatment). In $PGF_{2\alpha}$ treated ewes resulted in low pregnancy rate, possibly due to aging of the sperms in vivo. This was confirmed by Fukui and Roberts (1977) who obtained higher pregnancy rates through insemination at 70 and 78 h after $PGF_{2\alpha}$ treatment. In contrast, LH peak in ewes treated with progestagen sponges occurs at 36 h after sponge removal (Lewis *et al.*, 1974) and ovulation, regularly, follows after 24 h from the occurrence of the LH peak (Cumming *et al.*, 1973). Moreover, PMSG was known to shorten the interval from sponge removal to ovulation (Killeen and More, 1970; Boshoff *et al.*, 1973; Colas, 1975a; Evans and Robinson, 1980).

The pregnancy rates, observed in the current study, with $PGF_{2\alpha}$ was higher than those reported by Fukui and Roberts (1977) and Haresig and Acritopoulou (1978), where as progesterone recodoed comparable results to those of Lewis and Inskeep (1973), but higher then these reported by Rommel (1978).

Using oral megesterol acetate. However, treatment with progesterone PMSG gave higher pregnancy rate when compared with those of Langford *et al.* (1982).

Differences between the non-return rate and the pregnancy rates in the control group (D) and in the $PGF_{2\alpha}$ treated group (B) were due to occurrence of two cases of pseudopregnancy in the first one and one case in the other. This confirms the findings of Heap *et al.* (1981).

However, other infertility problems that lead to anoestrus such as persistent corpus luteun or early embryonie mortality could not be excluded in this study.

Fertility, fecundity and prolificacy rates have indicated for better reproductive performance with treatment C and D compared to treatment A and B. The lambing percentage with $PGF_{2\alpha}$ was lower than that observed by Wolf *et al.* (1990) with cloprostenol. Never the less, the lambing percentage recorded for progesterone is consistent with that obtained by Rommel (1978), but lower than that reported by Lewis and Inskeep (1973). The addition of $PGF_{2\alpha}$ to progesterone treatment, in this study , resulted in lambing percentage that conforms well with that of Kuksova and Stroumova (1972), but the prolificacy was lower than that noted by Hackett *et al.* (1982).

CONCLUSION

This study confirms that all the treatment employed can induce and synchronize oestrus in Sudanese Desert ewes, with luprostiol showing more efficiency in oestrous response and resulting in longer oestrous duration ($p<0.05$) compared to the other treatments. Treatment with progestagen significantly higher pregnancy rate to first insemination, than Luprostiol or progestagen alone. However, the overall pregnancy rates obtained with progesterone sponge treatments with or without PMSG were not significantly different, but they superceded that produced by Luprostiol.

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