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Research article

REPRODUCTIVE RESPONSE OF SAVANNA BROWN DOES TO EXTRACTS OF MISTLETOE

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Abstract

Thirty six nulliparous SB does aged 4-6 months were used to study the effect of mistletoe extract and clomid® on the reproductive response of Savanna Brown (SB) does.. They were randomly assigned into three groups of twelve does; each group treated with mistletoe, clomid® while the third group served as control. They were further subdivided into three subgroups of split (250 mg /kg mistletoe extract and 0.35 mg/kg of clomid® administered twice in a day), full (500 mg/kg of mistletoe extract and 0.7 mg/kg of clomid® administered once) and double dose of 1000 mg/kg of mistletoe extract and 1.4 mg /kg of clomid® administered once. The animals were not allowed to graze and were housed with two healthy intact bucks. All does (100 %) in the mistletoe treated group, 25 % and 33.33% of does in the clomid® and control groups conceived and carried their pregnancy to term. The birth weight of kids were better (1.90 kg) in the does drenched with mistletoe compared with those on clomid® (1.80 kg) and control(1.20 kg)., however, the hormone profile was better in does on both split (250 mg/kg) and normal (500 mg/kg) doses of mistletoe extract particularly in the 4^{th} – 20^{th} week of the study . It was concluded that though both mistletoe and clomid® were effective in enhancing reproductive activities in SB dose, Split doses should be administered for optimum performance.

Key words: clomid®, mistletoe, savanna brown does, reproduction.

Introduction

Effort has been made in the past to improve goat reproductive efficiency through improved nutrition (Fasanya *et al.*, 1992 a, b) and use of exogenous hormones like prostaglandin F_2 alpha (Alemede and Fasanya, 1999). However, the use of clomiphene citrate (clomid®) and herbs to improve fertility in animals is still uncommon. For thousand of years, knowledge of the herbs and wild plant that could increase fertility was the secret of the European village wise women (Susun, 1999) but following the holocausts against this women and their virtual extermination, this knowledge virtually disappeared and many people erroneously believed that primitive people had no means of controlling the likelihood of pregnancy. Many common plants can be used to influence fertility. These include the red clover (*Trifolium pratense*), life root (*Senecio aureus*), wild carrot (*Daucus carota*) and wild yam (*Dioscorea villosa*). Others are mistletoe (*Phragmanthera nigritana*) and tropical Nestle weed (*Fleurya aestuans*), some of which grow in the wild while others are easy to cultivate (Akobundu and Agykwa, 1998; Susun, 1999).

Mistletoe is a unique semi-parasitic plant that has extensive historical use as a medicine and also as a ubiquitous religious and poetic symbol, it has also been said to offer a cure to all ailments (Tibor, 1986; Osadebe and Uzochukwu, 2006). As a first step to it's full introduction and usage in all species of livestock, this study was designed to compare the effect of clomiphene citrate (clomid®) and mistletoe (*Phragmanthera nigritana*) extract on reproductive performance of Savanna Brown goat, thereby attempting to proffer natural fertility herbs solution to the small ruminant farmers in particular and livestock farmers in general.

Materials and method

Thirty six (36) nulliparous Savanna Brown does, aged 4-6 months old were used in this study. The animals were allowed a pre-treatment period of two weeks to enable them acclimatize. During this period, they were checked to ensure that they were not pregnant by dipping pregnancy test strip in urine samples collected from the does and properly labeling the animals for identification. They were also treated against endo- and ecto- parasite using a bolus of albendazole per 50 kg body weight orally and 1 % ivomectin injection at a recommended dose volume of 0.5 ml per 25 kg body weight subcutaneously, respectively. They were also injected intra-muscularly with long-acting Oxy- tetracycline broad spectrum antibiotics at a dose of 1 ml per 10 kg body weight in a single dose as a precautionary measure against bacterial infections.

The animals were managed under the intensive system and were fed maize bran, groundnut hay, salt lick and concentrate *ad libitum*. Ample amount of drinking water was provided. They were randomly assigned into three groups of mistletoe, clomid® and control (Water) and were further subdivided into three subgroups of split (250 mg /kg of mistletoe extract and 0.35 mg /kg clomid® administered twice in a day), full (500 mg /kg of mistletoe extract and 0.7 mg /kg of clomid® administered once) and double dose of 1000 mg /kg of mistletoe extract and 1.4 mg /kg clomid® each with four animals per group. The does were housed alongside two intact and healthy bucks for oestrus detection and subsequent mating and were observed daily for signs of oestrus, copulation and pregnancy. The type of birth, body weight of dam and kids were recorded.

About 5 mls of blood were obtained by vena puncture technique from each animal two days before treatment, then monthly thereafter. The blood sample was emptied into plain sterile bottles without EDTA (ethylene diamine tetra-acetic acid), cooled immediately in an ice chest and transferred to the laboratory where the serum was separated by centrifugations at 4000 rpm and stored for hormonal assay (progesterone and follicle stimulating hormone determination).

Result

Significant (P≤0.05) differences were observed in the gestation length, percentage of dam that kidded, percentage of kids born and birth weight of kids particularly in those administered split doses of either mistletoe or clomid®. 100 % of does administered with 250 mg/kg (split dosages) of mistletoe extract kidded compared to 25 % and 33.33 % in the group that had split doses of clomid® and the control, respectively (Table 1). 125 % of kids were born (due to the presence of twins) with a significantly higher mean birth weight of 1.93 kg in the 250 mg/kg mistletoe treated group compared with the does treated with split doses of clomid® (1.80 kg) and control (1.20 kg). Gestation length was significantly (P ≤ 0.05) shorter in the control group (145days) while more female were produced in the mistletoe treated groups. Generally, better reproductive response were observed in the groups on split dose of mistletoe while the worst results were obtained in the group that received double doses of either mistletoe or clomid®. Administration of double doses of mistletoe extract resulted in abortion in the only animal that conceived in this group

A summary of follicle stimulating hormone and progesterone changes from 0-20 weeks for does on mistletoe and clomid ® is presented in Tables 2 and 3. The follicle stimulating hormone (FSH) values for does on mistletoe extract were significantly (P<0.05) higher at day O, week 12 (split dose) and weeks 12 and 20 (normal dose) than clomid® and control. Values for FSH in goats treated with clomid® was significantly(P<0.05) higher at weeks 4, 16 and 20 (split dose), weeks 12, 16 and 20 (normal dose) and day 0 (double dose) than does on mistletoe and control. Progesterone level was higher on the 4th week of the experiment for does administered with various dosages.

Table 1: The reproductive parameters of the Savanna Brown (S.B) does administered with various dosages of mistletoe (*phragmantera nigritana*) and Clomiphene citrate (Clomid®)

	Misteltoe ±SEM	**Clomid ® ±SEM**	**Control ±SEM**	**LS**
Split Does				
Gestation length (days)	150.00^{a}±0.01	150.00^{a}±0.01	145.00^{b}±0.05	*
Dam that kidded (%)	100.00^{a}±0.00	25.00^{b}±0.47	33.33^{b}±0.18	*
Kids born (%)	125.00^{a}±0.25	25.00^{b}±0.25	33.33^{b}±0.08	*
Mean Birth weight (kg)	1.93^{a}±0.13	1.80^{b}±0.13	1.20^{b}±0.14	*
Type of birth (%)				
Single	75.00±0.25	25.00±0.25	33.33± 1.08	NS
Twin	25.00±0.25	00.00±0.00	0.00±0.00	NS
Sex (%)				
Male	50.00±0.87	25.00±0.50	33.33±1.08	NS
Female	50.00^{a}±0.87	0.00±^{b}0.00	0.00^{b}±0.00	*

Normal Dose				
Gestation length (days)	150.00±0.00	150.00±0.00	150.00±0.00	NS
Dam that kidded (%)	50.00±0.87	50.00±0.87	33.33± 0.87	NS
Kids born (%)	50.00±0.87	75.00±0.50	33.33±1.08	NS
Mean Birth weight (kg)	1.70±0.10	1.70±0.10	1.20±0.10	NS
Type of birth (%)				
Single	50.00±0.87	25.00±0.50	33.33±1.08	NS
Twin	0.00±0.00	25.00±25.00	0.00±0.00	NS
Sex (%)				
Male	25.00±0.50	50.00±0.87	33.33±1.08	NS
Female	25.00±0.50	0.00±0.00	0.00±0.00	NS
Double Dose				
Gestation length (days)	60.00±00.00**	150.00[a]±0.00	145[a].00±0.00	*
Dam that kidded (%)	0.00[a]±0.00**	25.00±0.50	33.33±1.08	NS
Kids born (%)	25.00±21.00**	25.00±21.00	33.33±1.08	NS
Mean Birth weight (kg)	0.50±0.10**	1.70[a]±0.00	1.20[b]±0.14	*
Type of birth (%)				
Single	25.00±1.00**	25.00±1.00	33.33±1.08	NS
Twin	0.00±0.00**	0.00±0.00	0.00±0.00	-
Sex (%)				
Male	25.00±21.00**	25.00±21.00	33.33±21.08	NS
Female	0.00±0.00**	0.00±0.00	0.00±0.00	-

[abc]: Mean values on the same row with different Superscript are significantly different(P<0.05)
LS: Level of significance *: Significant different(P<0.05) NS: Not significant(P<0.05)
** Abortion: Parameters were measure based on the expelled foetus.

Table 2: Follicle Stimulating Hormone (FSH) from 0-20 weeks of administering mistletoe and Clomid ® to Savanna Brown goats.

	Mistletoe	**Clomid®**	**Control**	**±SEM**	**LS**
Split dose					
2 days b/4	4.20	6.50	5.82	0.68	NS
Day 0	5.80[a]	5.10[ab]	4.13[b]	0.29	*
Day 2	3.95	8.50	6.48	0.96	NS
Day 4	4.06	7.53	4.93	0.89	NS
Week 4	2.00[b]	5.00[a]	4.47[a]	0.57	*
Week 8	2.74[b]	0.00[c]	6.00[a]	0.95	*
Week 12	4.59[a]	0.00[b]	0.00[b]	0.97	*
Week 16	3.27[b]	8.00[a]	0.00[c]	1.24	*
Week 20	4.40[b]	5.80[a]	0.00[c]	0.68	*
Normal dose					
2 days b/4	3.28	3.78	5.82	0.58	NS
Day 0	3.55	3.27	4.13	0.26	NS
Day 2	2.87	4.50	6.48	0.81	NS
Day 4	3.71	4.43	4. 93	0.78	NS
Week 4	2.26	3.15	4.47	0.49	NS
Week 8	2.10[b]	3.80[ab]	6.00[a]	0.66	*
Week 12	2.26[a]	3.40[a]	0.00[b]	0.59	*
Week 16	2.13[ab]	3.15[a]	0.00[c]	0.56	*
Week 20	2.90[a]	3.00[a]	0.00[b]	0.04	*
Double dose					
2 days b/4	5.10	6.60	5.82	1.27	NS
Day 0	5.00[b]	10.00[a]	4.13[b]	0.93	*
Day 2	8.45	8.30	6.48	0.73	NS
Day 4	4.47	6.27	4.93	1.00	NS
Week 4	6.73	5.00	4.47	1.04	NS
Week 8	3.33	4.00	6.00	0.65	NS
Week 12	3.33	0.00	0.00	0.73	NS
Week 16	0.00	0.00	0.00	0.00	NA
Week 20	0.00	0.00	0.00	0.00	NA

[abc]: Mean Values with different superscripts on the same row are significantly different (P<0.05) SEM: Standard Error of Mean LS: Levels of significance *: Significant different *P<0.05) NS: Not significant (P<0.05) NA: Not Available.

Table 3: Progesterone (P4) changes 0- 20 weeks after administration of various dosages of mistletoe (*phragmanthera nigritana)* to Savanna Brown (SB) does

Duration	**Treatment**				
	250mg/kg	**Mistletoe 500mg/kg**	**1000 mg/kg**	**Control**	**LS**
2days before	0.49±0.00[b]	1.93±1.07[a]	0.00±0.00[c]	0.68±0.00[b]	*
Day 0	9.22±0.30[b]	0.50±0.30[b]	0.00 ±0.00[c]	0.00±0.00[c]	*
Day 2	0.00±0.00[b]	2.19±0.82[a]	0.00±0.00[b]	0.00±0.00[b]	*
Day 4	0.44±0.31[ab]	0.52±0.00[a]	0.19±0.00[b]	0.00±0.00[c]	*
Week 4	19.39±1.18[a]	0.79±0.24[c]	0.00±0.00[d]	2.70±0.00[b]	*
Week 8	5.50±0.50[a]	3.75±0.20[a]	0.00±0.00[b]	0.00±0.00[b]	*
Week 12	2.09±1.51[a]	0.54±0.06[b]	0.00±0.00[c]	0.00±0.00[c]	*
Week 16	2.75±1.85[a]	1.00±0.00[b]	0.00±0.00[c]	0.00±0.00[c]	*
Week 20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	NA

[abcd]: Mean Values with different superscripts on the same row are significantly different (P<0.05). LS: Levels of significance *: Significant different *P<0.05) NA: Not Available.

Discussion

Both mistletoe extract and clomid ® initiated reactions in the does which caused the animal to abort and start an entirely new reproductive cycle which gave rise to the high levels of FSH observed. However, Adashi et al.(1981) suggested that clomid® and its enclomid isomer, unlike taximofen, exert a direct oestrogenic rather than antioestrogenic effect on cultured pituitary cells by enhancing the gonadotropins releasing hormones (GnRH) to stimulate the release of gonadotropins. Huang and miller (1983), reported that 17 beta oestradiol alters gonadotropin secretions in ovine pituitary cell cultures by argumenting the Luteotrophic response to LHRH and inhibiting the basal secretion of FSH.

The higher progesterone level of 19.39 mg/ml (Table 3) showed that many animals were pregnant at that point and this was reflective in the 100 % dam that Kidded (Table 1). This was in agreement with the report that mistletoe being a strong luteotropic agent stimulate the secretion of progesterone (Herb- Mistletoe, 2011). According to Meylan, (2008), a sustained “plateau” of 12-14 days (following ovulation) of basal body temperature is indicative of a good progesterone secretion from the corpus luteum of 4 mg/ml in the peripheral blood.

The significant (P<0.05) difference observed in the number of dam that kidded being 100 % in does administered with 250 mg/kg split dosages of mistletoe extract (Table 1), the 125 % of kids born (due to the presence of twins), the mean birth weight of 1.93 kg which is the highest compared with the does treated with clomid® and the control agrees with Al-amoudi, (2012) who reported that treating animals with mistletoe extract doses of 50 and 100 mg/ kg body weight caused elevation in serum cholesterol and tryglycerides level as well as significant changes in the total protein levels leading to increase in body weight.

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