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Short communication

Oestrus synchronization and superovulation in the red Sokoto doe (RSD) in Sokoto, Nigeria

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ABSTRACT

A dose determination study for the prostaglandin analogue cloprostenol and the gonadotrophic hormones; equine chronic Gonadotrophin (ECG) and human chorionic Gonadotrophin (HCG) was conducted in the Red sokoto doe (RSD). 24 RSD were divided into 4 groups of 6 animals. Each group was synchronized with cloprostenol, a two dose, 11 days a part regimen was used; the first group is the untreated control receiving 0µg of the cloprostenol, the second, third and fourth groups were given 50µg, 75µg and 100µg of the drug respectively. No oestrus response was observed in the control. All the animals in group 2, 3 and 4 were seen to come on heat between the 48th and 96th hours after the second injection, all the Does in these groups manifested heat (100% response). In the superovulation experiments; 12 doses were divided into 3 groups of 4 animals each. Group I was given 1000i.u. of eCG and 25i.u. HCG. Group II was given 1000 i.u. eCG only, while the third group (III) was given 250 i.u. HCG only, all following cloprostenol treatment, the fourth group (IV) was the untreated control. Superovulatory response was estimated from corpora lutea count on the ovaries. A total of 59, 40, 15 ovulations were recorded for group I, II and III respectively. This study demonstrates that 50µg, 75µg and 100µg of cloprostenol are effective doses for the synchronization of oestrus in

the RSD. It has also demonstrated that the combination of eCG and HCG produce quantitatively more ovulations than either eCG or HCG alone. These findings above were discussed in the paper.

1. Introduction

Studies in controlled reproduction or manipulation of reproduction in goats in Nigeria started with studies on the nature of oestrus and oestreous cycles (Kirkpatrick and Akindele, 1974., Orji, 1985). Molokwu and Igono, 1978 studied the reproductive performance and reproductive pattern in the brown of Nigerian Savanna Zone. Not until recently, most of the reproduction researches in Nigerian goats were reported on the West African Dwarf goats (Molokwu and Igono, 1978., Orji and Steinbach, 1979a., Orji, 1985).

Oestrus synchronization and related studies unlike superovulation and embryo transfer have received considerable research attention probably due to the fact that synchronization is an essential part of the artificial insemination protocol. Oestrous synchronization, superovulation and embryo are techniques used for the manipulation and control of reproductive processes. This study examines some aspects of oestrus synchronization and superovulation in the RSD.

2. Materials and methods

The prostaglandin analogue was given to the animals at a dose rate of 0µg, 50µg and 100µg. The animals were divided into 4 groups of six animals each. The animals were given the dose on day one and they were also given a second dose eleven days apart. The animals were observed for heat from 24 hours after the second cloprostenol injection. Observations were made for (1) the time from the second cloprostenol injection to onset of estrus. (2) The duration of the heat period and (3) the overall response of does to the synchronization agent.

Table 1
The synchronization regime of cloprostenol (2 doses; 11 days apart regime).

Dose	1st dose Cloprostenol(µg) Day 0	2nd dose Cloprostenol (µg) Day 11
Group 1(n = 6)	50	50
Group 2 (n = 6)	75	75
Group 3 (n = 6)	100	100
Group 4 (n = 6)	0	0

2.1. Superovulation

Twelve (12) RSDs, between 2-3 years of age were used in these trials. The does were divided into 3 groups of 4 does each. Pregnant Mare Serum Gonadotrohin (PMSG)/Equine chorionic Gonadotrophin (eCG) marketed as Folligon®. Each pack contains five vials of the solute with a solvent for dilution (the solvent is sterile phosphate buffered water for the injection, each vial containing 1000 i.u. Ph. Eur. Manufactured by invervet UK Ltd. Science Park, Milton road, Cambridge CB4 4FP. The hormone below was used for superovulation as described in experiments.

(i) eCG
(ii) Human Chorionic Gonadotrophin alone
(iii) eCG in combination with Human Chorionic

Super ovulation trials on Does using eCG (folligon®) and Human chorionic gonadotrophin (profasi) singly or in combination.

Table 2
Comparative superovulatory treatment.

S/NO	Treatment Group N=4	Hormone Regimen	Dose (i.u.)
1	GROUP 1	eCG	1000
2	GROUP 2	HCG	500
3	GROUP 3	eCG+HCG	500+250

In the superovulation experiment, 12 RSDs were put into 3 groups of 4 animals each. Three superovulation regiments used were as shown on table 2. Group 1, received 1000 i.u. eCG alone and group 2 received HCG alone, group 3 received eCG and HCG combination. All the animals in the three superovulation regimes were synchronized using 75µg of cloprostenol (given in the two doses, 11 days apart regimen model). The animals were given the particular superovulatory regimen 24 hours to the second cloprostenol injection 48 hours after the cloprostenol injection. Three days post breeding; the animals were prepared for surgery to evaluate the paralumbar or flank surgical approach. Estimation of the superovulatory responses was by corpora lutea count on the ovaries.

3. Results

3.1. Induction of oestrus using cloprostenol

Table 3 presents the response to cloprostenol induction No estrus response was observed in the control group that was given 0µg of the cloprostenol. In the second group given 50µg of the drug, animals had not shown oestrus by 24 hours after the second dose of the injection. Two (2) of the goats were on heat by 48 hours, while the other exhibited estrus by 72 hours. By 96 hours after the second injection all the does in this group given 75µg cloprostenol, three animals were on heat by 48 hours, 5 by 72 hours and all the six had come into heat by 96^{th} hours after injection. In the fourth and final group received 100µg of the hormone 3 were on heat, by 48 hours after the second injection and by the 72^{nd} hour all the goats in this group have manifested estrus.

Group I treated with 1000 i.u. eCG produced a total of forty ovulations (40, Sixteen on the right and twenty four on the left. Group II however yielded fifteen ovulations (seven on the right and eight on the left). However the third group treated. With a combination of eCG and HCG provided fifty nine ovulations (twenty one on the right and thirty seven on the left). From table 4; Doe number one produced thirteen ovulations (five on the right and eight on the left. Eight embryos were recovered after flushing. In the second doe sixteen ovulations were recorded seven on the right and nine on the left**).**nine embryos were also recovered. Also in the third doe eleven ovulations were observed, four on the right and seven on the left, while the fourth doe was seen to produce fourteen ovulations; four on the right and ten on the left. A total of eight embryos were recovered as a whole the does put together produced seventy three ovulations (twenty tree ovulations on the right and fourty seven on the left) forty tree embryos were recovered amounting to fifty eight point nine percent (58.9%) embryo recovery.

4. Discussion

Estrumate and most of the different commercial preparations of cloprostenol are prepared for use in large animals i.e. cattle and horses. However, there is equally a pressing need to use these preparations for the control of reproduction in the small ruminates (sheep and goats) classified as minor livestock species (Wildeus, 1999). This therefore brings about the need for what is called the “extra-label” use of these drugs, due to non-availability of pharmaceuticals for ES. Pharmaceuticals for ES are not readily available and most applications currently used required the extra-label application of products developed for the major livestock species i.e. cattle, swine, equines (Wildeus, 1999). As a result of extra-label use, there are no available standardized protocols and doses and a variety of synchronization protocols and product combinations have been described (Romano, 1998a; Greyling and Van Niekerk, 1991; Baril, *et al.,* 1992; Freitas *et al.,* 1996).

Table 3
Oestrus synchronization response due to cloprostenol (estrumate) induction in the RSD.

Dose level	Hour After 2nd Injection	No. of Does	% Response on heat	Cumulative Response
0µg	24	-	0	0
Control	48	-	0	
(n=6)	72	-	0	
Group I	96	-	0	
50µg	24	-	0	100
(n=6)	48	2	33.3	
Group II	72	2	33.3	
	96	6	100	
75µg	24	-	0	100
(n=6)	48	3	50	
Group III	72	5	83.3	
	96	6	100	
100µg	24	-	0	100
(n=6)	48	3	50	
Group IV	72	6	100	
	96	6	100	

Table 4
Estimation of super ovulation response from ovarian corpora lutea in RSD in the superovulation experiments.

Grouping N=4	hormone regime	dose (i.u.)	Right ovary	Left ovary	Total ovulations
GROUP I	eCG	100	16	24	40
GROUP II	HCG	500	7	8	15
GROUP III	eCG	1000	21	37	59
	HCG	500			

Oestrous synchronization studies in Nigeria are very few. They are much fewer in the caprine species Synchronization studies in the RSD are similarly very scarce. (Ogunbiyi et al. 1980; Akusu and Egbunike 1984).in the present study (Table 3) experiments to estimate the percentage oestroues response of the RSD and the time interval between 2nd injections to estrus at different doses of cloprostenol was used. In the control group no animal showed signs of heat during the study period, while Does in groups II, II and IV were seen to manifest heat within 48 hours of the second dose of the injection of the various test doses. All the animals in groups II, III and IV were seen to manifest profound standing heat by the 69th hour. A 100% response was recorded for does in Group II (50µg) III (75µg) and IV (100µg) by the 95th hour after the second hormone injection.

Findings in this study are in agreement with reports on daily goats and Nubian goats in which Nuti et al., (1992) observed no difference in estrus response and duration of estrus in goats treated with cloprostenol (125µg) on day 6 and 12 of the oestrous cycle. Different doses of cloprostenol have been used successfully to induce estrus ranging from 50µg (Baril et al., 1998; 62.5µg (Greyling and Van Niekerk 1991); 100µg (Baril, 1992). These dosages were used in combination with other gonadotrophin co-treatment. It is noteworthy that in the present study only cloprostenol was used in combination with the presence of a vasectomosed buck .a 100% estrus response was obtained in all the treatment groups without any gonadotrophin co-treatment. It is very possible to obtain good response with the use of doses lower than the 50µg used in these trials. The use of gonadotrophin co-treatment with reduced concentration of the synchronizing agent was shown to produce positive synchronization responses. Furthermore, Romano,(1998) observed no difference in the onset and duration of estrus in Nubian goats given 62.5 and 125µg cloprostenol injected once between day 8 and 15 of the oestrous cycle. Similarly, no difference

was observed between 50µg, 75µg and 100µg of cloprostenol in percentage estrus response and onset of estrus in the RSD (Table 3).

In cyclic Nubian goats a double injection of PGF induced estrus in a higher ($p<.05$) percentage of does (100%) than in sponge treated does (70%)(Ahmed et al., 1998). This finding closely relate with findings in this study (Table 4.0.6) of 100% in double injection 11 days apart from cloprostenol and 62.5% in sponge treated does. Back *et al*. (1993) compared the double injection system (125µg cloprostenol 11days apart) with a single injection and a combination of short-term progestagen treatment (MAP 5d) with a cloprostenol injection at sponge removal in clum Forest ewes. They found a 100% estrus response in the double injection on MAP-PGF combination treatment, whereas estrus response was reduced in the single injection group (52.9%) $p<.05$). Our findings using the double injection regime agree with above mentioned double injection regime.

4.1. Super ovulation in the RSD

There is virtually little research work on super ovulation in the RSD's in Nigeria; some few works have been reported in other small ruminants i.e. the yankasa sheep (Oyedipe *et al.,* 1991). Moreover, there is no published report to our knowledge on super ovulation in any of the local breed of goats in Nigeria. Published reports on super ovulation in the red Sokoto doe are similarly hard to come by. However, reports relating to super ovulation of the sheep are numerous (Radford *et al.,* 1984; Ryan *et al.,* 1984). Super ovulation is a necessary part of the embryo transfer and associated techniques and in genetic engineering, so will interest in super ovulation increase. Some few reports relating to super ovulation of goats in other parts of world however are available (Gonzalez Bulnes *et al*., 2004).

HCG has a luteinising hormone like effect and there are a number of reports suggesting a possible superovulatory activity for this hoemone (Braden, *et al.,* 1960).in experiment VII (table 4) it could be seen that a mean of 4, 5 and 6 ovulations of treatment groups 1, 2, and 3 treatment groups was recorded, a mean of 5.33 ovulations for all the groups was recorded. Nevertheless, in an experiment using Human chorionic Gonadotrophin in sheep, Radford *et al.,* (1984) observed significant increases in ovulation rate 1.68 – 2.04 as against 1.12 in control. Finding in this study tends to agree with those of Redford et al., (1984) who in preliminary experiments showed that, when infused after luteolysis, HCG does bring about increase in ovulation rate. However, if as mentioned above the super ovulation regime should include a luteolytic substance like cloprostenol or PGF_2 followed by HCG. Some super ovulatory response could be expected. This perhaps explained the increases in super ovulation response due to this hormone in this study. Increases in superovulatory response were obtained most probably, because we used 100µg cloprostenol as our synchronizing agents. As such our findings closely agree with those of Redford *et al.,* 1984. Table 4 present super ovulation responses and embryo recovery in goats stimulated with equine chorionic Gonadotrophin. A total of 40 ovulations with average ovulations of about 13.33 for the three groups a mean ovulation rate of 5.3 and 8 were recorded for the right and left ovaries respectively.

There are significant increases in ovulations when ovulations from the control and those of the treatments are compared. Ovulation rates produced by a hormone combination of HCG and eCG produced significantly better ovulation rate than Equine chorionic Gonadotrophin alone of HCG alone. However, several workers have reported the existence of several persistent unovulated follicles which fail to rupture when higher doses of PMSG are used. Human chorionic Gonadotrophin is known to be a luteinising hormone analogue which precipitates the rupture of Graafian follicles, as such a gonadotrophin co-treatment using PMSG and HCG, provide a good superovulatory regiment. This study is in agreement with reports suggesting the use of gonadotrophin co-treatment for improved superovulatory response (Moore 1980; Ryan *et al.,* 1984). It appeared this treatment brings about significant increases in ovulation rate (Table 4). In a study of ovarian responses to PMSG/oestradiol/HCG combination in beef heifers and the use of PMSG alone 18 heifers for each group were found to produce a mean ovulation rates of 16.3 and 15.6 respectively (Gordon, 1983) Multiple ovulations were recorded in all the treatment groups. The combination of eCG and HCG gives better superovulatory response compared with eCG alone or HCG alone (a total of 58 ovulations for all the 3 groups and mean ovulation rate of 19.3 as against a total of 40ovulatiobs with a mean ovulation rate of 13.33 in the eCG treated group and a total of 15 and an average of 3 ovulations per doe. Other workers have induced Super ovulation in the sheep by the administration of pituitary extracts e.g. horse anterior pituitary extracts HAP (Moore and Shelton, 1964). A number of reports have indicated that combination hormone treatments tend to produce greater superovulatory response than the use of a single superovulatory hormone in sheep (Ryan *et al.,* 1984; Hancock and Hovell, 1961).

5. Conclusion

The study demonstrated that cloprostenol at 50µg; 7µg and 10µg could all produce a 10% estrus synchrony, with profound manifestation of heat signs. This study further observed that the latent period after the second cloprostenol injection in the 2 dose eleven days a part regimen was about 24 hours. Other observations were on RSDs treated with cloprostenol, at 0µg, 50µg, 75µg and 100µg. by the 96^{th} hour all the animals in the 50µg 75µg and 100µg treatment groups were on heat (table 3) similarly PMSG/eCG was seen to induce multiple ovulations in the RSD. Human chorionic Gonadotrophin did produce but slight increases in the superovulatory rate. However, a combination treatment using eCG and HCG produced better superovulatory results.

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