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ESTRUAL CHARACTERISTICS OF WEST AFRICAN DWARF GOAT DOES UNDER THREE ESTRUS INDUCTION PROTOCOLS

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Estrual characteristics of three estrus induction protocols were investigated using thirty multiparous West African dwarf goats with average weight 16.2±3.14 kg. Three aproned bucks were used to determine estrus. Does were assigned equally and randomly to protocols: Chloprostenol (PGG), Ovsynch (OvG), and Estradiol (EG). Goats were fed elephant grass *ad libitum*, concentrate and clean fresh water. Goats in: PGG received 125 mcg chloprostenol (double injection, i/m), ten days interval; OvG received 50 mcg Gonadorelin® on day 0, 125 mcg Chloprostenol on day 7, 50 mcg Gonadorelin® on day 9; EG received 1 mg Super Estradiol® (double injections), two days interval. Onset of estrus (OE), proportion of does in estrus (PDE), duration of estrus (DE), reproductive hormones, vaginal cytology, and vulva diameters were studied. The OE, PDE, DE for PGG, OvG and EG were 48 hour, 90%, 2.32±3.84; 0 hour, 100%, 1.62±0.28; 0 hour, 100%, 5.00±0.00, respectively. Across protocols, differences in titers for LH, FSH, and Estradiol were significant ($P<0.05$) while progesterone was not ($P>0.05$); SEC (%) for the period 24-96 hours were significant ($P<0.05$); vertical and horizontal vulva diameters (VDs) were not significant ($P>0.05$); ratios vertical:horizontal VDs i.e. 1.29:1 (PGG), 1.53:1 (OvG) and 1.37:1 (EG) differed. In conclusion, the protocols synchronized estrus in WAD does; OvG and EG had shortest OE and highest PDE; OvG had least DE; hormone titers (except progesterone) and SEC varied; changes in VDs were similar but, vertical:horizontal VDs was highest in OvG.

Key words: Estrual characteristics, WAD goat does, chloprostenol, ovsynch, estradiol.

Efficient estrus detection is a crucial task in the management of reproduction in livestock (3). This is partly because the estrual period, during which normally cycling females only accept the male, represents a relatively short proportion, compared with other stages of the sexual and/or reproductive cycle (21). Apart from this, owing to the 'multiplicative' association among the determining factors of reproduction efficiency in any breeding station, low performance in any factor is never compensated for by high performance in any, or other factors (1). For some other animals also, the inter-estrous interval is wide (e.g. bitch), implying that the gap between mating (which only occurs at estrus), pregnancies and parturition will be correspondingly wide. Every successful breeding program must therefore, among other factors, prioritize efficient estrus detection, especially in cases where females are bred via artificial insemination. One way by which research has assisted in this regard is by the discovery of various protocols of estrus induction and synchronization which are most often, components of livestock breeding programs. In each of these protocols, with or without timed artificial insemination, it is reported that heat detection always factor in higher pregnancy rates (9). Several protocols, using single agents, as well as combinations have been established in literature for the cow, leading to enhanced beef (2) and dairy (27) production. In goats, the duration of estrous cycle as well as estrus are variable, and detection of estrus is almost impossible in the absence of a buck (15). This attribute makes estrus synchronization of paramount importance in goat breeding stations. Although, goats were described as the most

prolific of all domesticated ruminants in the tropics (20), the meat goat has received only little scientific attention (7) compared to dairy goats as well as other meat-producing species and breeds. According to (5), commonest method for estrus synchronization in the goat is by treatment with a progestagen for 9-11 days, followed by administration of a luteolysin at 36 h prior to removal of the progestagen. The use of a luteolysin alone either as a single- (28) or double-dose (18, 19) protocol had been reported to be as well effective. It has also been reported that although, gonadotropin has no significant effect on onset of estrus in goats, it reduces the interval from sponge removal to luteinizing hormone (LH) surge and ovulation, as well as, improves synchrony of LH surge and ovulation (25). As interest in goat breeding, either for research or other purposes continue to grow, there will be need to conduct further studies focused on manipulating its estrous cycle to enhance better understanding, so as to maximize its reproductive potential. This study was therefore designed to compare estrual characteristics of West African Dwarf (WAD) goat does treated with Chloprostenol, a combination of Gonadorelin acetate, and Estradiol benzoate. The findings will add to existing knowledge in the area of assisted reproductive techniques with the goat doe.

MATERIALS AND METHODS

Animals and management:

Three bucks, from the University's small ruminant farm, that were recently passed as satisfactory potential breeders and thirty multiparous does, all West African dwarf, with average weight 16.2±3.14 kg, and aged between 2 and 4 years (23) were used for the study. The goats were kept under intensive management at the small ruminant unit of the department of Veterinary Surgery and Reproduction, University of Ibadan, Nigeria. They were fed *ad libitum* with elephant grass and concentrate, under unrestrained access to clean fresh water. The does were equally and randomly grouped into three Experiments i.e. 1, 2 and 3. The bucks were kept individually in separate pens, apart from those for the does.

Experimental Procedures:

Experiment 1: Chloprostenol group (i.e. PGG)

The does in this group were administered via double injection (intramuscular), ten days apart, with 125 mcg Chloprostenol (Estroplan®, Parnell Australia PTY Ltd.). Blood for hormone assay was collected via jugular venipuncture for 4 days beginning 24 hours after the 2nd PGF2α injection. The blood was collected into labelled Bijou bottles and allowed to coagulate on the laboratory bench in a slanted position. Serum was decanted and centrifuged for 10 minutes at 3000g. The supernatant was stored in Eppindorof tubes under layers of ice-block in a chest freezer until all the samples were complete. Enzyme Immunoassay kits (Fortress Diagnostics Ltd. UK) were purchased and used to assay Luteinizing hormone (LH), Follicle stimulating hormone (FSH), progesterone and estradiol, under strict adherence to manufacturer's instructions. Other data were collected from 24 hours prior to administration of Chloprostenol. Vaginal smear was collected daily at 8:00 a.m. for cytology, throughout the study with the aid of a vaginal swab as earlier described (19). Briefly, this involves restraint of the doe in standing position by an assistant with both hind limbs raised at 45^{o}, gentle anterior vaginal mucosa swabbing, evaluation, and categorization of the Giemsa-stained exfoliated vagina cells under x400 of microscope. The vertical (i.e. distance between superior and inferior commissure) and horizontal (i.e. at the broadest horizontal curvature) diameters of the vulva were measured using a measuring tape.

Experiment 2: Ovsynch group (i.e. OvG)

The Ovsynch protocol as earlier described (26) was modified in Experiment 2. Does were administered with 50 mcg Gonadorelin acetate (Gonadorelin®, Parnell Australia PTY Ltd.) via intramuscular route on day 0, followed by 125 mcg Estroplan® on day 7, and a second 50 mcg Gonadorelin acetate on day 9. Blood was collected via jugular venipuncture for hormone assay (as for Experiment 1), beginning from 24 hours post PGF2α injection, for 4 days and analyzed as in Experiment 1. Similarly,

vaginal smear for cytological study was collected, and vulva diameters were evaluated as in Experiment 1.

Experiment 3: Estradiol group (i.e. EG)

The does were administered, via intramuscular injection, with two doses of 1 mg Estradiol benzoate (Super Estradiol®, Hebei New Century Pharm. Co. Ltd., China),at two days interval. Blood was collected via jugular venipuncture for hormone assay (as in Experiment 1), beginning from 24 hours post second dose of estradiol, and for 5 days. Other parameters: vaginal cytology and vulva diameters were carried out and analyzed as in Experiment 1.

Determination of estrus

Twice daily i.e. 07:00 hours and 17:00 hours, beginning the day after the last injection in the three Experiments, and for 5 days, each doe was introduced to each of the aproned bucks for 5 minutes in order to observe the does' response to being mounted. Standing estrus was recorded as acceptance of mounting for not less than 1 minute.

Guide on Research Conduct: The study was conducted under strict adherence to the principles of the care and use of farm animals in research, teaching and testing of the Canadian Council on Animal Use.

Data analysis

Parameters evaluated include:

- Onset of estrus post treatment: Time lapsed (hours) between second injection with Chloprostenol (in PGG), or Gonadorelin (in OvG), or Estradiol (in EG) and acceptance of mounting by the buck.
- Proportion of does in estrus: Number of does in estrus/total treated x 100 for each Experiment.
- Mean duration of estrus: Average time lapsed during which standing estrus occurred.

The mean percentage and standard error of means were calculated .One way Analysis of Variance (ANOVA) and Duncan multiple comparison test of the Statistical Package for Social Science (SPSS 19) were used to establish any significant difference at 95% Confidence Interval. P-values less than 0.05 were regarded as significant.

RESULTS

As shown in Table 1, the onset of estrus, proportion of does in estrus, and mean duration of estrus following treatments in the study were 48 hour, 90% and 2.32±3.84 (PGG); 0hr, 100% and 1.62±0.28 (OvG); and 0 hour, 100% and 5.00±0.00 (EG). Table 2 shows the differences in the mean titers for the hormones investigated-luteinizing hormone (LH), follicle stimulating hormone (FSH), Progesterone and Estradiol under different treatments (i.e. PGG, OvG and EG). The differences among the mean titers for LH under PGG (15.50±1.10), OvG (12.25±1.35) and EG (27.90±1.30) were significant (P<0.05). The differences among the mean titers for FSH under PGG (12.75±1.05), OvG (7.50±0.5) and EG (12.0±1.1) were also significant

Table 1: Estrual Characteristics of WAD goat does in the Experiments.

Parameter (s)	PGG	OvG	EG
Onset of estrus (hr) post treatment	48	0	0
Proportion of does in estrus (%)	90	100	100
Mean duration of estrus	2.32±3.84	1.62±0.28	5.00±0.00

Table 2: Hormone Concentrations during the Estrual Period in the Experiments.

Hormones	PGG	OvG	EG	P-value
LH	15.50±1.10	12.25±1.35	27.90±1.30	P<0.05
FSH	12.75±1.05	7.50±0.50	12.00±1.10	P<0.05
Progesterone	5.25±0.25	4.25±0.25	5.00±0.00	P<0.05
Estradiol	37.85±1.55	27,90±1.30	34.00±1.00	P<0.05

Table 3: Mean Percentage superficial epithelial cells during the estrual period in the Experiments.

Hours	PGG (%)	OvG (%)	EG (%)	P-value
24 hours	0.00±0.00	79.50±9.50	100.00±0.00	P<0.05
48 hours	11.00±1.00	87.50±12.50	75.00±25.00	P<0.05
72 hours	73.50±1.50	69.50±19.50	100.00±0.00	P<0.05
96 hours	98.50±1.50	55.00±45.00	100.00±0.00	P<0.05

Table 4: Mean±S.D for vulva diameters during the estrual period in the Experiments.

Vulva diameter (cm)	PGG	OvG	EG	P-value
Vertical	2.25±0.25	2.60±0.30	2.40±0.40	P>0.05
Horizontal	1.75±0.05	1.70±0.10	1.75±0.40	P>0.05
P-value	P<0.05	P<0.05	P<0.05	-

Table 5: Rate of change between horizontal and vertical vulva diameter.

Protocol	Mean horizontal vulva diameter	Mean vertical vulva diameter	Significance	Rate: Vertical / Horizontal vulva diameter
PGG	1.75	2.25	P < 0.05	2.25/1.75 = 1.29
OvG	1.70	2.60	P<0.05	2.60/1.70 = 1.53
EG	1.75	2.40	P<0.05	2.40/1.75 = 1.37
P-value	P>0.05	P>0.05	-	-

(P<0.05). However, the differences among the mean titers for progesterone under PGG (5.25±0.25), OvG (4.25±0.25) and EG (5.00±0.00) were not significant (P>0.05). The differences among the mean titers for estradiol under PGG (37.85±1.55), OvG (27.90±1.30) and EG (34.00±1.00) were significant (P<0.05). The changes in the mean percentage (%) superficial epithelial cells during 24, 48, 72 and 96 hours post treatment are presented in Table 3. The mean values for the PGG were 0.00±0.00, 11.00±1.00, 73.50±1.50, 98.50±1.50, respectively. For OvG, the superficial epithelial cells (%) were 79.50±9.50, 87.50±12.50, 69.50±19.50, 55.00±45.00, respectively. In the EG, the superficial epithelial cells (%) were 100.00±0.00, 75.00±25.00, 100.00±0.00, 100.00±0.00, respectively. These differences were significant (P<0.05). Plates 1, 2 and 3 show vaginal cell types obtained during estrus under the three protocols. The plates show an abundance of superficial cells with clumping or folding edges. Table 4 shows that the differences among the mean vertical diameters under the three estrus induction protocols i.e. 2.25±0.25 (PGG), 2.60±0.30 (OvG) and 2.40±0.40 (EG) were not significant (P>0.05). The differences among the mean horizontal diameters i.e. 1.75±0.05 (PGG), 1.70±0.10 (OvG) and 1.75±0.40 (EG) were also not significant (P>0.05). In Table 5, ratios of the changes in vertical:horizontal vulva diameters were 1:1.29 (PGG), 1:1.53 (OvG), and 1:1.37 (EG).

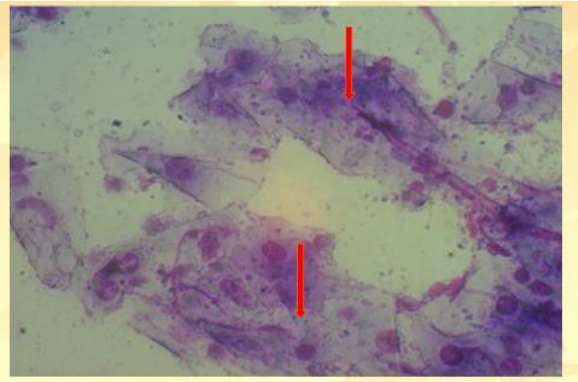

Plate 1: Exfoliated vaginal cells of WAD goat does during the estrual period in Experiment 1
(PGG) showing predominantly superficial cells with high degree of clumping. (x400).

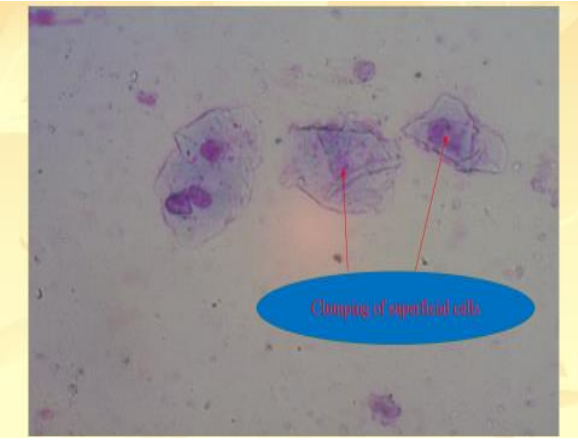

Plate 2: Exfoliated vaginal cells of WAD goat does during the estrual period in Experiment 2
(OvG) showing few superficial cells with clumping. (x400).

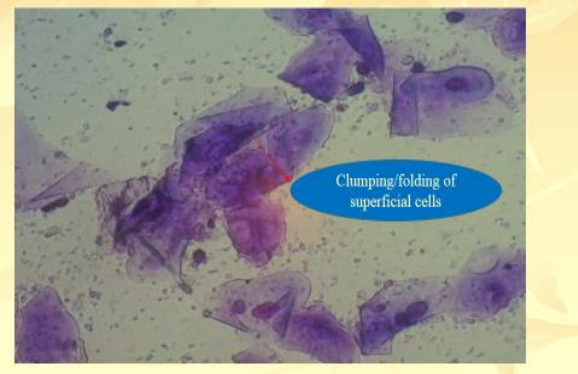

Plate 3: Exfoliated vaginal cells of WAD goat does during the estrual period in Experiment 3
(EG) showing predominantly superficial cells with clumping and folding. (x400).

DISCUSSION

The three protocols used in this study have both induced, as well as synchronized estrus in WAD goat does. Our earlier report (19) as well as those of others (10, 18) agreed with this finding with respect to the group treated with Chloprostenol (PGG). The observation with the Ovsynch experiment (OvG) was similar to the result earlier obtained (8) in Karagouniko ewes, as well as a second (13) in Boer goats. The earlier report (8) was perhaps the first application of the Ovsynch protocol in small ruminants. Our finding in the current study therefore supports more, the claim of the second (13), due to similarity of species. In the third experiment (i.e. EG), elevation in circulating level of estradiol benzoate may have activated the hypothalamic surge center, leading to increased expression of estrus (17), which was probably signified by the longer mean duration of estrus, as well as to ovulatory LH surge (4). Although, literature appeared to be scanty on the duration of estrus following ovsynch treatment, the mean duration of estrus obtained in this study captured the periods when insemination was carried out in the ewe (8) and the goat (13) i.e. 16-24 hours and 16 hours after second GnRH injections, respectively. Although, it was not clear why there were disparities in the mean durations of estrus in the three experiments, differences in the mechanisms of action of the different pharmacological agents used, as well as sites of activity, may be responsible. These facts may also explain why there were differences in the number of hours that lapsed, post treatment, before the onset of estrus occurred in Experiment 1 (PGG). Also, Chloprostenol appeared to induce estrus in the least proportion (90%) of does in the study. Although, this proportion was higher than the 83.3% reported in our earlier study in which two injections of prostaglandin were administered 7 days apart (19), both reports are higher than 60-70% synchronization rate reported for cattle (31), suggesting that prostaglandins are more effective in goats compared with cattle. The basic rule guiding the mechanism of action of prostaglandin however subsist in that treated animals needed to possess a functional corpus luteum (11, 16) to be effective.

The results on hormone assay during the estrual period appears to require careful interpretation. Looking at the columns with respect to each experimental group, the mean titers for FSH were lower compared to that for LH. The mean progesterone titers were lowest compared to other hormones in all experimental groups, and estradiol titers were similarly highest. These trends seem to follow normal pattern in the goat (12, 29). It is also likely that the high estradiol titers across the experimental groups was that necessary to produce the LH surge required for ovulation (21). The result on hormones also showed that except for progesterone, the differences among each other hormone across experimental groups were significant ($P<0.05$). One suggestion of this finding is

that the titer of each hormone following synchronization depends on the protocol used. This is more so since the focus of this report was on the estrual period. The similarity in progesterone titers, regardless of protocol, also suggest that progesterone could be an indicator of the estrual period in the WAD goat.

The pattern of vaginal cytological changes with respect to superficial epithelia cells (SEC) is also interestingly informative. Our earlier work (19) as well as those of others (22, 24) have established that increasing proportion of SEC is associated with approaching estrus in the goat. For the PGG (Plate 1), the proportion of SEC picked after 48 hours, this event perhaps, may be coincidental with the observation in Table 1 where the onset of estrus in this group occurred 48 hours after treatment. Whereas the SEC for OvG (Plate 2) increased from 24-48 hours and remained high during these periods, decreasing only thereafter, the SEC (EG) remained at 100% during the study (Plate 3), except for a decline at 48 hours; these observations suggest that, for OvG, estrus begins to wane after 48 hours and, the finding with estradiol treated goats (i.e. EG) may underline the sustained estrus in that group. Additionally, the observation with EG may be underlined by an elevated secretory activity of estradiol on the goat genitalia (6, 14, 30).

The observations on vulva diameters (vertical and horizontal) were not significant ($P>0.05$) when estrual values under the three experimental protocols were compared. This finding suggest that changes in vulva diameters were similar during the estrual period and occurred at comparable rates irrespective of the estrus induction/synchronization protocol used. However, the mean vertical diameters for the three protocols were significantly ($P<0.05$) higher than the corresponding horizontal diameters. This finding suggest that the changes in the vertical diameter occurred at a higher rate compared with that of its corresponding horizontal diameter. For instance, in Table 5, OvG had the highest ratio i.e. for every 1 cm increase in horizontal diameter, a 1.53 cm increase (i.e. 1:1.53) occurred in the vertical diameter. This was followed by EG with 1: 1.37, and the least (i.e. PGG) with 1:1.29, respectively. It is not clear what factors could have led to variations in these rates, but the authors presume that OvG had the highest rate due to its involvement with multiple hormones, whereas, the other two protocols involved the use of single hormones.

We conclude as follows: that the three protocols i.e. PGG, OvG an EG induced and synchronized estrus in WAD goat does; that ovsynch and estradiol had both the shortest onset to estrus as well as highest percentage estrus synchronization; that OvG had the least duration of estrus, followed by PGG and EG; that estrual titers of hormones depend on method of synchronization, and that progesterone values appear to be similar regardless of protocol used; that after 48 hours, SEC picked in PGG but declined in OvG while apart from the decline at 48 hours in EG, it remained at maximum level all through; that the three protocols produced comparable changes in vulva diameter, and that the ratios of change differs between corresponding vertical and horizontal vulva diameters depending on the estrus induction/synchronization protocol.

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