



# Early diagnosis of pseudopregnancy in goats by ultrasonography and response to treatment using cloprostenol sodium<sup>#</sup>

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## Abstract

*Pseudopregnancy is a state of temporary infertility in goats characterised by the accumulation of sterile fluid in the uterus in the absence of a foetus with persistent corpus luteum. B-mode ultrasonographic examination of goats on day 60 post-breeding was conducted for differential diagnosis of pregnancy, non-pregnancy and pseudopregnancy. Pseudopregnant does diagnosed on day 60 (day 0) post-breeding (Group I, n=8) were treated with three doses of 125µg of cloprostenol sodium (i/m) 10 days apart and were bred by natural service on induced oestrus after the third dose of treatment. The response to treatment was evaluated and compared with normal cycling does (Group II, n=8) which were mated on spontaneous oestrus. The uterine wall thickness measured ultrasonographically on day 0 in group I and on day 60 post-breeding in pregnant does of group II were 0.63 ± 0.02 and 0.66±0.03 cm, respectively (P >0.05). Serum progesterone concentration estimated on day 0, day of the evacuation of uterine fluid, days 10 and 20 in group I and on day 60 post-breeding in group II were 2.73 ± 0.19, 0.78± 0.19, 1.67 ± 0.10, 1.72 ± 0.19 and 3.64± 0.16 ng/mL, respectively. A statistically significant difference was observed between day 0 in group I and day 60 in group II; within group I, between day 0 and the day of evacuation and between the day of evacuation and day 10. Pregnancy diagnosis on day 60 post-breeding revealed a conception rate of 50 and 75 per cent in groups I and II, respectively (P>0.05).*

**Keywords:** Goat, pseudopregnancy, ultrasonography, cloprostenol sodium

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Pseudopregnancy is one of the major causes of infertility in adult goats characterised by the presence of persistent corpus luteum and accumulation of sterile fluid in the uterus without any conceptus or placentomes. In goats, the duration of pseudopregnancy usually lasts as same as the gestation length or even more. In some cases, corpus luteum (CL) undergoes spontaneous lysis which results in the voiding of a large amount of cloudy fluid from the uterus denoted as a 'cloud burst' (Noakes *et al.*, 2009).

Ultrasonographic examination is the best tool for the differential diagnosis of pseudopregnancy from pregnancy in goats. Ultrasonographic examination during pregnancy revealed the presence of the foetus or its body parts, foetal heartbeats and placenta, whereas, the presence of anechoic fluid in the absence of the placenta or foetus was reported in the case of pseudopregnant goats (Hesselink, 1993). The optimal stage for conducting an ultrasonographic examination to differentiate pregnancy and pseudopregnancy in a goat was reported to be about 70 days after insemination (Wittek *et al.*, 1998). More advanced cases of pseudopregnancy revealed the presence of a large amount of anechoic clear fluid accumulation in the uterus without placentomes or foetal structures and the lumen was separated by hyperechoic trabeculae (Farliana and Yimer, 2016). Additionally, the progesterone concentration was found to be significantly lower between days 10 and 55 in pseudopregnant does compared to pregnant does and hence, it could be used for differential diagnosis (Kornalijnslijper *et al.*, 1997).

The treatment regimens for pseudopregnancy using single, double or multiple doses of PGF<sub>2</sub>α have been reported. Treatment using a single dose of cloprostenol administration (50 µg i/m) in pseudopregnant goats resulted in luteolysis of one or more CLs, the onset of uterine drainage and return to oestrus (Lopes Junior *et al.*, 2004). Evacuation of uterine contents after the first and second doses of dinoprost (5 mg i/m) was reported as 57.90 and 89 per cent, respectively (Souza *et al.*, 2013; Reddy *et al.*, 2014). This demonstrated the effectiveness of a second dose of PGF<sub>2</sub>α

for better results in terms of preventing fluid re-accumulation and enhancing future reproductive performance in pseudopregnant does. Recently, treatment protocols using three doses of prostaglandin were found to be more effective for the complete evacuation of uterine fluid and tissue debris than single or double-dose administration (Maia *et al.* 2018). Pseudopregnancy if left undetected and maintained for a longer period, might irreversibly damage the endometrium leading to serious forms of infertility. Hence, early diagnosis and treatment of pseudopregnancy are important to regain fertility in such does.

## Materials and methods

### Selection of animals and observations

A total of 254 goats were presented at Teaching Veterinary Clinical Complex, Pookode, for pregnancy diagnosis from January'21 to July'22. Eight does were confirmed as pseudopregnant on day 60 post-breeding (Group I) using B-mode transabdominal ultrasonography and eight normally cycling does (Group II), were selected for this study. The does in Group I were treated with three doses of PGF<sub>2</sub>α analogue (Inj. Cloprostenol sodium-125µg, i/m) at 10 days intervals. The pseudopregnant does were observed on the day of confirmation (day 0), day of evacuation, day 10 and day 20 and were bred with locally available bucks on the day of onset of induced oestrus after the last PGF<sub>2</sub>α administration. Whereas, the does in Group II were bred naturally on spontaneous oestrus and were subjected to sonographic examination on day 60 post-breeding.

Physiological parameters like body temperature, colour of the mucous membrane, respiration rate and heart rate were recorded in all the animals.

### Ultrasonographic examination of pseudopregnant does

The does in Group I were subjected to detailed B-mode transabdominal ultrasonographic studies (Aeroscan Ultrasound CD Elite, Konica Minolta Healthcare, India) with respect to uterine wall thickness, echogenicity of

uterine fluid and diameter of compartments on day 0 and on the day of the uterine evacuation, day 10 and day 20. The does in Group II were studied for uterine wall thickness on day 60 post-breeding.

### **Blood collection and serum progesterone estimation**

Blood was collected from group I animals, on day 0, day of the uterine evacuation, day 10 and 20 and in group II does, on day 60 post-breeding, for estimating serum progesterone concentration by radioimmunoassay (RIA) using commercial RIA kits (IM1188 Beckman Coulter RIA PROGESTERONE, France).

### **Statistical analysis**

Parameters of uterine wall thickness and progesterone values were compared using repeated measures ANOVA and between groups comparison was done using independent t test. Conception rate was compared by using Z test for two proportions.

### **Results and discussion**

A total of 254 goats were presented for pregnancy diagnosis from January'21 to July'22 and out of which, 15 does of various breeds were confirmed as pseudopregnant. The occurrence of pseudopregnancy cases in different cross-bred goats observed in the present study was 5.90 per cent, whereas, Devalal (2018) observed 1.97 per cent incidence of pseudopregnant cases.

Pseudopregnant does were clinically normal with a body temperature of  $101.51 \pm 0.27^\circ\text{F}$ , pink colour of the mucous membrane, the respiration rate of  $22 \pm 0.92$  per min and heart rate of  $77 \pm 1.73$  bpm and their abdomen was distended as in pregnant does.

### **Ultrasonographic observations of the uterus**

Ultrasonographic examination of the uterus on day 0 in group I does revealed distended uterine horns filled with anechoic fluid. The fluid-filled uterus was divided into different compartments by thin double-layered

hyperechoic trabeculae providing a honeycomb appearance to the uterus (Fig.1). The uterine compartments measured  $3.21 \pm 0.42$  cm, whereas, Devalal (2018) reported  $0.20 \pm 0.004$  cm in pseudopregnant does on 90 days post-breeding. The difference in thickness could be due to the variation in the time of diagnosis, as the diagnosis in this study was performed earlier, i.e., 60 days.

$\text{PGF}_2\alpha$  administration resulted in the dilation of cervical os, contraction of myometrium and evacuation of uterine content (Bisla *et al.*, 2019). The mean time taken for the onset of uterine fluid evacuation post-treatment was observed to be  $60 \pm 3.93$  h. On the day of the evacuation, moderate ( $>3$  mm anechoic region within the uterus) and minimal (0-3mm) fluid retention could be seen in 75 and 25 per cent cases, respectively. On day 10, before the second dose of  $\text{PGF}_2\alpha$  administration, moderate and minimum fluid retention was observed in 62.50 and 37.50 per cent of cases, respectively. The findings were in agreement with Souza *et al.* (2013) and Reddy *et al.* (2014), who reported that a single dose of prostaglandin injection was not sufficient enough for the complete evacuation of uterine fluid in pseudopregnant goats.

On day 20, before the third treatment dose, 12.50 per cent (1/8) cases revealed a moderate quantity of fluid still present in the uterus. These findings were in accordance with Maia *et al.* (2018) who reported that three doses of d-cloprostenol at an interval of 10-12 days were required for complete evacuation of uterine fluid from pseudopregnant uteri of does.

In this study, the echogenicity of uterine fluid in pseudopregnant does were observed on day 0, and it was found that 62.50 per cent of the cases presented were with anechoic fluid accumulation in the uterus. Whereas, in 37.50 per cent of cases anechoic fluid with hyperechoic free floating contents was observed. The same observations were reported by Gonzalez- Bulnes *et al.* (2010) in goats and they mentioned that hyperechogenic particles moving in the anechoic uterine fluid originated from desquamated endometrial

cells.

Measurement of uterine wall thickness on different days of observation and treatments, revealed statistically significant ( $P$ -value=0.005) changes within group I (Table 1). The reduction in uterine wall thickness observed from the day of uterine evacuation to day 10 and from day 10 to 20 of treatment were statistically significant. However, there was only a marginal reduction from day 0 to day of evacuation. Between the groups, a marginal ( $P$ -value=0.347) difference in uterine wall thickness on day 0 in group I and on day 60 post-breeding in pregnant does of group II were observed.

The uterine wall thickness observed on day 0 was  $0.63 \pm 0.02$  cm. In the contrary, Devalal (2018) reported uterine wall thickness of  $0.20 \pm 0.004$  cm in pseudopregnant does 90 days post-breeding. The presence of thin uterine walls in pseudopregnant does at five to seven months post-breeding has been reported by Maia *et al.* (2018) and Bisla *et al.* (2019). The difference in the uterine wall thickness observed in the present study could be due to the variation of the day of diagnosis and as the condition progresses, the thickness of the uterine wall would have reduced.

#### Serum progesterone estimation

The mean  $\pm$  SE of serum progesterone concentration (ng/mL) of pseudopregnant does



**Fig. 1.** Honey comb compartmentalisation of the uterus with anechoic fluid accumulation in pseudopregnant doe on the day of confirmation (day 0).

on day 0 was  $2.73 \pm 0.19$  which decreased to  $0.78 \pm 0.19$  on the day of evacuation of uterine fluid ( $P < 0.01$ ). The value increased marginally from  $1.67 \pm 0.10$  to  $1.72 \pm 0.19$  ng/mL on day 10 and 20, respectively (Table 1).

The mean  $\pm$  SE of serum progesterone concentration observed in pregnant goats at 60 days post-breeding was  $3.64 \pm 0.16$  ng/mL. The difference between progesterone values in pregnant and pseudopregnant does 60 days post-breeding was found to be highly significant ( $P$ -value = 0.0042).

The increased level of serum progesterone concentration on day 0 was due

**Table 1.** Uterine wall thickness and serum progesterone concentration (ng/mL) in pseudopregnant and pregnant goats

Reproductive status	Uterine wall thickness (cm) Mean $\pm$ SE		t-value (P-value)	Serum progesterone concentration (ng/mL) Mean $\pm$ SE		t-value (P-value)
	Group I (n=8)	Group II (n=6)		Group I (n=8)	Group II (n=6)	
Day 60 post breeding	$0.63^a \pm 0.02$	$0.66 \pm 0.03$	0.9713 (0.347 <sup>ns</sup> )	$2.73^a \pm 0.19$	$3.64 \pm 0.16$	3.517 (0.0042 <sup>**</sup> )
Day of uterine evacuation	$0.62^a \pm 0.02$			$0.78^b \pm 0.19$		
Day 10	$0.60^b \pm 0.03$			$1.67^{bc} \pm 0.10$		
Day 20	$0.58^c \pm 0.03$			$1.72^c \pm 0.19$		
F-value = 7.934 P-value = 0.005 <sup>**</sup>				F-value = 10.615 P-value = <0.01 <sup>**</sup>		

<sup>\*\*</sup> Significant at 0.01 level; ns- non-significant

Means having different alphabets as superscript differ significantly

to the presence of persistent CL responsible for pseudopregnancy. The findings correlated with ultrasonographic examination with the presence of CL in ovaries on day 0 in pseudopregnant does. The findings were similar to Wittek *et al.* (1998) who reported that the plasma progesterone level in pseudopregnant does on the day of diagnosis was  $4.97 \pm 2.94$  ng/mL and ranged between 0.00 and 12.33 ng/mL (Maia *et al.*, 2018).

The reduction in the serum progesterone value on the day of evacuation when compared to day 0 might be due to the luteolysis caused by the administration of the first dose of PGF<sub>2</sub>α. The findings were in agreement with Wittek *et al.* (1998) and Devalal *et al.* (2019) who reported that the serum progesterone value in pseudopregnant does reduced to less than 1ng/mL on the day of evacuation after PGF<sub>2</sub>α administration.

On day 10, the initial PGF<sub>2</sub>α administration resulted in regression of the CL followed by follicular dominance, ovulation and development of a new CL. Romano *et al.* (2017) reported that the luteolytic action of exogenous prostaglandin initiated from day three of oestrus cycle. Zarkawi and Soukouti (2001) reported that early luteal phase in goats lasted for 2-5 days followed by luteal phase with mean progesterone values of  $0.28 \pm 0.34$  and  $5.36 \pm 1.76$  ng/mL, respectively. Hence it can be assumed that the increase in the level of progesterone on day 10 could be due to the development of new CL. Maia *et al.* (2018) also reported that the serum progesterone concentration on the day of the second dose treatment (day10) with d-cloprostenol was  $1.32 \pm 0.38$  ng/mL.

On day 20, as in the case of day 10, the development of new CL following ovulation could be the reason for the increase in the level of serum progesterone. The findings were similar to Maia *et al.* (2018) who also reported that the serum progesterone value on day 20 was higher than day 10 and was recorded as  $3.24 \pm 0.75$  ng/mL.

On day 60 post-breeding in pregnant does of group II, the serum progesterone value was  $3.64 \pm 0.16$  ng/mL. Alwan *et al.* (2010)

reported that serum progesterone concentration during the second month of gestation was  $7.75 \pm 0.75$  ng/mL. According to Khanum *et al.* (2008) mean concentration of progesterone followed no trends for a continuous increase or decrease throughout pregnancy in goats.

### **Response to treatment**

In the present study, evacuation of uterine fluid started within 24 h of initial treatment. But ultrasonography of the uterus on the day of evacuation and the day of the second cloprostenol injection revealed moderate fluid retention in all cases. The first dose of PGF<sub>2</sub>α treatment could initiate uterine evacuation in all does, but oestrus was expressed only in 12.50 per cent (1/8) does. After the second treatment, two does (25%) expressed oestrus signs. All the goats (8/8) expressed oestrus signs after the third dose of treatment. The Mean±SE time taken for the onset of expression of oestrus signs after the first, second and third treatments were 48,  $84 \pm 36$ ,  $99 \pm 26.61$  h, respectively. The variation in the time denotes differences in the age of CL and the status of uterine evacuation. All does were bred by natural service and pregnancy diagnosis was done on 60 days post-breeding.

### **Conception rate**

In the present study, the first service conception rate in group I was found to be 50 per cent (4/8). The does that did not conceive on the first service were bred again on the next observed oestrus but did not conceive. Hence the overall conception rate remained at 50 per cent. The first service and overall conception rates obtained for the control group were 75 (6/8) and 87.50 (7/8) per cent, respectively. The conception rates in treatment and control groups were comparable ( $P > 0.05$ ), indicating that the treatment protocol followed was successful for the management of pseudopregnancy in does.

### **Recurrence of pseudopregnancy**

After treatment and breeding of eight pseudopregnant does in group I, recurrence of pseudopregnancy was noticed in one doe with an incidence rate of 12.50 per cent. The diagnosis was made 60 days post-breeding



**Fig. 2.** Ultrasonographic image of uterus with non-viable embryo on day 60 post breeding in a doe that showed recurrence of pseudopregnancy



**Fig. 3.** Ultrasonographic image of the uterus with anechoic fluid-filled compartments and hyperechoic free-floating particles on day 90 post-breeding in a doe that showed a recurrence of pseudopregnancy

where the ultrasonographic image revealed a dead embryo/foetus of almost 30 days of age (Fig.2). The goat was re-examined on day 90 post-breeding where typical pseudopregnancy characteristics of anechoic fluid-filled uterus without foetus or placentomes was observed (Fig.3). The findings were in accordance with Chemineau *et al.* (1999) who reported that 50 per cent of the occurrences of hydrometra in does were caused by late embryonic mortality at a gestational age of 40 to 60 days.

### Conclusion

It can be concluded that pseudopregnancy can be differentially diagnosed on day 60 post-breeding using B-mode ultrasonography and can be effectively treated and brought back to fertile status using three doses of cloprostenol sodium at 10 days interval.

### Conflict of interest

The authors declare that they have no conflict of interest.

### References

Alwan, A.F, Amin, F.A.M. and Ibrahim, N.S. 2010. Blood progesterone and estrogen hormones level during pregnancy and after birth in Iraqi sheep and goat. *Basrah J. Vet. Res.* **9**:153-157.

Bisla, A., Kumar, B., Yadav, D., Kurhe, R.S., Khan, J.A., Ngou, A.A., Rautela, R. and Kumar, H. 2019. Ultrasonographic Diagnosis and Clinical Management of Pseudopregnancy in Goats. *Theriogenology Insight.* **9**:13-18.

Chemineau, P., Baril, G., Leboeuf, B., Maurel, M.C., Roy, F., Pellicer-Rubio, M., Malpoux, B., Cognie, Y. 1999. Implications of recent advances in reproductive physiology for reproductive management of goats. *J. Reprod. Fert. Supplement.* **54**: 129-142.

Devalal, K. 2018. Early therapeutic management of Pseudopregnancy in goats with Prostaglandin F<sub>2</sub> alpha. *M.V.Sc. Thesis.* Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, 109p.

Devalal, K., Joseph, M., Kurien, M.O., Aravind, A. and Ajithkumar, S., 2019. Serum progesterone and oestradiol profile in pseudopregnant and pregnant goats below 3 months post-service. *J. Vet. Anim. Sci.* **50**: 63-67.

Farliana, M. D. N. and Yimer, N. 2016. Pseudopregnancy in a doe and its hormonal therapy. *Int. J. of Livestock Res.* **6**: 90-95.

Gonzalez-Bulnes, A., Pallares, P. and Vazquez, M. 2010. Ultrasonographic imaging in

- small ruminant reproduction. *Reprod. Domest. Anim.* **45**: 9-20.
- Hesselink, J.W. 1993. Incidence of hydrometra in dairy goats. *Vet. Rec.* **132**:110-112.
- Khanum, S.A., Hussain, M. and Kausar, R. 2008. Progesterone and estradiol profiles during estrous cycle and gestation in dwarf goats (*Capra hircus*). *Pakist. Vet. J.* **28**: 1-4.
- Kornalijnslijper, J.E., Kemp, B., Bevers, M.M., Van Oord, H.A. and Taverne, M.A. 1997. Plasma prolactin, growth hormone and progesterone concentration in pseudopregnant, hysterectomized and pregnant goats. *Anim. Reprod. Sci.* **49**: 169-178.
- Lopes Junior, E.S., Cruz, J.F., Teixeira, D.I.A., Lima Verde, J.B., Paula, N.R.O., Rondina, D. and Freitas, V.J.F. 2004. Pseudopregnancy in Saanen goats (*Capra hircus*) raised in Northeast Brazil. *Vet. Res. Commun.* **28**: 119-125.
- Maia, A.L.R.S., Brandao, F.Z., Souza-Fabjan, J.M.G., Veiga, M.O. and Balaro, M.F.A. 2018. Hydrometra in dairy goats: Ultrasonic variables and therapeutic protocols evaluated during the reproductive season. *Anim. Reprod. Sci.* **99**: 169-178.
- Noakes, D.E., Parkinson, T.J. and England, G.C. 2009. *Veterinary Reproduction and Obstetrics*. (9<sup>th</sup> Ed.). W. B. Saunders, Elsevier, USA, 950.30p.
- Reddy, R., Arunakumari, G., Anil, K.R., Muralimohan, K. and Sunil, A. 2014. Efficacy of cloprostenol therapy in hydrometra goats. *Indian J. Anim. Reprod.* **35**: 39-41.
- Romano, J.E., Alkar, A. and Amstalden, M. 2017. Onset of luteolytic action of exogenous prostaglandinF-2 $\alpha$  during estrous cycle in goats. *Theriogenology.* **92**:45-50.
- Souza, J.M.G., Maia, A.L.R.S., Brandão, F.Z., Vilela, C.G., Oba, E., Bruschi, J.H. and Fonseca, J.F. 2013. Hormonal treatment of dairy goats affected by hydrometra associated or not with ovarian follicular cyst. *Small Rum. Res.* **111**:104-109.
- Wittek, T., Erices, J. and Eelze, K. 1998. Histology of the endometrium, clinical-chemical parameters of the uterine fluid and blood plasma concentrations of progesterone, estradiol-17  $\beta$  and prolactin during hydrometra in goats. *Small Rum. Res.* **30**: 105-112.
- Zarkawi, M. and Soukouti, A. 2001. Serum progesterone levels using radioimmunoassay during oestrous cycle of indigenous Damascus does. *New Zealand J. Agricultural Res.* **44**:165-169.