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Hydrometra in dairy goats: Ultrasonic variables and therapeutic protocols evaluated during the reproductive season

A.L.R.S. Maia[a,*], F.Z. Brandão[a], J.M.G. Souza-Fabjan[a], M.O. Veiga[b], M.F.A. Balaro[a], L.G.B. Siqueira[c], O. Facó[d], J.F. Fonseca[d]

[a] Faculdade de Veterinária, Universidade Federal Fluminense, Av. Vital Brazil Filho, 64, CEP 24230-340, Niterói, RJ, Brazil
[b] Departamento de Medicina Veterinária, Universidade Federal de Lavras, Av. Doutor Sylvio Menicucci, 1001, CEP 37200-000, Lavras, MG, Brazil
[c] Embrapa Gado de Leite, Av. Eugênio do Nascimento, 610, CEP 36038-330, Juiz de Fora, MG, Brazil
[d] Embrapa Caprinos e Ovinos, Estrada Sobral/Groaíras, km 04, CP 145, CEP 62010-970, Sobral, CE, Brazil

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ABSTRACT

Hydrometra is characterized by the accumulation of fluid within the uterus due to the persistence of corpus luteum. The diagnosis of this disorder occurs with an ultrasonic exam. This study evaluated uterine drainage and fertility rates in goats after the use of d-cloprostenol in association or not with Gonadotropin-releasing hormone (GnRH) treatment. Twenty Saanen goats, diagnosed with hydrometra, received three 37.5-μg doses of d-cloprostenol laterovulvarly at 10-day intervals. On D5, the goats were assigned into two groups receiving 1 mL of GnRH or saline solution intramuscularly. Ultrasonography (US) was performed from D0 to D25. An US approach was used to rank hydrometra in scores. The pregnancy rate was assessed 45 and 90 days after the end of treatment. The uterine fluid was totally drained after the first and second administration of d-cloprostenol in 50% and 95% of the goats, respectively. In one female, full emptying of the uterus occurred only after D20. US performed at 45 and 90 days after the end of treatment indicated there was a pregnancy rate of 45.0% and 55.0%, respectively. Fertility did not differ between the GnRH-treated and control goats. Those goats not pregnant at 45 days had a follicular cyst, hydrosalpinx or hydrometra. At 90 days, no change was observed in the hydrosalpinx, and four goats had hydrometra. The use of three doses of d-cloprostenol 10 days apart was efficient for induction of draining the contents of the uterus, resulting in a relatively acceptable pregnancy rate. This treatment associated with the US approach can be important when applied in the field.

1. Introduction

Brazil ranks 22nd in goat breeding worldwide, with approximately 9 million animals (FAOSTAT, 2015). In the Southeast Region, dairy goat herds are composed of specialized European breeds kept in semi-intensive or intensive systems (Lôbo et al., 2010). For profitability in milk production, a need exists to improve breeding systems and control reproductive indexes such as the conception rate, fertility, kidding intervals, kidding rate and gestational losses (Fonseca, 2006). Ultrasonography (US) is an efficient diagnostic method for use in assessing the genital tract. Furthermore, this technique enables rapid and rational reproductive management of the herd (Gonzalez-Bulnes et al., 2010).

Eventually, female goats have a pathological condition of the uterus characterized by a varying amount of fluid which

* Corresponding author.
E-mail address: maia.alrs@gmail.com (A.L.R.S. Maia).

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accumulates in the uterine lumen due to the persistence of a corpus luteum (CL). This phenomenon is known as pseudopregnancy or hydrometra (Pieterse and Taverne, 1986; Duquesnel et al., 1992). Although several studies have been conducted to gain a greater understanding of the disease in goats, its etiology was not completely elucidated (Pieterse and Taverne, 1986; Wittek et al., 1998). It is also unknown whether in goats a persistent luteal function with plasma progesterone concentration > 2.0 ng/mL invariably leads to the fluid accumulation in the uterus (Kornalijnslijper et al., 1997). Thus, the presence of fluid in the uterus seems to be the result, but not the cause, of a prolonged progesterone secretion by the CL (Taverne et al., 1994). The affected goats may have an anestrous condition, reduction in milk production, and sometimes the distension of the abdomen similar to what occurs during pregnancy (Martel, 2001). Clinical manifestations are not clear, and there are no deviations in the hematological and blood biochemical parameters (Yotov et al., 2009). Normally, spontaneous expulsion of the fluid ("cloudburst") occurs without fetuses and/or placental remnants being present (Pieterse and Taverne, 1986). Since the introduction of US for pregnancy detection, the goats are easily diagnosed with hydrometra, but without this technique, females with marked abdominal distension are recognized with hydrometra only because of lack of parturition occurred by the expected date when parturition should occur (Pieterse and Taverne, 1986; Smith and Sherman, 2009). The development of hydrometra due to embryonic mortality also occurs (Humblot et al., 1995; Wittek et al., 1998; Brice et al., 2003).

In the southeast region of Brazil, hydrometra was the most prevalent disorder of the reproductive tract of dairy goats, occurring in herds with an average incidence of 10.0% (Maia et al., 2017). This occurrence was similar to 9% (ranged from 3.0% to 20.8%) previously reported by Hesselink (1993) in a study conducted for 3 years in herds of Saanen goats in the Netherlands. Desire et al. (2018) also found an approximate 10% (904/8642) of hydrometra incidence from records of crossbred dairy goats of the three original breeds (Alpine, Saanen, and Toggenburg) in the United Kingdom.

Although no other implications in female health have been observed, hydrometra certainly impairs the reproductive efficiency (Martel, 2001). Furthermore, it is considered the most frequent noninfectious cause of infertility or subfertility in dairy goats (Kornalijnslijper et al., 1997). The treatment of hydrometra involves emptying of the uterus usually by one or two administrations of prostaglandin F2α (PGF2α), (Pieterse and Taverne, 1986). In some females, however, complete drainage of the fluid was not achieved after the use of two doses of PGF2α. Also, in a part of the cases, the re-initiation of reproductive cycles does not occur using this therapy (Pieterse and Taverne, 1986; Martel, 2001; Souza et al., 2013). Furthermore, Souza et al. (2013) reported the presence of follicular cysts concomitant with hydrometra, observed after drainage of the uterine contents, suggesting a relation between these disorders in goats. This finding may suggest the inclusion of drugs such as gonadotropins, in association with prostaglandins, for the treatment of hydrometra due to the possible involvement of ovarian follicular cysts.

This study was conducted to evaluate the effectiveness of a hormonal treatment with three doses of d-cloprostenol with inclusion of GnRH administration for dairy goats affected with hydrometra for clinical-reproductive treatments. Furthermore, there was an aim to establish ultrasonic parameters for the diagnosis of this pathology.

2. Materials and methods

2.1. Ethics and animal care

The Animal Care Committee of the Universidade Federal Fluminense approved the study design (protocol number #678/2015), and it was conducted under the principles of the Brazilian Society of Laboratory Animal Science, which regulates conditions for experiments involving animals.

2.2. Location and experimental conditions

The study was conducted in the breeding season in March and April 2015 on a dairy goat farm (21°27′S and 43°07′W) in the Minas Gerais State, Brazil. According to the Köppen classification, the climate is type Cwa, characterized by a dry winter and hot summer (Peel et al., 2007).

The goats were managed in an intensive production system, confined in group pens, and fed corn silage. A balanced concentrate supplement was provided on demand (National Research Council, 2007). Mineralized salt (Caprinofós® Tortuga, São Paulo, Brazil), and drinking water was available *ad libitum*.

2.3. Animals and treatments

Of 184 goats evaluated, 20 (9 pluriparous, 8 primiparous and 3 nulliparous) were diagnosed using US as having hydrometra. These goats ranged from 1 to 7 years of age, body weight (BW) from 45.0 to 109.0 kg (71.2 ± 4.1 kg) and body condition score (BCS) between 2.75 and 4.75 (scale 1–5) (Detweiler et al., 2008). Sixteen goats were lactating (nine pluriparous and seven primiparous) and only six goats had previously been mated (four primiparous and two nulliparous). Goats were monitored throughout the period of the disease treatment.

All females received three applications of 37.5 μg d-cloprostenol (Prolise®, Tecnopec LTDA, São Paulo, Brazil), laterovulvarly at intervals of 10 days (D0, D10 and D20) for reduction of the uterine contents. On D5, the goats were randomly assigned to two groups to receive either 1 mL (25 μg) of GnRH (Gonadorelin - Gestran Plus®, Tecnopec LTDA, São Paulo, Brazil) (G_{GnRH}) or 1 mL of saline solution ($G_{Control}$) intramuscularly (Fig. 1). At the end of the treatment (D20), the goats were placed with fertile bucks for 5 days. After this period (D20–D25), the goats that returned to estrus were again placed with the respective bucks.

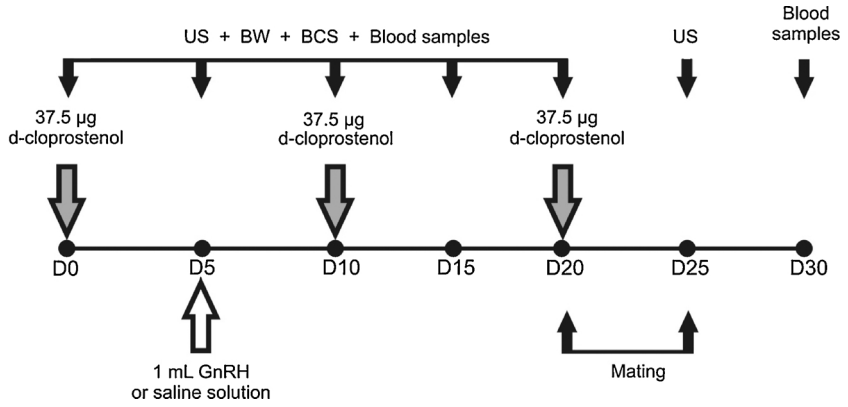


Fig. 1. Schematic representation of days and procedures performed for the treatment of hydrometra in dairy goats during the breeding season (US: Ultrasonography; BW: Body Weight; BCS: Body Condition Score).

2.4. Ultrasonographic evaluation

Transrectal ultrasonographic assessment of the uterus was performed from the first day of d-cloprostenol administration and continued every 5 days until the end of treatment (D0, D5, D10, D15, D20 and D25; Fig. 1). The genital tract was also evaluated in the same period described above for the diagnosis of ovarian follicular cysts or any other reproductive disorder.

All examinations were conducted by a single operator using a B-mode, transrectal ultrasonographic scanner (Mindray®; M5Vet, Shenzhen, China), equipped with a linear 5.0 MHz transducer taped to a PVC tube to facilitate its use in small ruminants (Souza et al., 2013). During the US evaluations, the goats remained standing, while being restrained by an assistant. To prevent the spread of pathogens among the female goats, plastic sanitary sleeves developed to fit the US rectal transducer (Camisinha para probe retal; NTB Indústria e Comércio de Produtos para Pecuária LTDA, São Paulo, Brazil) were used, with a new sleeve being applied to the US rectal transducer after each examination. A syringe was used to deposit 10 mL of carboxymethylcellulose gel (Carbogel UTL; Carbogel Indústria e Comércio LTDA, São Paulo, Brazil) into the goat's rectum for lubrication and to increase the contact surface between the transducer and the wall of the rectum.

At D0 an ultrasonography score between 0 and 4 (Grade 0: absence of liquid and Grade 4: full of liquid) (Fig. 2) was assigned to rank the uterine fluid drainage resulting from the treatment. This score was based on the visualization of the uterine sections (hypoechoic portions), filled or not with different amounts of liquid (anechoic portions). Initial (Grade 1) and moderate (Grade 2) degrees of hydrometra were indicated when the amount of fluid viewed could be mistaken for the beginning of pregnancy. For these scores, the ovaries containing one or more CL could be observed. Medium (Grade 3) and advanced (Grade 4) degrees of hydrometra were detected by US when the uterine walls were observed as being thin with undulations of the uterine wall with the uterus containing a large amount of liquid.

In addition to the US evaluation of the uterine fluid score, there was assessment of whether the hydrometra was due or not due to a gestational loss (Fig. 3).

2.5. Weighing and evaluation of the body condition score

The goats were weighed, and the BCS was evaluated to estimate the amount of fluid lost throughout the treatment (Fig. 1) until D25. No change occurred in the nutritional management during the study period.

2.6. Hormonal variables (P_4, A_4 and E_2)

On days 0, 5, 10, 15, 20 and 30, blood samples from each goat were collected for the determination of plasma progesterone (P_4), estrogen (E_2) and androstenedione (A_4) (Fig. 1). Blood collections were conducted in the morning (08:00 to 09:00 am) by jugular vein puncture using heparinized vacuum tubes (BD Vacutainer® Plus with Heparin, São Paulo, Brazil). The samples were immediately centrifuged at 2000 x *g* for 15 min for separation of the plasma, which was then divided into two aliquots, placed into microtubes and conditioned at −20 °C. Analysis of the plasma P_4, E_2 and A_4 was performed using commercial radioimmunoassay (RIA) kits for use with solid phase (P_4, E_2) and liquid phase (A_4) (ImmuChem Coated Tube, ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA) plasma in a Wizard radiometric detector (PerkinElmer of Brazil Ltda). The blood samples from each animal were evaluated in a single assay. The intra-assay coefficients were 8.3%, 10.1% and 9.2%, for P_4, E_2 and A_4, respectively. All concentrations determined were between the minimum and maximum points of the curve.

2.7. Pregnancy diagnosis

The pregnancy diagnosis was verified by transrectal US at two stages of gestation. The first diagnosis was performed 45 days from

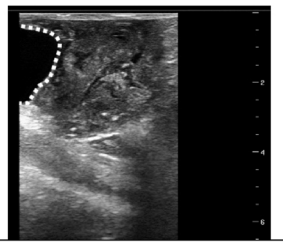
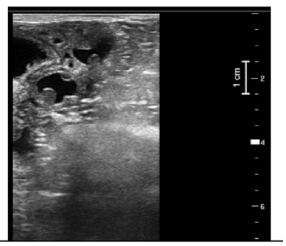
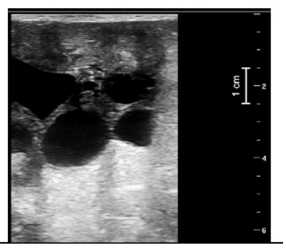
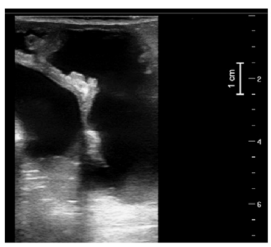
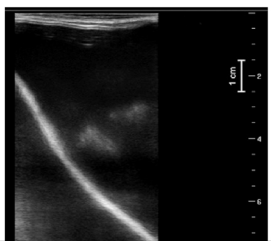
Hydrometra score and features	Ultrasonic images
Grade 0 Absence of fluid Normal Uterus Cross sections of the uterine horn (next to the urinary bladder - dashed line) are shown with the characteristic echogenicity feature of the organ (hypoechoic tissue)	
Grade 1 Presence of small fluid filled sacs (anechoic) Initial hydrometra: similar to the image of ≈ 20 days of pregnancy Note: at this stage of pregnancy it is not common to see the embryo The cross sections of the uterine horn can be checked	
Grade 2 Presence of fluid filled sacs (anechoic) Moderate hydrometra: similar to the image of ≈ 27 days of pregnancy Note: at this stage of pregnancy it would be possible to see the conceptus heart beat The cross sections of the uterine horn can still be checked	
Grade 3 Presence of a large amount of fluid (anechoic) Medium hydrometra: similar to the image of ≥ 60 days of pregnancy. Note: at this stage of pregnancy it would be possible to see conceptus heart beat and placentomes The uterine walls appear as hyperechoic lines formed by a double layer of adjacent tissues	
Grade 4 Presence of a large amount of fluid (anechoic), in almost the entire image Advanced hydrometra: The uterine walls appear as hyperechoic thin lines	

Fig. 2. Scores and ultrasonic characterization of the uterine aspect from images of goats with or without hydrometra.

the end of the emptying protocol (D20). A second diagnosis was performed at 90 days after D20 to verify gestational losses or new pregnancies. Pregnancy was diagnosed in females by observation of the embryonic vesicle for a viable placenta and fetus having a heartbeat.

2.8. Statistics

Descriptive statistics for all the data were calculated. The Lilliefors test was used to verify the data normality. Non-normal quantitative data were analyzed by the Kruskal-Wallis test and means compared by the Dunn test. Frequency data were assessed by

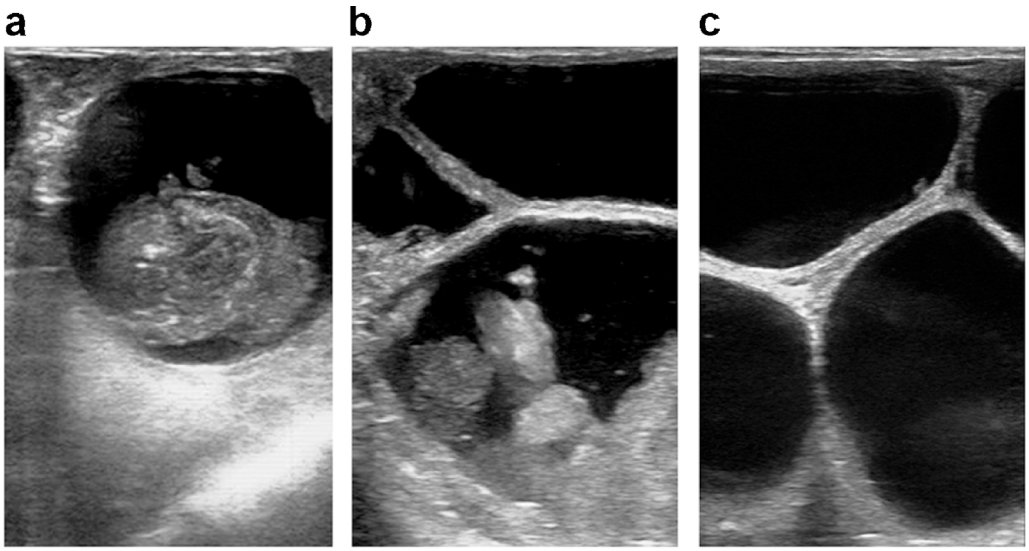


Fig. 3. Ultrasonographic images of goat uteri: (a) early detection of gestational loss, characterized by a misshapen conceptus with homogeneous echogenicity and compact aspect (no occurrence of hydrometra); (b) late detection of gestational loss, characterized by scattered, mobile materials (with different echogenicities) present in one of the segments separated from the uterine wall by anechoic fluid; (c) typical hydrometra, unrelated to the loss of the conceptus, characterized by fluid-filled segments (anechoic), divided by thin uterine walls (hyperechoic).

the Fisher's exact test or chi-square test. For all tests, a computational package (SAEG®, Funarbe, Viçosa, Brazil) was used at a significance level of 5%.

3. Results

3.1. Treatment

There were no differences were obtained between the experimental groups treated with GnRH and the control ($P > 0.05$) (detailed in the topic “Pregnancy diagnoses”). The data, therefore, were grouped regardless of the treatment.

3.2. Ultrasonic evaluation

At the first US evaluation (D0), 20.0% (4/20) of the females there were diagnoses of hydrometra as result of a conceptus death, with content that varied in echogenicity (from hypoechoic to hyperechoic) and with contents being amorphous, and with a changing position as a result of the movement of the goat´s abdomen. In fact, among these four goats, two (Grade 3) had a 90 day pregnancy and two (Grade 2) had a 50 day pregnancy. It is noteworthy that in one goat (1/20), a follicular cyst was detected concomitant with hydrometra (Grade 2).

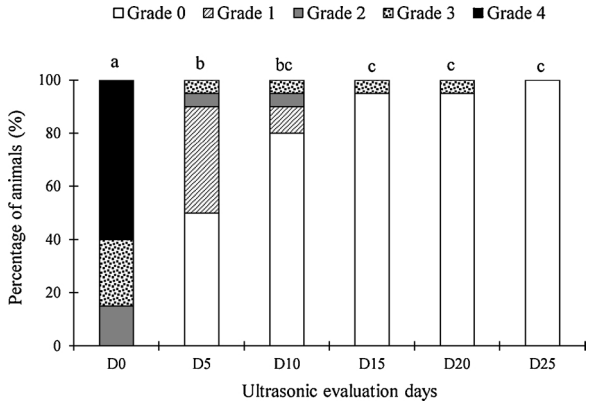


Fig. 4. Uterine drainage based on the uterine fluid ultrasonic score ranging from Grade 0 (absence of liquid) to Grade 4 (advanced amount of fluid) of goats affected by hydrometra and administered three doses of 37.5 μg d-cloprostenol on days 0 (D0), 10 (D10) and 20 (D20).
[a, b, c] Percentages with different superscript letters within columns differed (Fisher’s exact test; $P < 0.05$).

Table 1
Comparison between body weight recorded at the first (D0) and subsequent days (D5, D10, D15 and D20) ($P > 0.05$) due to treatment for uterine drainage with three applications of d-cloprostenol (D0, D10 and D20) in goats ($n = 20$) affected by hydrometra (Mean ± SE).

	Day of treatment			
Variables	D5	D10	D15	D20
Percentage of body weight reduction[a] (%)	10.6	10.3	9.8	8.8
Average body weight reduction[a] (kg)	7.5 ± 1.5	7.3 ± 1.6	6.9 ± 1.6	5.7 ± 1.9
Body weight range	0.0 kg to −29.2 kg	− 0.3 kg to −30.7 kg	+ 0.9 kg to −30.0 kg	+ 2.0 kg to −30.3 kg

[a] Compared with D0 = 71.2 ± 4.1 kg.

As depicted in Fig. 4, in 60.0% (12/20) of the goats, a large amount of uterine contents rated as Grade 4 was detected while 25% (5/20) and 15% (3/20) were Grade 3 and 2, respectively. There was a difference ($P < 0.05$) in the amount of fluid in the uterus observed among the days of treatment (0, 5, 10, 15 and 20).

On D5, there were no females with Grade 4 hydrometra. After the first and second application of d-cloprostenol, a percentage of 50.0% (10/20) and 95.0% (19/20) of the goats respectively, had total drainage of the uterine contents. Importantly, after the uterine emptying, a unique follicular cyst was visible in one goat on D5 and in two goats on D20.

3.3. Weighing and evaluation of the body condition score

Data on the variation of BW, which was compared from D0 to each of the 5 days of treatment (D5, D10, D15 and D20), are shown in Table 1. The greatest BW loss occurred after the first application of d-cloprostenol (D0 compared with D5), due to the large amount of liquid drained from the goats with advanced hydrometra (Grade 4). Only one goat maintained its body weight throughout the experiment. In the D0 to D10 interval, one goat with uterine fluid rated as Grade 4 had a reduction of 30.7 kg in body weight due to expulsion of the uterine contents. Beginning with D10, most females (11/20; 55.0%) began to exhibit a minor gain in weight (1.2 ± 0.2 kg) compared with the previous weights. Furthermore, throughout the experiment, there were no significant change in BCS.

3.4. Hormonal variables (P_4, A_4 and E_2)

There was no treatment effect (GnRH compared with control) was observed on the hormonal concentrations ($P > 0.05$). The data, therefore, were grouped and evaluated as a function of time (days 0, 1, 15, 20 and 30). Plasma concentrations of the three hormones analyzed are listed in Table 2.

On the first day of treatment (D0), 90.0% (18/20) of the goats had plasma P_4 concentrations greater than 1.0 ng/mL. The remaining two goats (2/20; 10%) had a concentration of $P_4 < 1.0$ ng/mL (Grades 2 and 4). The goat with the follicular cyst concomitant to hydrometra (Grade 2) had a P_4 concentration of 1.6 ng/mL and E_2 concentration of 16.2 pg/mL, with both concentrations being greater than the basal concentration. Although the number was small, there was no correlation ($P > 0.05$) between the amount of fluid in the uterus (Grades 0–4) and the concentrations of plasma P_4.

There was a decrease in the plasma concentration of P_4 in response to the first administration of d-cloprostenol (D0) (3.51 compared with 0.31 ng/mL, $P < 0.05$). After the second administration of d-cloprostenol, there was no decrease in the P_4 values (1.32 compared with 1.19 ng/mL, $P > 0.05$). When the administrations were evaluated individually, on D10 and D15 there were 65.0% (13/20) and 50.0% (10/20) of the goats, respectively, with P_4 concentrations < .1.0 ng/mL.

There was no difference ($P > 0.05$) in average plasma concentrations of E_2 throughout the treatment period. In addition, the average plasma E_2 was greater than basal concentrations of the hormone. Nevertheless, goats that were diagnosed as pregnant at 45 days after the third application of d-cloprostenol had an average plasma E_2 on the D20 that was greater than the value in the nonpregnant goats (36.16 ± 11.26 compared with 5.18 ± 4.52 pg/mL, $P > 0.05$). Similarly, the average plasma A_4 on D20 (1.70 ± 0.84 compared with 0.73 ± 0.50 ng/mL, $P > 0.05$) was also greater in pregnant when compared with nonpregnant goats

Table 2
Plasma concentrations of progesterone, androstenedione and estrogen (Mean ± SE) in goats affected by hydrometra after receiving three doses of d-cloprostenol at 10-day intervals (Days 0, 10 and 20) on days 0, 5, 10, 15, 20 and 30 of the uterine emptying protocol of goats ($n = 20$).

Days	Progesterone (ng/mL)	Androstenedione (ng/mL)	Estrogen (pg/mL)
0	3.51 ± 0.66[a]	0.11 ± 0.02[bc]	18.50 ± 7.15[a]
5	0.31 ± 0.11[b]	0.02 ± 0.01[c]	24.45 ± 3.88[a]
10	1.32 ± 0.38[b]	0.19 ± 0.06[bc]	11.28 ± 2.96[a]
15	1.19 ± 0.32[b]	1.22 ± 0.49[abc]	24.21 ± 5.67[a]
20	3.24 ± 0.75[a]	1.32 ± 0.51[ab]	24.57 ± 8.25[a]
30	3.20 ± 0.31[a]	3.11 ± 0.83[a]	41.26 ± 17.74[a]

[a, b, c]Means with different superscripts within columns differed (Kruskal − Wallis test; $P < 0.05$).

Table 3
Ultrasonography of the uterine fluid score as assessed on D0 and pregnancy diagnoses conducted at 45 and 90 days after uterine emptying in goats (n = 20) affected by hydrometra.

Ultrasonography uterine fluid score (D0)	Number of animals	Pregnancy rate (%)	
		45 days[a]	90 days[b]
Grade 1 (initial)	0	–	–
Grade 2 (moderate)	3	33.3 (1/3)	33.3 (1/3)
Grade 3 (medium)	5	60.0 (3/5)	40.0 (2/5)
Grade 4 (advanced)	12	41.7 (5/12)	667 (8/12)
Total	20	45.0 (9/20)	55.0 (11/20)

[a] Diagnosis at 45 days after the third treatment with d-cloprostenol.
[b] Diagnosis at 90 days after the third treatment with d-cloprostenol.

45 days after the end of treatment.

3.5. Pregnancy diagnoses

The conception rate verified at 45 days after the end of treatment was 40% (4/10) and 50% (5/10) in the GnRH and saline groups, respectively ($P > 0.05$). As a result of there being no difference between the groups, the data were organized by the degree of hydrometra and diagnosis of pregnancy (Table 3).

At the time of the first US diagnosis 45 days after the third application of d-cloprostenol, 45.0% (9/20) and 55.0% (11/20) of the goats were diagnosed as pregnant and nonpregnant, respectively. In four nonpregnant females (4/11, 36.4%), alterations in the reproductive tract related to subfertility or infertility were identified: follicular cyst (1/11, 9.1%), hydrosalpinx (2/11, 18.2%) and recurrence of hydrometra (1/11, 9.11%). The results with the second US assessment at 90 days after the end of the treatment, indicated there was a percentage of 55.0% (11/20) and 45.0% (9/20) of pregnant and nonpregnant goats, respectively. Notably, two of the three females that had an image of follicular cyst during the course of treatment [D5 (P_4 = 1.9 ng/mL) and D20 (P_4 = 3.5 ng/mL)] were positively diagnosed as pregnant at 90 days. The goat diagnosed with a follicular cyst at 45 days developed hydrometra. Two goats had a fetal death at 90 days; however, only one of the does had hydrometra. In addition, one other goat had a recurrence of the disease. No change was observed in the females diagnosed at 45 days with regard to detection of hydrosalpinx and hydrometra.

4. Discussion

In the present study, there was detection of one case of hydrometra concomitant with the present of a follicular cyst at D0, after the first and second administration of d-cloprostenol. Due to the uterine emptying, there could be ultrasonic observations of follicular cysts and there were occurrences in one goat at D5 (G_{GnRH}) and two goats at D20 ($G_{Control}$), respectively. Goats were considered to have a follicular cyst(s) if an anechoic structure > 10 mm in diameter was detected in the absence of CL in three ultrasonic examinations performed at 5-day intervals (Medan et al., 2004). According to Gonzalez-Bulnes et al. (2010) with ultrasonic assessments, follicular cysts have the same appearance as follicles but with a diameter from 10 to 30 mm, and with little or no visible luteinization. The administration of 25 μg of GnRH on D5 occurred in an attempt to luteinize any possible ovarian follicular cysts that were concomitant to the hydrometra cases, but there was not improvement in the pregnancy rate as a result of this treatment. Souza et al. (2013) also reported the occurrence of follicular cysts in goats with hydrometra and used 500 IU of hCG, 7 days after the first prostaglandin administration, without an improvement occurring in the conception rate between the experimental groups. Regassa et al. (2009) observed a greater prevalence of disorders in the uterus of ewes with ovarian abnormalities than in females without ovarian abnormalities. It was noted that the possibility of uterine abnormalities was six times greater when there was a pathological condition of the ovaries. In this context, the presence of one goat diagnosed with an ovarian follicular cyst and recurrence of hydrometra at 45 and 90 days after the end of treatment is noteworthy.

As a result of US assessments performed on D15, 95% of the goats had complete uterine emptying after the second application of d-cloprostenol. In one goat classified as Grade 4 on D0, however, the complete emptying of the uterus (Grade 0 – visualized in D25) occurred only after the third application of d-cloprostenol on D20. This fact verifies that some females do not have total uterine emptying, confirming the hypothesis in the present study that an additional application of PGF2α is required to assure complete uterine emptying in all goats. If US capacity is available, therefore, the need of the third dose can be evaluated on D15. For some studies, it was also reported that there was only a partial uterine emptying using just one or two doses of PGF2α (Pieterse and Taverne, 1986; Martel, 2001; Souza et al., 2013). Notably, even when the hydrometra was classified as Grade 4 on D0 and the need existed for a third administration of d-cloprostenol, the goat recovered reproductive functions, and a pregnancy was confirmed at 90 days after the end of treatment.

Because no change occurred in the diet during the study, at D10, all goats had a decrease in body weight of 0.3–30.7 kg due to the expulsion of uterine fluids. In fact, the few goats that had a reduction in BW reduced in of less than 1 kg (n = 2), all of these also had a reduction in the grade of hydrometra (from Grade 2 and 3 at D0 to Grade 0 at D10). Wittek et al. (1998) reported a loss of 0.25–8.2 L of liquid, and Souza et al. (2013) also observed a decrease in the BW of 84.2% (16/19) of females ranging from 1.1 to 17.0 kg. In the

present study, some goats had an increase in body weight after D10, possibly due to the greater feed intake as a result of the rumen no longer being compressed by the uterine contents. Martel (2001) reported a decrease in the milk production due to hydrometra. This fact may be related to the lesser food intake due to rumen compression, similar to the condition that occurs at the end of pregnancy (Pugh, 2002). In addition to being one of the major causes of subfertility in dairy goats (Hesselink, 1993) pseudopregnancy with uterine fluid from Grade 3 to 4 can also interfere with animal feeding and impair other important productive indexes such as milk yield.

According to hormonal analyses, on D0, the average plasma P_4 was 3.51 ng/mL, ranging between 0.00 and 12.33 ng/mL. Several researchers also reported values > 2.0 ng/mL in goats with hydrometra (Pieterse and Taverne, 1986; Duquesnel et al., 1992; Hesselink, 1993). Taverne et al. (1994) reported that a "cloudburst" occurs when the concentration of P_4 is < 1.0 ng/mL. The presence of an active CL, therefore, would allow for the maintenance the hydrometra. Kornalijnslijper et al. (1997) induced the retention of the CL to experimentally develop pseudopregnancy and found individual relevant variations among the goats. There was a reported fluid expulsion that occurred when the concentration of P_4 decreased to less than 0.5 ng/mL. In addition, in one goat, ovarian cyclicity ceased soon after the immunization against PGF2α, but the plasma P_4 concentrations reached > 1.0 ng/mL only 4 months later. Even though there are differences between studies, in the luteolysis mechanism, the P_4 reduction to < 1.0 ng/mL are the very first changes (24 h) induced by PGF2α treatment. This response occurs because of a disruption of the steroidogenic pathway as a result of a decreased availability of P_4 precursors by reduction of blood flow to the CL which reduces plasma P_4 concentrations (Arashiro et al., 2010). Consistent with the variable findings reported by Kornalijnslijper et al. (1997), in the current study there were two goats with lesser concentrations of P_4 on D0. In one of the goats, a Grade 4 uterine score and concentration of P_4 = 0.34 ng/mL was observed. With this goat three doses of d-cloprostenol had to be administered to induce complete uterine emptying. Considering the second goat, there was a Grade 2 uterine score and when P_4 concentrations were considered there were indications luteolysis was occurring that contributed to the spontaneous elimination of the uterine fluid before the treatment began.

In addition to monitoring the response to the hormones used during the treatment, the analysis of the E_2 and A_4 were used to endocrine milieu associated with follicular cysts because these cysts may be related to estrogen or androgen concentrations. Cases of hyperestrogenism and virilism have already been related to the occurrence of follicular cysts and hydrometra in cows (Nascimento and Santos, 2011). In the present study, the concentrations of A_4 were considered within the normal values. The mean plasma E_2 on D0 was 18.50 ± 7.15 pg/mL. Similar values were reported by Wittek et al. (1998) who observed that, in goats affected by hydrometra, despite the persistence of CL, 17β-estradiol concentrations were relatively greater on D0 (mean = 13.63 pg/mL). In the present study, one goat had relatively greater concentrations of estrogen (D0 and D20), followed by the development of follicular cyst and hydrometra.

Because the present study was conducted throughout the breeding season, the return of some goats to having estrous cyclicity with the occurrence of an estrus during which there was breeding and a resulting conception after the third administration of d-cloprostenol, or in the subsequent cycle. Overall, the treatment led to fertility of 55.0% (11/20) of the females affected by hydrometra. These results were indicative of a greater improvement than the 20.0% reported by Souza et al. (2013), when hydrometra was related to the presence of ovarian follicular cysts and treated the goats with two doses of 5 mg dinoprost (D0 and D10) and 500 IU hCG (D7). Moraes et al. (2007), however, using one (10/13) or two (3/13) administrations of 0.5 mg dinoprost (D0 and D11) reported that all females were diagnosed positive for pregnancy between 30 or 35 days after mating. In the present study, from the 45.0% (9/20) of the females that did not become pregnant, two were identified with hydrosalpinx, four had hydrometra recurrence (one diagnosed at 45 and three at 90 days after treatment), and three had US images indicating there was a healthy reproductive tract. A recurrence of uterine fluid accumulation has been reported in several studies (Hesselink, 1993; Batista et al., 2001). Thus, the recurrence in females of hydrometra in does that have been previously evaluated with this condition needs to be ascertained (Souza et al., 2013). In the present study, there was verification of the occurrence of hydrosalpinx concomitant to hydrometra in 10.0% (2/20) of the goats. The fetal loss likely is related to both diseases; hydrosalpinx could be a possible cause of the inadequate implantation of the conceptus (Strandell, 2000; Margalioth et al., 2006), and hydrometra the consequence of this condition (Humblot et al., 1995; Brice et al., 2003) due to the retention of the CL. In the present study, among the females that had hydrosalpinx, one developed hydrometra due to the death of the concept.

5. Conclusions

In conclusion, the use of three doses of d-cloprostenol 10 days apart was effective inducing the draining of the uterine contents of goats with hydrometra. The use of GnRH 5 days after the first prostaglandin administration did not benefit the pregnancy rate. The need for a third application of d-cloprostenol is emphasized, especially in goats classified as Grade 4. The ultrasonography approach used in the present study allowed for the classification of hydrometra in goats with categorizations occurring based on scores that related to the characteristics of uterine content. This approach was useful for understanding the reason that hydrometra develops and can be applied in the field especially in farms where US diagnoses are not frequently performed.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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