Short communication

Hormonal treatment of dairy goats affected by hydrometra associated or not with ovarian follicular cyst

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ABSTRACT

Hydrometra is considered a very important pathological condition, because it represents one of the main causes of temporary infertility in dairy goats. The objective was (i) to evaluate a protocol for the treatment of hydrometra associated ($n = 2$) or not ($n = 17$) with follicular ovarian cyst in 19 dairy goats and (ii) to assess its reproductive efficiency after treatment. For this purpose, 10 mg of dinoprost (PGF) divided in two equal doses were administered to all animals intravulvosubmucosally on Days 0 and 10. In addition, 500 IU hCG were administered on Day 7. Ultrasound exams were performed in all females from Days 0 to 3, 7 and 10 to 13 of treatment, in order to evaluate uterus drainage after each treatment. Goats were monitored for estrus after both treatments and mated after the second dose of PGF. Blood samples were collected from 11 goats to determine plasma progesterone concentrations before, during and after treatment. Of the 19 goats treated, 16 lost weight after the first dose, probably due to uterine discharge. Complete drainage of uterine fluid was observed in 11/19 (57.9%) and 17/19 (89.5%) after the first and second doses, respectively. Afterwards, we diagnosed 2 more goats with follicular cysts, for a total of 21.1% (4/19) of animals exhibiting hydrometra and ovarian cyst concomitantly. In one doe the diagnosis was on Day 2 and in the other on Day 11. All does showed progesterone concentrations superior to 1 ng/mL at Day 0, with an average of 10.6 ± 1.4 ng/mL. Out of the 10 goats mated, only two became pregnant after treatment, corresponding to 10.5% of the total (2/19). Although prostaglandin was effective to drain the uterine fluid and led to the onset of estrus, it did not improve the pregnancy rate. The use of hCG in female goats was not effective in luteinizing the cysts. It can be concluded that hydrometra alone or associated with ovarian follicular cyst may adversely affect goat reproductive performance.

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1. Introduction

The goat population in Brazil has reached approximately 10 million animals, making it the seventh largest goat herd worldwide (FAO, 2009). The importance of goats as providers around the world of essential food in meat and dairy products has been well reported. In Brazil, this situation is reasonably the same and in order to optimize goat production, the reproductive efficiency has been intensely studied. Among the major dairy goat diseases that can affect the reproductive efficiency, hydrometra or pseudopregnancy is considered one of the most important

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as it can cause subfertility or temporary infertility (Smith, 1980) and it is thus economically relevant for breeders (Kornalijnslijper et al., 1997). Although Smith (1980) proposed a difference between pseudopregnancy and hydrometra, most authors consider them synonyms (Pieterse and Taverne, 1986; Martel, 2001).

Hydrometra occurs mainly in dairy goats and is characterized by excessive accumulation of sterile fluid (Wittek et al., 1998) in the uterus in the absence of fetuses and placentomes. The typical ultrasonographic image shows non-echogenic fluid compartments separated by double-layered thin tissue walls (Hesselink and Taverne, 1994). Apparently the presence of fluid is the result, but not the cause, of a prolonged progesterone secretion (Taverne et al., 1988), probably due to a failure in the luteolytic mechanism. According to Brice et al. (2003), there are two important mechanisms for hydrometra establishment: one is the spontaneous persistence of the corpus luteum (CL) after an ovulation without fertilization and the other is the persistence of the CL after an early embryonic mortality. Chemineau et al. (1999) stated that at least 50% of the cases of hydrometra in goats occur as a consequence of embryo mortality that apparently takes place at a gestational age of about 40 days (Wittek et al., 1998). Some other causes could be associated with indiscriminate use of hormones or mating outside the breeding season (Pugh, 2002). However, this can also be observed in goats with spontaneous or synchronized ovulation and also whether or not does have been mated (Pieterse and Taverne, 1986; Wittek et al., 1998), being difficult to establish a profile. Nonetheless, Hesselink and Elvin (1996) identified a genetic influence on its occurrence. The hormonal and physiological changes (mostly do not present estrus and usually show an increase in abdominal volume) are consistent with pregnancy and breeders are convinced the goat is pregnant. Therefore, the disease affects the interval between births, reducing the number of lactating females and consequently reducing the milk production of the herd (Martel, 2001).

Prostaglandin F2α (PGF) or a synthetic analog is indicated for the treatment of hydrometra, leading to luteolysis of one or more CLs, uterine drainage and return of estrous onset (Wittek et al., 1998; Lopes Junior et al., 2004). In sheep, the prevalence of hydrometra was significantly higher in ewes with ovarian cyst, although authors did not differentiate between follicular or lutein when both pathological conditions were present (Regassa et al., 2009). According to Gordon (1997), PGF is also effective for the treatment of animals showing luteal cyst or even a combination of both persistent CL and ovarian luteal cysts. Conversely, for treatment of a follicular cyst, the application of gonadotropin-releasing hormone, hormonal preparation that has similar activity or that resembles the luteinizing hormone, or application of human chorionic gonadotropin (hCG) are indicated for goats (Smith, 1980). The knowledge of ovarian cysts in goats is still incipient and little is known about its causes and incidence (Tanaka et al., 2007).

Literature is still contradictory in relation to reproductive efficiency of females after hormonal treatment of hydrometra. Therefore, the aim of this study was to: (i) evaluate the efficacy of a treatment for hydrometra associated or not with follicular cyst in dairy goats using prostaglandin and hCG; (ii) characterize their plasma progesterone (P_4) profile and (iii) evaluate their subsequent reproductive efficiency.

2. Materials and methods

2.1. Location and experimental conditions

This study was conducted during June and July of 2009 (end of breeding season), in a commercial goat milk farm, in the rural area of Piau, located in the state of Minas Gerais, Brazil (latitude 21°35′S, longitude 43°15′W and altitude of 435 m). The climate is characterized as mesothermal Cwa, according to Köppen classification, dry winter and hot summer (Peel et al., 2007). The average minimum and maximum temperatures during the study were 18.8 and 27.7 °C, respectively. The average daily rainfall was 8.75 mm^3 and relative humidity ∼75%.

The goats were kept in an intensive system, within pens 15 m long by 2 m wide which housed 10 goats each, providing 3 m^2 per animal, allowing a high degree of animal welfare (Ribeiro, 1997). Goats were fed corn silage and *Pennisetum purpureum* as forage. Furthermore, a balanced concentrate supplement was fed according to milk production (National Research Council, 2007). Mineralized salt (Salminas Goats®, Nutriplan, Juiz de Fora, MG, Brazil) and drinking water were available *ad libitum*. This research was reviewed and approved by the Animal Care Committee of Fluminense Federal University (UFF/0048-08).

2.2. Experimental design

As experimental animals, 19 goats with hydrometra (15 Toggenburg and 4 Saanen breed; 17 pluriparous and 2 nulliparous), of which 2 with concomitant ovarian cysts were used. Does were between 1 and 8 years old, had an average weight of 56.4 ± 3.1 kg and mean body condition score (BCS) of 3.5 ± 0.1 (range 1–5). Both hydrometra and ovarian cysts were diagnosed by ultrasound. Follicular cyst diagnosis was made when a non-echoic structure >10 mm in diameter was detected during 15 days (Medan et al., 2004). All examinations were conducted with a B-mode transrectal ultrasonographic scanner with 5 MHz transducer (Aloka SSD 500®, Tokyo, Japan). To facilitate manipulation of the transducer, it was taped to a PVC tube. Does were maintained in a standing position, fecal pellets were removed manually (with a finger), and 20 mL of carboxymethylcellulose gel was placed into the rectum with a syringe. Animals that showed fluid in the uterine lumen in the absence of fetuses and placentomes were diagnosed with hydrometra. Ovaries were located as previously described (Ginther and Kot, 1994).

All animals were treated with two doses of 5 mg of dinoprost (Lutalyse®, Pfizer Animal Health of Brazil, São Paulo, Brazil), intravulvosubmucosally (Mellado et al., 1994), with a 10 day interval (Day 0 and Day 10). Then, for the treatment of ovarian follicular cyst, on Day 7, animals were treated with 500 IU hCG (Vetecor®, Calier Laboratories Ltd., Brazil). All animals diagnosed with hydrometra were also treated with hCG as they could have a non-diagnosed ovarian cyst concomitantly to hydrometra.

Ultrasound scans were performed from Days 0 to 3, on Day 7 and from Days 10 to 13 of treatment with the aim of monitoring the emptying of the uterus and also ovarian cyst involution. In addition to ultrasound examination, the animals underwent evaluation of body weight and BCS on Days 0, 3, 10 and 13. After each dinoprost treatment, estrus was monitored from Days 0 to 3 and from Days 10 to 13 with the use of bucks twice a day (07:00 and 19:00 h) and females were considered to be in estrus when allowed to be mounted. After the second treatment of prostaglandin, does displaying signs of estrus were mated by fertile bucks at the onset of estrus and 24 h later if they were still in estrus. Pregnancy rate and ovarian cyst involution were diagnosed 30 days after natural mating by ultrasonography.

2.3. Plasma progesterone concentrations (P_4)

Blood samples were collected from 11 goats randomly selected by jugular veinpuncture, into tubes containing EDTA at the following times: concurrent with the first PGF administration (Day 0); five days after (Day 5); at the day of hCG administration (Day 7); concurrent with the second PGF (Day 10) and three days later (Day 13). Only 11 out of 19 does were selected since 10–11 animals should be enough to demonstrate a progesterone behavior, like we demonstrated in other studies we have published (Souza et al., 2011; Pinna et al., 2012). Tubes were immediately

placed on ice, transported to the laboratory, and centrifuged at $2000 \times g$ for 15 min. Plasma was removed and stored at $-20\,°C$ pending determination of plasma P_4 concentrations with a commercial solid phase radioimmunoassay (RIA) kit (Coat-a-Count® progesterone kit, DPC, Diagnostic Products Corporation, Los Angeles, CA, USA), used according to the manufacturer's instructions. The mean intra- and inter-assay coefficients of variation were 8.8 and 9.7%, respectively, and the analytical detection limit was 0.08 ng/mL.

2.4. Variables

The following end points were determined: estrus response (number of does in estrus/number of treated does × 100); interval to estrus (from the first or second PGF to first acceptance of mounting); estrus duration (interval from the first to last acceptance of mounting); pregnancy rate (number of pregnant does/number of does treated); and plasma P_4 concentration. The results are described as mean ± SE.

3. Results

3.1. Sexual behavior and ultrasonography end points

In the dairy goat herd studied, 19 animals out of a total of 154 were diagnosed with hydrometra (incidence of 12.4%) after a routine pregnancy diagnosis. Very large fluid compartments were seen and although not precisely quantified a representative part of goats also had membranes in the

uterine fluid, suggesting embryonic reabsorption. Within the 19 goats treated, 16 lost weight (1.1–17 kg with an average of 4.3 ± 0.9 kg per doe) after the first dose of PGF, presumably due to uterine discharge (Fig. 1). As expected, no difference was observed in the BCS before and after treatment. Complete drainage of uterine fluid was observed in 11/19 (57.9%) by ultrasound from 48 to 72 h after the first dose of PGF. Following the second dose, six of the eight remaining goats showed normal uterine imaging. Therefore, the treatment for hydrometra was efficient in 17/19 (89.5%), since just two does showed fluid retention in the uterus after two doses of PGF. After uterus drainage it was possible to diagnose 2 more goats with follicular cysts (Fig. 2). In one doe the diagnosis was on Day 2 and in the other on Day 11. Therefore, 21.1% (4/19) of animals diagnosed with hydrometra showed simultaneously ovarian cyst. The use of 500 IU hCG was not effective to luteinize the cyst in all 4 goats.

After the first dose of PGF, only 36.8% (7/19) of the dairy goats showed estrus and after the second dose three more does manifested estrus, making up a total of 52.6% (10/19). The interval to estrus and duration of estrus was 39.4 ± 5.0; 29.1 ± 5.8 h (after first PGF) and 40.8 ± 5.4; 30.0 ± 6.3 h (after second PGF), respectively. Only two of the 10 goats
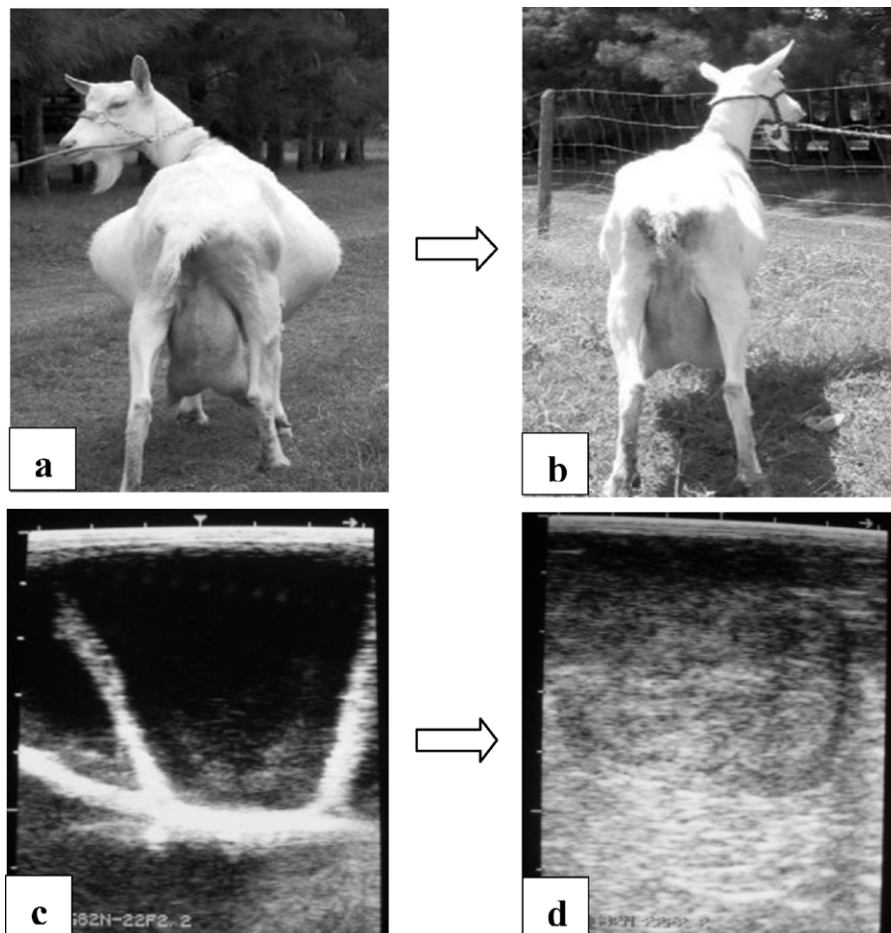

Fig. 1. (a) Saanen doe presenting hydrometra before, (b) 72 h after treatment and (c and d) corresponding ultrasonography images taken, respectively.
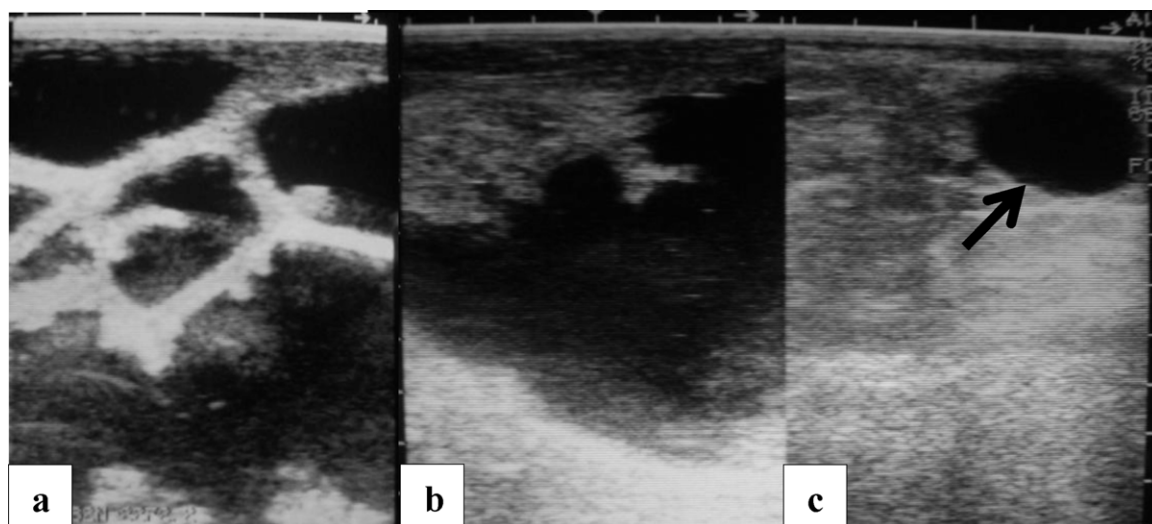
Fig. 2. (a) Goat presenting well-developed hydrometra before treatment, (b) 72 h after prostaglandin, partial uterus drainage, making possible to diagnose and (c) the ovarian follicular cyst (arrow).

mated (20.0%) became pregnant after treatment; considering the total number of goats treated, the percentage is lower (2/19; 10.5%). Regarding the nine goats which did not show estrus, four had ovarian cyst, two did not have their uterus emptied and three had normal imaging of both uterus and ovaries.

3.2. Plasma progesterone concentration

All 11 does evaluated showed concentrations superior to 1 ng/mL at Day 0, with an average of 10.6 ± 1.6 ng/mL. One female did not have her uterus emptied and maintained high progesterone concentrations throughout all the experimental period, even after both PGF doses (average of 9.9 ± 2.3 ng/mL in the five analyses). The other 10 does showed a deep decrease in P_4 from the first (Day 0) to the second analysis (Day 5). All goats that had estrus detected after the first dose presented concentrations superior to 1 ng/mL at Day 5 or 7, probably as a result of a new ovulation. Both females that were identified with ovarian cyst
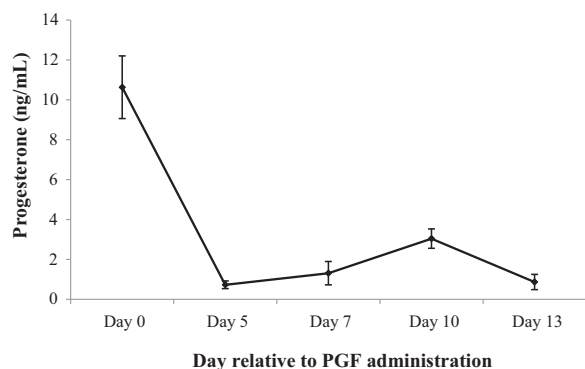

Fig. 3. Plasma progesterone concentration in 10 goats diagnosed with hydrometra, before (Day 0), after the first dose of prostaglandin (Day 5), before (Day 10) and after the second dose of prostaglandin (Day 13).

before treatment showed no differences in their progesterone profile in comparison with the others. Only one goat had P_4 supraluteal concentrations (4.25 ng/mL) after the second dose whereas in all the others P_4 decreased to subluteal concentrations at Day 13. The P_4 standard profile (10 goats) before, during and after treatment is shown in Fig. 3.

4. Discussion

In this study, an incidence of hydrometra of 12.4% was detected; comparable to what has been previously reported, varying from 3% (Martel, 2001) to 30% (Lopes Junior et al., 2004) in dairy goat herds. A higher number of Toggenburg goats compared to Saanen were diagnosed with hydrometra, but there were more of the Toggenburg breed in the commercial farm evaluated, and as a result, no difference was detected in the prevalence of this disease concerning both breeds. It is well known that hydrometra is associated with the presence of at least one CL in the ovary. However, according to the consulted literature, this study is the first report of goats having both hydrometra and ovarian follicular cyst concomitantly, i.e., a CL in one ovary and a cyst in the contra-lateral ovary. Two animals were diagnosed with both disorders before the treatment and, just after the drainage of the uterus fluid, it was possible to diagnose two more does; therefore, 21.1% (4/19) of animals diagnosed with hydrometra also showed ovarian cysts. The uterine dilation promoted by the uterine pathological condition could lead to difficulties diagnosing ovarian cyst, which may be resolved by drainage. Therefore, we suggest that the treatment for hydrometra should be complemented with a treatment for ovarian cysts, because even if they are not diagnosed at the same time, a high number of goats can present both disorders. In sheep, the prevalence of hydrometra was significantly higher in ewes with ovarian cyst, although authors did not report if cysts were follicular or luteal (Regassa et al., 2009). This indicates

that both pathological conditions may often be associated with small ruminants.

Regarding the hormonal protocol proposed, our results demonstrated that a single administration of PGF is not a satisfactory therapy for hydrometra (57.9 and 89.5% of efficiency in draining uterus contents after the first and second dose, respectively). This corroborates with Hesselink (1993) who reported that after the first PGF, only 20 of 49 (41%) does showed estrus and after the second dose, all the remaining 29 goats came into estrus. Conversely, Salles and Araújo (2008) treated dairy goats with a single dose of PGF and demonstrated that it was sufficient to induce uterus drainage in all animals. Another treatment was proposed by Taverne et al. (1988), using bromocryptine injections in affected goats to chronically suppress prolactin secretion. This was able to decrease P_4 concentrations by CL due to the diminished luteotrophic effect of prolactin. However, the same group reported later that there was no correlation between the concentration of prolactin and the development or the presence of a persistent CL (Hesselink et al., 1995). The data in the current experiment and the literature confirm that the double dose of PGF or its analogs are the best option for the treatment of hydrometra.

After the use of PGF, drainage of the uterine fluid occurs between 24 and 48 h (Wittek et al., 1998) or most often from 48 to 72 h, as in this study. However, it has been reported that even when the uterus drainage is performed, it is fairly common to have a recurrence developing in the next year or after the first estrus treatment (Pieterse and Taverne, 1986; Hesselink, 1993; Wittek et al., 1997; Leboeuf et al., 1998; Lopes Junior et al., 2004). Although using PGF to promote luteolysis in the present study was confirmed, it was observed that 500 IU hCG was not effective in luteinizing the ovarian cysts and restoring the ovarian function and other protocols should be proposed and evaluated at a later date. Some observations suggest that the etiology of follicular cysts in small ruminants is partially different from that of cows (Tanaka et al., 2007) and possibly that there are different mechanisms mediating the occurrence of follicular cysts between cows and goats.

Most of the females returned to estrus after treatment in the present study, but only a few of them became pregnant, indicating that the reproductive efficiency was severely affected. The literature is contradictory regarding the reproductive efficiency after treatment. Pieterse and Taverne (1986) suggested that even in the case of hydrometra the uterine wall is still capable of releasing prostaglandins as the does had a drop of the P_4 levels after the treatment with oxytocin. The authors described that six goats from a total of eight treated became pregnant some weeks after treatment. Moraes et al. (2007) observed that all female goats ($n = 11$), diagnosed with hydrometra and treated with PGF showed estrus within 120 h, were mated and were positive for pregnancy after 30 days. Conversely, goats treated with PGF and then submitted to artificial insemination had an average fertility rate of 48%, lower than 73% obtained in goats without hydrometra in the same herds (Leboeuf et al., 1998). Hesselink (1993) cited that reproductive performance improves when a second treatment is applied. After the first PGF, only 3 out of 20 does

conceived but, after the second dose administered 12 days later, 14 out of 29 became pregnant. We believe that if the disorder is maintained for a long time it could irrevocably damage the endometrium, perhaps altering uterus capacity for hormone secretion and leading to subfertility in affected goats. The possibility of using consecutive services with higher interval from the end of treatment could improve pregnancy rate (Wittek et al., 1997) but, in commercial systems like in the current study, this management would not be profitable.

All 11 does displayed supraluteal progesterone concentrations at Day 0 as a result of the persistent CL. This is in accordance to Wittek et al. (1998) who described all affected goats with elevated levels of progesterone before treatment. Taverne et al. (1988) determined that the progesterone concentration in goat with hydrometra was always above 2 ng/mL. However, Wittek et al. (1998) reported that 5 out of 30 affected goats had concentrations between 1 and 2 ng/mL. It seems plausible to consider the progesterone concentration of 1 ng/mL as the baseline for the maintenance of hydrometra.

5. Conclusions

In adult female goats with bilateral abdominal distention, hydrometra should always be considered, due to its high incidence in this species. In goats diagnosed with hydrometra, prostaglandin had been effective to drain the uterine fluid and also stimulate the onset of estrus, although pregnancy rate was considerably low. The use of hCG in female goats was not effective to luteinizing the ovarian follicular cysts and restoring reproductive function of does with either hydrometra and ovarian cysts. Therefore, the profitability of maintaining these animals in the herd should be evaluated. If it is decided to treat the animals, it is essential to be aware of the diverse possibilities after prostaglandin administration: (i) if no uterus drainage occurs after subsequent attempts, this animal should be discarded; (ii) if the uterus was drained but goats do not show estrus, submit them to estrous induction if in the non-breeding season; (iii) if the uterus was drained and the doe was bred but is not pregnant, perhaps the implantation or pregnancy maintenance capacity of the embryo is impaired, and thus the female could undergo oocyte or embryo collection. It can be concluded that both hydrometra and ovarian cyst may adversely affect goat reproductive performance and when diagnosed, the female may be discarded or undergo treatment in case of high producing females.

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