



Hydrosalpinx in dairy goats: Occurrence, ultrasound diagnosis, macro- and microscopic characterization

A.L.R.S. Maia^{a,*}, F.Z. Brandão^a, J.M.G. Souza-Fabjan^{a,b}, M.C.C. Araújo^c, L.G.B. Siqueira^d, O. Facó^e, J.F. Fonseca^e

^a Universidade Federal Fluminense, Av. Vital Brazil Filho, 64, CEP 24230-340, Niterói, RJ, Brazil

^b Escola de Ciências da Saúde, Universidade do Grande Rio, Rua Prof. José de Souza Herdy, 1160, CEP 25071-202, Duque de Caxias, RJ, Brazil

^c Departamento de Morfologia e Histologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Rua José Lourenço Kelmer, s/n, CEP 36036-900, Juiz de Fora, MG, Brazil

^d Embrapa Gado de Leite, Av. Eugênio do Nascimento, 610, CEP 36038-330, Juiz de Fora, MG, Brazil

^e Embrapa Caprinos e Ovinos, Estrada Sobral/Groaíras, km 04, CP 145, CEP 62010-970, Sobral, CE, Brazil

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ABSTRACT

Hydrosalpinx is an oviduct disease that can compromise female fertility. This study describes the occurrence and characterization of hydrosalpinx in dairy goats and proposes a new approach for an accurate diagnosis of hydrosalpinx by using transrectal ultrasonography (US). The data were collected from 2579 goats subjected to US exams. Six goats had their reproductive tracts collected *post mortem* and macroscopically and microscopically examined. A total of 28 cases of hydrosalpinx (1.1%) were detected: 27 Saanen goats and one Alpine goat. Among these, 22.2% (6/27) had previously shown other reproductive disorders such as spontaneous abortion (3.7%), follicular cysts (3.7%) and hydrometra (14.8%). Only the oldest goat (12 years old) was pluriparous with history of previous reproductive success, while 27 nulliparous females ranged in age from one to eight years old. The US exams revealed a fluid-filled, rounded structure (> 24 mm) formed by the accumulation of fluid inside the oviduct lumen of the affected goats. The infundibulum was the most commonly affected region, and contained rounded structures up to 100 mm in diameter. The ampulla was the second most commonly affected structure, and minor changes were observed in the isthmus. In one of the 28 positive cases, the presence of bilateral hydrosalpinx was identified. Microscopically, at the infundibulum and ampulla, a decrease and/or absence of the mucosal folds with subsequent reduction of the epithelial surface area was noted. In conclusion, hydrosalpinx may be successfully diagnosed in goats via transrectal US. The occurrence rate of hydrosalpinx in this study reveals the importance of an accurate diagnosis and a strong possibility that hydrosalpinx has been underdiagnosed and neglected in this species.

1. Introduction

Brazil has more than nine million goats spread over different regions of the country. Approximately five million of these goats are raised for milk production (FAOSTAT, 2014). Increasing productivity is still essential for greater profitability and competitiveness of dairy goat milk production. In cattle, the incidence of reproductive disorders, advanced age at first calving and extended calving intervals are among the most important limiting factors for the expansion of livestock production (De Kruif, 1978). Studies carried out with slaughterhouse animals have identified diverse causative factors of subfertility or infertility in small ruminants, including abnormalities of the ovary, oviduct or uterus (Sattar et al., 1988; Santa Rosa, 1996; Palmieri et al., 2011).

Hydrosalpinx is an oviduct disease that can partially or fully compromise female fertility, depending upon whether the disease occurs unilaterally or bilaterally (Noakes et al., 2001; Purohit, 2014). Hydrosalpinx is characterized by a dilated lumen in any of the three portions of the oviduct and may affect up to 80% of the organ (Santa Rosa, 1996). The possible causes of hydrosalpinx include congenital malformations or an inflammatory process sequela (Grunert et al., 2005). Despite its detrimental effect on fertility, hydrosalpinx is generally identified only after slaughter, and some studies have addressed the occurrence of hydrosalpinx in goats found at slaughter (Sattar et al., 1988; Beena et al., 2015). However, the use of transrectal ultrasonography (US) allows the identification of changes or abnormalities in the female reproductive tract in a noninvasive, real-time manner,

* Corresponding author.

E-mail address: maia.analucia@gmail.com (A.L.R.S. Maia).

which provides immediate clinical answers (Medan and Abd El-Aty, 2010). The US examination of the reproductive tract of females with a history of infertility that reveals anechoic content in segments cranial to the urinary bladder may often lead to the diagnosis of hydrometra or cystic ovarian disease (Janett et al., 2001; Dawood, 2010). These ultrasonographic findings may be confounding factors that could hamper the correct diagnosis of hydrosalpinx. Currently, there is only one report in the literature describing the occurrence of hydrosalpinx in one live goat. Interestingly, the animal was previously treated for hydrometra. The laparoscopic procedure resulted in the correct diagnosis of hydrosalpinx (Janett et al., 2001).

Therefore, the objective of this study was to present the ultrasonographic diagnosis of hydrosalpinx in goats and to describe its macro- and microscopic characteristics. Additionally, a new approach for an accurate differential diagnosis of hydrosalpinx and other reproductive pathological conditions was proposed.

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 the experiment was conducted under the principles of the Brazilian Society of Laboratory Animal Science.

2.2. Location and study conditions

Data were collected between January 2015 and March 2017 from dairy goats of 22 commercial herds located in the Southeastern region of Brazil (States of São Paulo, Rio de Janeiro, Espírito Santo and Minas Gerais). In 19 farms, goats were raised in an intensive production system, confined in group pens, fed with chopped *Pennisetum purpureum* forage or corn silage and concentrate supplementation. In the three remaining farms, the production system was semi-intensive with access to pasture. Water and mineralized salt (Caprinofós[®]; Tortuga, São Paulo, Brazil) were available *ad libitum*.

2.3. Animals

Dairy breed goats (n = 2579) ranging from eight months to 12 years of age were subjected to transrectal ultrasonographic examination. The majority of the enrolled goats were Saanen (77.4%; 1996 of 2579), but other breeds included Alpine (14.0%; 361 of 2579), Toggenburg (6.7%; 172 of 2579), Anglo-Nubian (0.3%; 8 of 2579) and crossbreeds (1.6%; 42 of 2579).

2.4. Ultrasonography

The reproductive tract of each goat was examined 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 US evaluations, goats remained standing, 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 containing 10 mL of carboxymethylcellulose gel (Carbogel UTL[®]; Carbogel Indústria e Comércio LTDA, São Paulo, Brazil) was used to deposit lube into the goat's rectum for lubrication and to increase the contact surface between the transducer and the wall of the rectum.

After visualization of the urinary bladder, imaging of the uterus and ovaries were performed to evaluate reproductive soundness. Once an abnormality was observed on the reproductive organs, sonographic

representative images were saved for subsequent analysis. In cases of recent mating (< 30 days), positive pregnancy diagnoses were re-confirmed at a later stage of gestation (60 days). Females diagnosed with hydrometra and mucometra were treated as previously described (Maia et al., 2016) and re-evaluated by US 30 days later to confirm whether the uterine contents were expelled. Thus, during the study period, some females were examined by US more than once.

2.5. Macroscopic and microscopic analysis

Six goats presenting with histories of infertility and with US evaluations suggestive of hydrosalpinx were discarded by producers, and their reproductive tracts were recovered in the slaughterhouse. The tracts of four of these six goats were macroscopically examined, measured and photographed with a DSLR digital camera (Canon EOS Rebel XSi[®]; Tokyo, Japan), and the reproductive tracts were microscopically analyzed, as described below.

Tissue fragments of the three anatomical portions of the oviduct (infundibulum, ampulla, isthmus) and from both uterine horns were collected from two goats, which had no apparent abnormalities other than hydrosalpinx. The 2-cm³ tissue fragments were fixed in a solution of 10% formaldehyde buffered with sodium phosphate 0.1 M at 4 °C for 24–48 h. After fixation, a standard histological procedure (dehydration, diaphanization and inclusion in paraffin) followed by microtomy cuts with a thickness of 4 µm was performed after a 20-µm thinning of the specimen. The histology sections were mounted and stained with hematoxylin and eosin (H&E). The microscopic evaluations of tissue samples were performed using an optical microscope (Primo Star LED[®]; Carl Zeiss Microscopy GmbH, Göttingen, Germany) attached to a digital camera (Axio Cam ERc 5s[®]; Carl Zeiss Microscopy GmbH, Göttingen, Germany). Images were captured and analyzed using an image processing software (ZEN lite 2012[®]; Carl Zeiss Microscopy GmbH, Göttingen, Germany). Assessment of morphometric features was performed in six fields of each fragment by examining the epithelium, connective tissue and muscular layers (Wittek et al., 1998; Zhu et al., 2013).

2.6. Statistics

Data are presented in a descriptive form. The determined end points were overall hydrosalpinx occurrence rate, hydrosalpinx occurrence rate per breed, hydrosalpinx occurrence rate per category (either nulliparous or pluriparous) and hydrosalpinx occurrence rate associated with other reproductive disorders.

3. Results

3.1. Hydrosalpinx occurrence

Of the 2579 goats examined by ultrasonography, 28 cases of hydrosalpinx (1.1%) were detected. Twenty-seven of the goats with hydrosalpinx were Saanen (1.35%; 27/1996) and one was Alpine (0.27%; 1/361). Only the oldest goat (12 years old) was pluriparous with history of previous reproductive success. The majority of other 27 nulliparous females were between one and two years of age (23/27; 85.2%), with an overall age range of one to eight years old.

Anamnesis revealed that all the nulliparous goats had shown previous estrus, had been mated by fertile bucks, but never had successful pregnancies and therefore no history of kidding. Among these goats, 22.2% (6/27) had previously shown other reproductive disorders, either reported by the farmer [spontaneous abortion, (3.7%; 1/27)] or diagnosed via US in the present study [follicular cyst (3.7%; 1/27) and hydrometra (14.8%; 4/27)]. In the four goats diagnosed with hydrometra, visualization of oviductal alterations was only possible after treatment and complete emptiness of uterine content.

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