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Review

PREGNANCY DIAGNOSIS TECHNIQUES IN GOATS – A REVIEW

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Summary

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Several techniques for pregnancy diagnosis in goats are developed. Some of them are efficient and applicable in the field, but not enough accurate. Others are outlined with a high precision, but require equipment and specialised skills. Laparoscopy, laparotomy and vaginal biopsy are accurate but invasive and thus, inappropriate for the routine practice. Abdominal inspection, transabdominal palpation and increased live weight could be indicative for pregnancy in goats but they are reliable only after the second half of gestation. Udder examination and palpation are of low accuracy and their independent application for pregnancy diagnosis is not suitable. Blood, milk and faecal progesterone assays could indicate pregnancy in goats after the 21st post insemination day until the end of gestation. The detection of pregnancy associated glycoproteins (PAGs) in blood plasma or milk samples is an early pregnancy marker but requires laboratory equipment. Non-return to oestrus is a cheap, practical and widely used method for detection of pregnancy between post insemination days 17 and 21 and does without signs of oestrus are assumed to be pregnant. A-mode, B-mode and Doppler ultrasonography are alternative methods for pregnancy diagnosis. Their accuracy and practical application potential are different. The two-dimensional ultrasound is one of the most appropriate methods for pregnancy diagnosis in goats. The accuracy of the method is about 100% on post insemination day 25–30 using the transrectal approach, whereas via the transabdominal approach, this accuracy is attained between the 40th and the 45th day.

Key words: diagnostics, goats, pregnancy

Goats are seasonally polyoestrous animals. The beginning and duration of the breeding period depends on a number of climatic and physiological factors (latitude, climate, breed, production system). The average duration of the sexual cycle is 21 days and is determined by the interval between two successive ovulatory oestruses. The oestrus duration is about 36 h, ranging between 24 and 48 h depending on the age, breed, season and presence of male breeder. The fertilisation occurs in oviduct ampullae several hours after the ovulation. The embryo reaches the uterus 4–5 days after conception and is implanted 18–22 days after the beginning of the oestrus. The average gestation period is 149 days, but it could vary among breeds by several days (Fatet *et al.,* 2011).

The pregnancy diagnosis in goats is essential for better efficacy and management of reproduction (Doize *et al*., 1997), providing information about conception rates after artificial insemination (Matsas, 2007), gestation course (Amer, 2010), time for drying-off and parturition date (Doize *et al*., 1997; Gonzalez *et al*., 2004). The separation of pregnant and non-pregnant does in different groups reduces the losses from abortions, stillbirths or giving birth to non-viable offspring and optimises labour, feed and medication costs (Wani *et al*., 1998). The utilisation of an accurate and easily applicable method for pregnancy diagnosis allows the timely repeated insemination, breeding or culling of non-pregnant animals (Amer, 2010).

A number of techniques for pregnancy diagnosis have been implemented (Table 1), some of them are efficient and applicable under field conditions, but not enough accurate, others are distinguished with a high precision, but require specialised equipment and working skills (Ishwar, 1995; Medan *et al*., 2004; Amer, 2010). A method, easy to perform, reliable, not expensive and with high accuracy is preferable. The selection of a method depends on the stage of gestation, method's precision and the available equipment at the farm (Gonzalez *et al*., 2004). From the point of view of their practical application potential, Karen *et al*. (2001) divide the methods for pregnancy diagnosis in goats to less practical (non-return to oestrus, transabdominal palpation, laparoscopy, laparotomy, palpation of the caudal uterine artery, rosette inhibition test) and widely used (radiography, rectoabdominal palpation, hormonal assays, assay of pregnancy proteins, ultrasonography).

So far, there is no universally accepted classification of methods for pregnancy

Table 1. Comparative evaluation of the different methods for pregnancy diagnosis in small ruminants

Method for pregnancy diagnosis	Species	Gestation days	Accuracy	Reference
Non-return to oestrus	Sheep and goats	> 17–21	not reliable	Lindsay, 1973
Live weight increase	Goats	28	not reliable	Wani, 1982
	Sheep	120	not reliable	Domanski & Lipeska, 1966
Laparotomy and perineoscopy	Sheep and goats	28–56	97%	Lamond, 1963
Cervical palpation	Goats	–	–	Kutty, 1999
Radiography	Goats	>80	100%	West, 1986
		>70	100%	Memon & Out, 1980
Hormonal analysis. (progesterone and estrone sulfate assays)	Sheep and goats	21–28	80–100%	Murray & Newstead, 1998 Refstal *et al.*, 1991 Sousa *et al.*, 1999 Medan *et al.*, 2004
PAGs immunoassay	Goats	25–26	–	Gonzales *et al.*, 2004
Ultrasonography	Goats	15–30	100%	Medan *et al.*, 2004
		20–25	100%	Amer, 2008
		26	100%	Abdelghafar *et al.*, 2010
		30–35	100%	Omontese *et al.*, 2012

diagnosis in goats. Therefore, we believe that the existing classification in sheep could be applicable also to goats with several changes and additions. The methods of limited interest for the practice are the laparoscopy, laparotomy, vaginal biopsy, transabdominal palpation, abdominal inspection, udder evaluation and appearance of milk secretion, live weight increase, rectoabdominal method, method of Hulet, while more practical approaches include the non-return to oestrus, hormonal and pregnancy proteins assays, radiography and ultrasonography.

The purpose of the present review is to go over the main pregnancy diagnosis techniques in goats and to discuss in detail the methods most commonly used in the practice with emphasis on their advantages and weaknesses.

LAPAROSCOPY, LAPAROTOMY, VAGINAL BIOPSY

According to the studies of Phillippo *et al*. (1971) the precision of diagnosing early pregnancy in small ruminants through laparoscopy is 91%, between the 17th and the 28th day after breeding. The method is invasive, requires time, special equipment and skilled personnel, which makes it inapplicable under field conditions.

The pregnancy in goats could be detected by paramedian laparotomy and direct palpation of the uterus. The method is complex, non-ergonomic, expensive, reduces the chances for future conception and therefore, is inappropriate for practical application (Cutten, 1979).

Vaginal biopsy is based on changes occurring in the vaginal epithelium during the pregnancy. The method is used for pregnancy diagnosis in sheep (Richardson, 1972). A disadvantage outlined by Ishwar (1995) is that it could not detect twin pregnancy. The method is not suitable under field conditions, is expensive and requires time for specimen processing (Memon & Ott, 1980). No data about the use of this technique for pregnancy diagnosis in goats are available. Therefore, the three abovementioned techniques are accurate, but invasive and inapplicable in the practice (Goel & Agrawal, 1992).

ABDOMINAL INSPECTION AND PALPATION; LIVE WEIGHT MONITORING

The inspection of the abdominal region, transabdominal palpation of the uterus and foetuses and body weight increase are techniques for pregnancy diagnosis in goats with a low precision, applicable only during the second half of gestation (Taverne & Noakes, 2009). The live weight increase during pregnancy is rather variable, and therefore not enough reliable trait (Domanski & Lipecka, 1966).

UDDER EVALUATION AND MILK SECRETION INITIATION

The udder development and the appearance of colostrum is also used as a method for detection of pregnancy. Between gestation days 111 and 130, a moderate development of the udder occurs in 96% of sheep, and after the 130th day – a fully developed and enlarged udder is already observed in 84% of animals. The changes in udder consistency and its enlargement is an accurate and practical method for pregnancy diagnosis, but only by its end; moreover, it is consistent in primiparous ewes in which the udder development is more obvious (Watt *et al*., 1984). To the best of our knowledge, there are no reports about the applicability of this method in goats.

Udder inspection and palpation are not accurate for pregnancy diagnosis in small ruminants and should not be used independently for this purpose; they should be better combined with other techniques (Das *et al.*, 2011).

RECTOABDOMINAL TECHNIQUE

Rectal palpation, a routine technique for pregnancy diagnosis in large animals (cows and mares), is not pertinent to sheep and goats due to specific anatomic features (Ganaie *et al*., 2009). Kutty & Sudarsanan (1996) have developed a technique for pregnancy detection in small ruminants by palpation with both hands. The method includes palpation of the uterus through the rectum with one hand, while the other presses the abdominal wall. Pregnancy is diagnosed on the basis of cervical dilatation, altered position of the uterus, palpation of placentomes or parts of the foetus, asymmetry of uterine horns and impossibility for palpation of ovaries (Kutty, 1999).

HULET ROD METHOD

In small ruminants, pregnancy could be detected by careful passing a lubricated glass rod (1.5 cm diameter, 50 cm length) into the rectum after preliminary fixation of the animal in dorsal recumbency. The rod is manipulated with one hand and the other exerts a moderate pressure in the abdominal region, anterior to the udder. In non-pregnant animals, the tip of the rod is palpated through the abdominal wall (Hulet, 1972). Although the technique is easy, cheap and fast (150 animals per hour), it is not recommended in goats because of the risk for injury of rectal mucosa (Ott *et al*., 1981) and the low accuracy of the method in the beginning of the pregnancy (Chauhan *et al.*, 1991).

HORMONAL ASSAYS

The assay of steroid hormone concentrations (progesterone, estrone sulfate) at a determined time after insemination/mating aids the early detection of pregnancy (Worsfold *et al*., 1986; Murray & Newstead, 1988; Refstal *et al*., 1991; Sousa *et al*., 1999).

Progesterone assay

Various methods are used for analysis of blood, milk and faecal progesterone concentrations in small ruminants for evaluation of their endocrine status – immunoenzymatic (ELISA), radioimmune (RIA) and immunochemiluminescence analysis (Boscos *et al*., 2003; Gaafar *et al*., 2005; Capezzuto *et al*., 2009; Jack *et al*., 2012).

The analysis of blood plasma or serum progesterone is indicative for pregnancy after post insemination day 21 (Boscos *et al*., 2003; Medan *et al*., 2004; Capezzuto *et al*., 2009). Blood plasma concentrations >1 ng/mL between days 15–17 for nulliparous and days 18–22 in parous goats indicate pregnancy. The accuracy of the assay is 75–86% in pregnant and 90–100% in non-pregnant goats (Dawson, 1999; Boscos *et al*., 2003; Khadiga *et al*., 2005).

Milk progesterone ≥ 10 ng/mL on days 22–26 after mating indicate pregnancy with accuracy >86%, whereas concentrations ≤ 10 ng/mL denote lack of pregnancy with up to 100% accuracy (Dawson, 1999). False negative results could be due to pseudopregnancy, uterine or ovarian damages (Holdsworth & Davies, 1979).

Progesterone is mainly converted in the liver and excreted via the bile in faeces under the form of the water-soluble metabolite pregnandiol-3-glucuronide (IPD G) (Docke, 1994; Schwartzenberger *et al*., 1996). The experiments of Jack *et al*.

(2012) with goats indicate that the accuracy of the method for distinguish pregnant from non-pregnant animals on the 19th–20th day after mating was 100%.

Estrone sulfate assay

Estrone sulfate could be detected in blood plasma of goats and sheep about the 70th gestation day with concentrations ranging between 0.1–0.7 ng/mL, gradually increasing until 2 days before the parturition (Tsang, 1978). According to Murray & Newstead (1988) the presence of estrone sulfate in milk confirms pregnancy with accuracy of 82% and lack of pregnancy in 93% of cases.

ANALYSIS OF PREGNANCY ASSOCIATED PROTEINS

The detection of pregnancy in goats could be done by immunological analysis of some specific proteins, such as the early pregnancy factor. Specific antigens are detected by radioimmunological (RIA) (Zoli *et al*., 1992) or immunoenzymatic (ELISA) techniques using monoclonal (Green *et al*., 2005) or polyclonal antibodies between the 5th and 60th gestation days (Friedrich & Holtz, 2004; 2010).

Pregnancy associated glycoproteins

The detection of pregnancy associated glycoproteins (PAGs) in blood plasma or milk samples is a reliable method for early pregnancy diagnosis in goats (Batalha *et al.,* 2011; Zamfirescu *et al*., 2011; Shahin *et al*., 2013). According to Gonzalez *et al*. (2004) detectable blood plasma PAGs concentrations in goats appear around the 25–26 day of gestation. Shahin *et al*. (2013) reported statistically significant differences in PAGs levels by the 21st day post breeding between pregnant and non-pregnant goats. The presence of PAGs in milk samples is also a precise criterion for pregnancy after the 32nd gestation day (Gonzalez *et al*., 2004).

Pregnancy-specific protein B

Pregnancy-specific protein B (PSPB) was first detected in bovine placenta (Butler *et al*., 1982), secreted by binuclear cells of the foetal trophectoderm (Eckblad *et al*., 1985). PSPB is established in sheep and goats and could be also serve for diagnosis of pregnancy (Bearden *et al*., 2004)

NON-RETURN TO OESTRUS

The non-return to oestrus is among the oldest and commonly used methods for pregnancy detection in small ruminants. it is not expensive, practical and yields information about pregnancy at a very early stage after the mating (17th–21st day) (Goel & Agrawal, 1992). The non-return to oestrus evaluation is not recommended during the late breeding period and for animals, synchronised during the anoestrus (Memon & Ott, 1980). Very often, pregnant goats also exhibit signs of oestrus (Taverne & Noakes, 2009; Das *et al*., 2011), and the prevalence of early embryonic death in some herds is not small. Such animals could be hardly differentiated, even in the presence of a male breeder. That is why, the technique is not deemed reliable for detection of pregnancy in goats (Elmore, 1988).

RADIOGRAPHY

The radiography as a imaging method for pregnancy diagnosis is applicable after the 80th day post breeding, when the foetal skeleton is mineralised and could be visualised (West, 1986). The earliest term for radiographic detection of pregnant goats is the 58th day after insemination (Barker & Cawley, 1967). According to the au-

thors, the foetal skeleton is radiologically visible after the 65th gestation day. The expensive equipment and potential risks for both the operator and the patient are substantial drawbacks limiting the application of the technique.

ULTRASONOGRAPHY

Ultrasonography as a method for monitoring of the reproduction status in small ruminants is becoming increasingly important and popular (Medan & Abd El-Aty, 2010; Erdogan, 2012). Now, A-mode, B-mode and Doppler ultrasound equipment is used for pregnancy diagnosis in goats. Their accuracy and usefulness under field conditions is various (Goel & Agrawal, 1992; Ishwar, 1995).

A-mode ultrasonography

The early attempts of using ultrasound in animal reproduction are associated with type A (amplitude-depth) ultrasound equipment (Medan & Abd El-Aty, 2010). Their work principle is based on ultrasound wave reflection that determines the boundaries of organs filled with fluid, such as the pregnant uterus. The type A apparatuses possess a good sensitivity and specificity, exhibiting an accuracy of 80–85% between the 60th and 120th post breeding days (Dawson, 2002), but the proportion of false positive diagnoses could be considerable when the urinary bladder is full or in some inflammatory uterine diseases (hydrometra, pyometra) (Haibel, 1990; Karen *et al*., 2001).

Doppler ultrasonography

Doppler ultrasound scanners detect pregnancy through investigation of blood circulation in the median uterine artery, umbilical artery and foetal heartbeats (Ott *et al*., 1981; Wani *et al*., 2003). The technique is applicable after the 25th day after mating via the transrectal approach, but provided that soft faeces are present, a higher percentage of false negative diagnosis could be obtained. Some researchers report that the accuracy of Doppler ultrasonography for detection of pregnancy via the transrectal or transcutaneous approach was higher during the second or third gestation months (Lindahl, 1971; Wani, 1981; Watt *et al*., 1984; Serin *et al*., 2010). After using Doppler ultrasonography for pregnancy detection in goats, Wani *et al*. (1998) reported accuracy of 100%, 100% and 72%, for foetal heartbeats, umbilical artery and median uterine artery, respectively. According to Russel & Goddard (1995), Doppler ultrasound was not appropriate as a technique for gestational age determination.

B-mode (brightness) real-time ultrasonography

In veterinary practice, ultrasonography was introduced in the 1980s for diagnosis of pregnancy at the earliest gestational stage (Buckrell *et al*., 1988; Davey, 1986; Haibel, 1990). B-mode (brightness) real-time ultrasonography is the most commonly used diagnostic imaging techniques for detection of pregnancy in small ruminants (Wani *et al*., 1998; Langfort, 2003; Kähn, 2004; Sousa *et al*., 2004; Gardon, 2005; Johnson, 2005; Yotov, 2007). It is a modern, non-invasive, rapid and accurate method for early detection of pregnancy in animals (Abdelghafar *et al*., 2007; Santos *et al*., 2007). The share of false positive diagnoses is small (Ganaie *et al*., 2009). An experienced operator could achieve an accuracy of 91–100% (Romano & Christians, 2008).

Hesselink & Taverne (1994) were the first to report the use of diagnostic ultrasound to detect pregnancy in goats. Af-

terwards, B-mode ultrasonography has been applied by numerous researchers (Doize *et al*., 1997; Martinez et al., 1998; Gonzalez *et al*., 2004; Amer *et al*., 2010; Omontese *et al*., 2012). It is a reliable method for early diagnosis of pregnancy (Medan *et al*., 2004; Gonzales-Bulnes *et al*., 2010).

In goats, the transabdominal and transrectal approaches are most frequently used. They could be performed in standing position, without sedation of the animal (Vinoles-Gil *et al*., 2010). The appropriate time for transabdominal ultrasonography is between post breeding days 40–45 and for transrectal examination – between post breeding days 25 and 30 (Georgiev *et al.,* 2003; Buckrell, 1988; Mialot *et al*., 1991; Dawson, 2002). The accuracy could attain 100% with 3.5, 5.0, 7.5 MHz transducers (linear, sector or convex probes) (Vinoles-Gil *et al*., 2010).

The main criteria for positive diagnosis of pregnancy in goats are the visualisation of anechoic cross sections of the uterine lumen (embryonic vesicle), embryo/foetus or placentomes in the amniotic fluid. Embryonic vesicles could be detected between the 21st and 28th days, whereas the embryo could be visualised by the 28–35 gestation days using transrectal and transabdominal uterine scans respectively (Haibel, 1990; Hesselink & Taverne, 1994; Martinez *et al*., 1998; Abdelghafar *et al*., 2010; Amer, 2010)

The pregnancy diagnosis in goats is a primary factor for improving the reproductive performance in herds and achieving high economic efficiency. During the years, multiple techniques for pregnancy detection in small ruminants have been developed. Each method has advantages and drawbacks. Some of them are invasive or of low accuracy hence of limited practical use. Others are precise but require equipment and specialised skills. The practical implementation of user-friendly, accurate and non-invasive methods for pregnancy detection in goats compatible with animal welfare standards would result in optimisation of the reproductive performance.

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