## **REVIEW ARTICLE** PREGNANCY DIAGNOSIS IN SHEEP: REVIEW OF THE MOST **PRACTICAL METHODS**

A. KAREN<sup>1</sup>, P. KOVÁCS<sup>2</sup>, J. F. BECKERS<sup>3</sup>, O. SZENCI<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Reproduction, Faculty of Veterinary Science, Szent István University, Hungary; <sup>2</sup>Awassi Rt., Bakonszeg, Hungary

<sup>3</sup>Department of Physiology of Reproduction, Faculty of Veterinary Medicine, University of Liege, Belgium

Received September 19, 2000 Accepted May 28, 2001

#### Abstract

Karen A., P. Kovács, J.F. Beckers, O. Szenci: Pregnancy Diagnosis in Sheep: Review of the Most Practical Methods. Acta Vet. Brno 2001, 70: 115-126.

Various practical methods have been used for pregnancy diagnosis in sheep. Both pregnancy and fetal numbers are accurately diagnosed by using radiography after day 70 of gestation. Rectal abdominal technique detects pregnancy with an accuracy of 66 to 100% from d 49 to 109 of gestation, however, it has a low (17 to 57%) accuracy for determining multiple fetuses. Progesterone assays have a high sensitivity (88% to 100%) and a low specificity (60% to 72%) at d 16 to 18. Estrone sulphate assay accurately detects pregnant ewe at d 30 to 35. Ovine pregnancy specific protein B (PSPB) assay accurately (100%) detects pregnancy from d 26 after breeding onwards. The accuracy of progesterone, estrone sulphate and oPSPB assays for determining fetal numbers is relatively low. A-mode and Doppler ultrasonic techniques accurately detect pregnancy during the second half of gestation. Fetal numbers cannot be determined by A-mode ultrasound, while the Doppler technique needs experience to achieve high accuracy. Transrectal B-mode, real time ultrasonography identifies the embryonic vesicles as early as d 12.8 after mating, but the sensitivity of the technique for pregnancy is very low (12%) earlier than 25 d after mating. Transabdominal B-mode ultrasonography achieved high accuracy for pregnancy diagnosis (94% to 100%) and the determination of fetal numbers (92% to 99%) on d 29 to 106 of gestation. Realtime, B-mode ultrasonography appears to be the most practical and accurate method for diagnosing pregnancy and determining fetal numbers in sheep.

Pregnancy diagnosis, ewe, radiography, rectal-abdominal palpation, hormonal assays, pregnancy proteins, ultrasonography

Early detection of pregnancy is of considerable economic value to sheep industry. Nonpregnant ewes could be sold, reducing feed expenses, while non-pregnant lambs could be marketed at higher price than they would bring as mature ewes (Gearhart et al. 1988). Separation of the sheep herd into pregnant and non-pregnant ewes might reduce reproductive and production losses in form of abortions, stillbirths and production of weak lambs (Wani et al. 1998).

Predictions of the number of fetuses would allow appropriate nutritional management of the ewes in late gestation that will prevent pregnancy toxemia (Ford 1983), minimize prelambing feeding costs, optimize birth weight, weaning weight and survivability of lambs and reduce the incidence of dystocia (Gearhart et al. 1988). In addition, the accurate information on the stage of gestation would be useful to dry off lactating females at adequate period and to monitor the females near term (Doize et al. 1997).

Methods of pregnancy diagnosis

Various methods have been used to diagnose pregnancy in sheep. These methods can be classified as less practical such as the management method (non-return to estrus), abdominal

Address for correspondence:

Aly Karen

Aly Karen Department of Obstetrics and Reproduction, Faculty of Veterinary Science, Szent István University, István ut., H-1400 Budapest, P. O. Box 2, Hungary

Phone: +361-4784206 Fax : +361-478-4207 e-mail: akaren@univet.hu palpation and ballotment, palpation of the caudal uterine artery, laparotomy, peritoneoscopy and rosette inhibition test reviewed by Ishwar (1995), and the most practical methods such as radiography, rectal abdominal palpation, hormonal assays, pregnancy protein assays and ultrasonography. In the present review, only the most practical methods are discussed.

## 1. Radiography

Ford et al. (1963) examined 322 ewes by radiography and reported 100% and 90% accuracy for diagnosing pregnancy and determination of the fetal number, respectively, after 70 days of gestation. Grace et al. (1989) reported 94 to 100% accuracy of radiography for determining fetal numbers in 13 sheep flocks. Besides the accuracy, the technique is quick; 400 to 600 ewes can be tested per day under farm conditions. The cost of the equipment and the potential health hazard to the operator may limit its use in the field (West 1986).

### 2. Rectal abdominal palpation

Pregnancy diagnosis in sheep was determined by gentle insertion of a lubricated glass rod (1.5 cm in diameter and 50 cm long) into the rectum of ewe lying on its back. The free hand was placed on the posterior abdomen while the rod was manipulated with the other hand (Hulet 1972). At the early stage of pregnancy, the sensitivity of the technique for diagnosing pregnancy was low but it increased with progressing of the pregnancy reaching the highest accuracy (100 %) at Days 85 to 109 after mating (Hulet 1972; Chauhan et al. 1991; see Table 1). In contrast, others (Tyrrell and Plant 1979; Trapp and Slyter 1983) reported a lower sensitivity and specificity at d 60 to 96 after mating (Table 1). Although this technique is simple, cheap and quick (150 ewes can be examined per hour), it had a low accuracy in diagnosing multiple fetuses (Table 2) and was more hazardous with respect to rectal injury (Tyrrell and Plant 1979) and abortion (Turner and Hindson 1975; Ishwar 1995).

		-	-				-	-	-	
No. of	Days of	а	b	с	d	Se	Sp	+PV	-PV	Authors
animals	exam.					%	%	%	%	Autions
79	85 to 109	61	0	18	0	100	100	100	100	Hulet 1972
432	21 to 55					59				Tyrrell & Plant 1979
99	49 to 83					73				Tyrrell & Plant 1979
498	60 to 96	173	97	139	89	66	59	62	61	Trapp & Slyter 1983
14		10	2	2	0	100	50	82	100	Chauhan et al.1991

#### Table 1 Sensitivity (Se), specificity (Sp), and predictive (+PV, -PV) values of rectal abdominal technique for pregnancy diagnosis in sheep

a, correct positive (pregnant); b, false positive (non-pregnant); c, correct negative (non-pregnant); d, false negative (pregnant)

Table 2 Sensitivity (Se), specificity (Sp), and predictive (+PV, -PV) values of rectal abdominal technique in determination of fetal numbers

No. of animals	Days of exam.	а	b	с	d	Se %	Sp %	+PV %	-PV %	Authors
41	90 to 105	4	1	33	3	57	97	80	92	Hulet (1973)
12		1	1	5	5	17	83	50	50	Chauhan et al. (1991)

a, correct positive (multiple); b, false positive (single); c, correct negative (single); d, false negative (multiple)

The technique of bimanual palpation of small ruminants was developed by Kutty and Sudarsanan (1996). This method includes digital palpation per rectum combined with abdominal manipulation. By using this technique pregnant ewes (n = 9) were accurately diagnosed based on enlarged cervix, prepubic position of the uterus, palpation of placentomes and/or fetal parts, asymmetry and/or marked distension of uterine horns and inability to palpate the ovaries (Kutty 1999).

## 3. Hormonal assays

## 3.1. Assessment of progesterone

Measurement of blood progesterone concentration is a reliable indicator of the functional *corpus luteum*. Concentration of plasma progesterone samples was determined in ewes on Day 18 post-breeding by using enzyme immunoassay (EIA) and radioimmunoassay (RIA). The accuracy of both type of assays for detecting pregnancy was high, while it was low for diagnosing non-pregnancy (Amezcua-Moreno 1988; Susmel and Piasentier 1992; Gvozdic and Ivkov 1994; see Table 3). On the other hand, 100% accuracy for detecting non-pregnant ewes was achieved by using EIA at Day 16 (McPhee and Tiberghien 1987) and Day 21 after mating (Zarkawi 1997) or by using RIA at Days 17 to 18 (Zarkawi et al. 1999; see Table 3). Early embryonic death, uterine and/or ovarian pathology may be the source of the false positive cases. At Days 100  $\pm$  9 after breeding, the accuracy of progesterone assay for pregnancy diagnosis was 98% in ewe lambs and 99% in mature ewes (Schneider and Hallford 1996).

Days of exam.	No. of animals	a	b	с	d	Se %	Sp %	+PV %	-PV %	Authors
16 to 17	130	106	0	24	0	100	100	100	100	McPhee & Tiberghien (1987)
18	170					91	64			Amezcua-Moreno (1988)
18	112	80	9	23	0	100	72	90	100	Susmel & Piasentier (1992)
16 to 18	22	15	2	3	2	88	60	88	60	Gvozdic & Ivkov (1994)
21	16	16	0	0	0	100		100		Zarkawi (1997)
17 to 18	24	24	0	0	0	100		100		Zarkawi et al. (1999)

Table 3 Sensitivity (Se), specificity (Sp), and predictive (+PV, -PV) values of progesterone assay for diagnosing pregnancy in sheep

a, correct positive (pregnant); b, false positive (non-pregnant); c, correct negative (non-pregnant); d, false negative (pregnant)

EIA test for the measurement of faecal immunoreactive Pregnendiol-3-Glucuronide (iPd G), a progesterone metabolite, was a useful tool for diagnosing pregnancy in Bighorn sheep with 100% accuracy from about Day 60 of pregnancy until a few days before parturition. (Borjesson et al. 1996).

Concerning the estimation of the fetal number, serum progesterone concentration was significantly higher in ewes carrying two and three fetuses than those carrying one fetus (19.2 and 29.9 ng/ml, vs 9.2 ng/ml, respectively) (Chauhan and Waziri 1991). There was a positive relationship between the number of fetuses and the mean plasma progesterone concentrations (P < 0.001) after the second half of pregnancy (Kalkan et al. 1996). The number of fetuses was estimated with 88% accuracy in ewe lambs and with 74% accuracy in mature ewes on Days 100  $\pm$  9 after breeding (Schneider and Hallford 1996). In contrast, others reported a much lower accuracy (25%) for ewes carrying multiple fetuses

# (Chauhan et al. 1991; Sandabe et al. 1994).

Regarding the fetal sex, the plasma progesterone concentrations of ewes giving birth to male and female lambs were not significantly different (Kalkan et al. 1996).

## 3.2-Assessment of estrone sulphate

The presence of a viable feto-placental unit is accompanied by an increase in estrone sulphate concentrations in the peripheral plasma of ewes. Estrone sulphate was detectable around Day 70 of gestation with value ranging between 0.1 to 0.7 ng/ml, then its level increased steadily till 2 days before parturition when an upsurge was seen (15-50 ng/ml) (Tsang 1978). On Day 85 of gestation, there was a significant difference in the level of estrone sulphate between pregnant and non-pregnant ewes. However, due to considerable variation of the hormone levels between individuals, the accuracy for detection of non-pregnancy was only 44 % whilst for detection of pregnancy it was 87.9% using the cut-off value of 0.1 ng/ml (Worsfold et al. 1986). On the contrary, Illera et al. (2000) reported that the EIA test for the measurements of serum estrone sulphate concentrations gave an optimal accuracy for pregnancy diagnosis between Days 30 to 35 of gestation.

Regarding the fetal number, the concentration of serum estrone sulphate was significantly higher in ewes carrying multiple than those carrying single fetus from Days 80 to 124 of gestation (Illera et al. 2000). However, the determination of estrone sulphate concentrations in ovine blood might not be reliable for prediction of fetal numbers due to the high variation between individuals (Worsfold et al. 1986).

## 3.3. Ovine chorionic somatommamotrophin (oCS) or ovine placental lactogen (oPL)

Ovine placental lactogen (oPL) was studied and purified by Chan et al. (1978). RIA assay of oPL achieved 97% and 100% accuracy for diagnosing pregnant and non-pregnant ewes at Day 64 of gestation, respectively (Robertson et al. 1980).

- 4. Assessment of pregnancy proteins
- 4.1. Pregnancy-specific protein B (PSPB)

Pregnancy-specific protein B (PSPB) first detected in the bovine placenta (Butler et al. 1982), is secreted by binucleate cells of fetal trophoectoderm (Eckblad et al.1985). The physiological role of PSPB during pregnancy might be the maintenance of *corpus luteum* by stimulating prostaglandin E2 production (Vecchio et al. 1995).

Although the RIA test for the measurements of bovine PSPB accurately detects pregnancy (100%) and non-pregnancy (83%) in sheep from Days 26 to 106 of gestation (Table 4), ovine PSPB concentration cannot be measured quantitatively because ovine antigen cross-reacts only incompletely with antibodies to bPSPB (Ruder et al. 1988).

Table 4 Sensitivity (Se), specificity (Sp) and predictive (+PV, -PV) values of oPSPB assay for pregnancy diagnosis in sheep

Days of exam.	No. of animals	а	b	с	d	Se %	Sp %	+PV %	-PV %	Authors
26-96	33	30	2	1	0	100	33	94	100	Ruder et al. (1988)
35-106	180	159	2	19	0	100	90	99	100	Ruder et al. (1988)
Total	213	189	4	20	0	100	83	97	100	

a, correct positive (pregnant); b, false positive (non-pregnant); c, correct negative (non-pregnant); d, false negative (pregnant)

Willard et al. (1987) developed a quantitative RIA test for the measurements of ovine pregnancy specific protein B (oPSPB). oPSPB became detectable at  $19.7 \pm 0.1$  (Mean  $\pm$  SE) (Willard et al. 1987; 1995) and  $21.7 \pm 0.6$  days postmating (Wallace et al. 1997). Then, it increased steadily until Day 30 when it was  $10.8 \pm 0.4$  ng/ml. The concentration remained stable within a period of 20 d prepartum (Willard et al. 1995). After lambing, the concentration dropped rapidly and it was last detectable at  $12.8 \pm 2.3$  d (Willard et al. 1995) and  $3 \pm 0.1$  weeks postpartum (Willard et al. 1987).

By using the RIA test for the measurements of oPSPB, the accuracy for detecting ewes carrying single and twin lambs was 71% and 81%, respectively, from Days 60 to 120 of gestation (Willard et al. 1995). At the same time, oPSPB concentrations were not influenced by the sex of the fetus (Wallace et al. 1997).

PSPB might be a useful marker of placental development and function and provide a reliable indicator of fetal distress and adverse pregnancy outcome. Between Days 50 and 100 of gestation, PSPB concentrations were positively correlated with placental weight at term. In addition, the mass of the fetus in ewes that aborted during late pregnancy was highly correlated with PSPB concentrations up to Day 120 of gestation (Wallace et al. 1997).

### 4.2. Ovine pregnancy-associated glycoproteins (oPAGs)

Ovine pregnancy-associated glycoproteins (oPAGs) are synthesized by binucleate cells of trophoblast, and belong to aspartic proteinase family (Xie et al. 1991) and most of them are without enzyme activity (Xie et al. 1997). They have molecular weights between 43 to 67 kDa (Zoli et al. 1995; Xie et al. 1997).

The concentration of oPAG in Churra and Merino ewes was detectable in some (20/30) ewes at Week 3 and in all ewes on Week 4 after mating (Ranilla et al. 1994). The concentration of oPAG increased slowly from Weeks 3 to 9 of gestation. Thereafter, plasmatic profiles of oPAG varied among sheep breeds from Week 9 till Week17, however, oPAG concentrations increased in all studied breeds from Week 17 till lambing. After lambing, the oPAG levels decreased rapidly reaching the basal value at fourth week postpartum (Ranilla et al. 1994 and 1997; Gajewski et al. 1999).

The concentration of oPAG might be influenced by the fetal numbers and the sex of the fetus. Ewes carrying two fetuses had higher mean oPAG concentrations than those carrying a single fetus from Week 12 of gestation to lambing. This difference was only significant at Week 21 (Ranilla et al. 1997). Also, ewes carrying male fetuses had oPAG concentrations higher than those carrying female fetuses at Weeks 19, 20 and 21 of gestation (Ranilla et al. 1994).

Although bPAG and cPAG have been successfully used for detecting pregnancy in cattle (Zoli et al. 1992; Szenci et al. 1998) and goats, respectively, (Folch et al. 1993, Gonzalez et al. 1999), there is no data evaluating the accuracy of oPAG assays for diagnosing pregnancy in sheep.

## 5. Ultrasonography

In the past 20 years, three types of ultrasonographic systems were used for pregnancy diagnosis in small ruminants.

## 5.1. A-mode ultrasound (Amplitude-depth or echo-pulse)

In this system, the transducer containing one crystal emits ultrasound waves which penetrate the tissues under the skin and reflect when meet a high acoustic impedance interfaces (pregnant uterus or fluid-filled structures). The transducer receives the reflected echoes and converts them into peaks on oscilloscope with horizontal scale representing the depth of the reflecting structure or into audible signal.

Meredith and Madani (1980) used the reflection of ultrasound at depth 9 cm or greater as a positive sign of pregnancy in ewe and reported 96 % sensitivity and 87.5 % specificity

in the period from 61 to 151 days after mating. However, by the same approach, lower sensitivity (86.7%) and specificity (69%) were reported in the ewe lambs at Days 73 to 103 postmating (Madel 1983). By using echo-pulse detectors, the accuracy for detecting pregnant ewes averaged 91% from Days 69 to 112 of gestation (Trapp and Slyter 1983). However, Watt et al. (1984) reported 97% accuracy for diagnosing pregnancy from Day 51 of gestation to lambing. A-mode ultrasound is a quick, convenient and simple technique, but it cannot predict the fetal number and the viability of the fetus.

### 5.2. Doppler ultrasound

Doppler devices utilize the Doppler shift principle to detect the fetal heart beats and flow of blood in uterine and fetal vessels. Lindahl (1971) reported that the intrarectal Doppler technique could be used for diagnosing pregnancy at the beginning of the second third with an accuracy of 90 % or better. According to the work reported by Deas (1977) the accuracy of intrarectal Doppler transducer for diagnosing pregnancy and non-pregnancy was 82 % and 91 %, respectively, from Days 41 to 60 of gestation. After Day 71, the accuracy for diagnosing pregnancy and non-pregnancy ranged between 85 % and 94 %, respectively (Watt et al. 1984). In contrast, Trapp and Slyter (1983) reported 68 % and 84 % accuracy for diagnosing pregnancy and non-pregnancy from Days 60 to 96 of gestation. The use of an external Doppler transducer gave almost 100 % accuracy for diagnosing pregnancy after Day 111 of gestation (Watt et al. 1984).

Concerning the predictions of fetal numbers, the external Doppler technique, when used by skilled operator gave 83 % and 93 % accuracy for diagnosing single and multiple fetuses at Days 80 to 95 of gestation, respectively (Fukui et al. 1986). However, Fukui et al. (1984) reported 74% and 89% accuracy for ewes carrying single and multiples, respectively from Days 60 to 120 of gestation. Doppler devices have not been used successfully for estimating ovine gestational age (Russel and Goddard 1995).

## 5.3. Real-time, B-mode ultrasonography

Real-time B-mode ultrasonic scanning of the uterus in sheep appears to offer an accurate, rapid, safe and practical means for diagnosing pregnancy, determination of fetal numbers and estimation of gestational age.

### 5.3.1. Diagnosis of pregnancy

By using transrectal ultrasonography (7.5 MHz), embryonic vesicle of the pregnant Manchega dairy ewe was identified at Day 12.8 after mating, while the first visualization of

	ultrasonography for pregnancy diagnosis in sneep													
Days of	MHz	No. of	а	b	с	d	Se	Sp	+PV	-PV	Authors			
exam.		animal					%	%	%	%	Tutions			
25 to 50	5	64	33	1	25	5	87	96	97	83	Buckrell et al. (1986)			
0 to 25	5	26					12	100			Gearthart et al. (1988)			
26 to 50	5	26					65	100			Gearthart et al. (1988)			
24 to 26	5	91	17	3	62	9	65	95	85	87	Garcia et al. (1993)			
32 to 34	5	91	22	1	64	4	85	98	96	94	Garcia et al. (1993)			
0 to 60	7.5	117	94	8	13	2	98	62	92	87	Schrick & Inskeep (1993)			

Table 5 Sensitivity (Se), specificity (Sp) and predictive (+PV, - PV) values of using transrectal (5 MHz and 7.5 MHz) ultrasonography for pregnancy diagnosis in sheep

a, correct positive (pregnant); b, false positive (non-pregnant); c, correct negative (non-pregnant); d, false negative (pregnant)

the embryo was at Day 19 (Gonzalez et al. 1998) or Day 20 (Schrick and Inskeep 1993). By using 5 MHz transrectal probe, the first signs of pregnancy in form of circular and elongated anaechoic images located in utero cranial to bladder were observed in ewe on Days 17 to 19 (Garcia et al. 1993; Doize et al. 1997), while embryo could be detected on Day 25 after mating (Buckrell et al. 1986).

The specificity of 7.5 MHz transrectal ultrasonography for diagnosing non-pregnancy was low during the first two months of gestation (Schrick and Inskeep 1993; Table 5). The false positive diagnoses were attributed to embryonic or fetal death. The sensitivity of 5 MHz transrectal ultrasonography for detecting pregnant ewes was very low (12%) at less than Day 25 of gestation (Gearhart et al. 1988). Thereafter, the sensitivity increased with progressing the pregnancy and ranged between 65% and 87% at Days 25 to 50, depending on the breed, age and parity of the ewes and the technique of the examination (Buckrell et al. 1986; Gearhart et al. 1988; Garcia et al. 1993; Table 5).

By using transabdominal approach, pregnancy was first verified at Day 25 (Gearhart et al. 1988) or Day 30 after breeding (Bretzlaff et al. 1993). The sensitivity and specificity of the technique were high after Day 29 (Taverne et al. 1985) reaching approximately 100% from Days 46 to 106 of gestation (White et al. 1984; Fowler and Wilkins 1984; Davey 1986; Gearhart et al. 1988). However, Logue et al. (1987) reported a lower specificity on Days less than 40 to100 after mating (Table 6).

Table 6

Sensitivity (Se), specificity (Sp) and predictive (+PV, - PV) values of using transabdominal (3, 3.5 and 5 MHz) ultrasonography for pregnancy diagnosis in sheep

Days of exam.	MHz	No. of animal	а	b	с	d	Se %	Sp %	+PV %	-PV %	Authors
46 to 106	3.5	5530	5006	1	491	32	99	100	100	94	Fowler & Wilkins (1984)
46 to 93	3.5	554	520	0	34	0	100	100	100	100	White et al. (1984)
29 to 89	3	724	593	3	123	5	99	98	99	96	Taverne et al. (1985)
50 to 100	3.5	516	473	0	37	6	99	100	100	88	Davey (1986)
< 40 to > 100		2499	2331	21	141	6	100	87	99	96	Logue et al. (1987)
51 to 75	5	26	24	0	2	0	100	100	100	100	Gearhart et al. (1988)

a, correct positive (pregnant); b, false positive (non-pregnant); c, correct negative (non-pregnant); d, false negative (pregnant)

Table 7

Sensitivity (Se), Specificity (Sp) and Predictive (+PV, -P V) values of using transrectal (TR) and transabdominal (TA) ultrasonography for determination of fetal numbers in sheep

Days of exam.	Method of exam.	No. of animals	а	b	с	d	Se %	Sp %	+PV %	-PV %	Authors
46 to 106	TA	5039	1328		3577		94	99	99	98	Fowler & Wilkins (1984)
46 to 93	TA	520	327	1	190	2	99	99	100	99	White et al. (1984)
45 to 77	TA	210	142	5	53	10	93	91	97	84	Taverne et al. (1985)
50 to 100	TA	479	118	0	349	12	91	100	100	97	Davey (1986)
<40 to > 100	TA	2348	1216		1006		96	94	95	94	Logue et al. (1987)
26 to 50	TR	24					5	80			Gearhart et al. (1988)
51 to 75	TA	24					97	100			Gearhart et al. (1988)

a, correct positive (multiple); b, false positive (single); c, correct negative (single); d, false negative (multiple)

# 5.3.2. Determination of the fetal number

By using transrectal ultrasonography (7.5 MHz), single and multiple pregnancies in sheep were accurately (15 of 17 ewes) detected on Day 25 (Schrick and Inskeep 1993). However, the accuracy of a 5 MHz transrectal ultrasonography for detecting ewes carrying two fetuses or more was disappointing (Gearhart et al. 1988; Table 7). By using transabdominal ultrasonography, the accuracy of experienced operator for determination both single- and multiple-bearing ewes was 99% from Days 46 to 93 of gestation (White et al. 1984). A similar accuracy for ewes carrying single fetus was reported by Fowler and Wilkins (1984), Davey (1986) and Gearhart et al. (1988), however, a lesser accuracy for ewes carrying multiples was reported by others (Table 7).

## 5.3.3. Estimation of gestational age

When the date of mating is unknown, monitoring fetal development allows estimation of gestational age.

## A-Embryonic vesicle

Gonzalez et al. (1998) measured the ovine embryonic vesicle from Days 12 to 29 of gestation by using 7.5 MHz transrectal ultrasonography and found a close correlation (r = 0.76) with the gestational age.

### B-Crown-Rump length

By using transrectal ultrasonography (7.5 MHz), Schrick and Inskeep (1993) measured the crown-rump length of the ovine fetus from Days 20 to 40 of gestation and described the relationship between the crown-rump length (x) and the gestational age (y) by the following equation,  $Y = 14.05 + 1.16x - 0.012x^2$ . By using the same approach, Gonzalez et al. (1998) reported a high (r = 0.94) correlation between the crown-rump length and the gestational age from Days 19 to 48 of gestation.

## C-Fetal head diameters

Fetal head diameters including the biparietal diameter, the occipito-nasal length and the diameter of the orbit were used to predict the stage of gestation in sheep.

Regarding to the biparietal diameter (BPD), Gonzalez et al. (1998) used the transrectal ultrasonography to measure the BPD of Manchega sheep from Days 32 to 90 and found a high correlation (r=0.96) between the measured diameters and the gestational age. Similar correlation was found by using transabdominal approach in Suffolk and Finn sheep from Days 40 to 95 (Haibel and Perkins 1989), in Booroola x South Australian Merino sheep from Days 49 to 109 (Sergeev et al. 1990) and in Swedish peltsheep from 10 weeks before lambing to birth (Aiumlamai et al. 1992).

Kelly and Newnham (1989) found the occipito-nasal length to be more accurate than BPD, showing a linear increase till Day 80. However, Sergeev et al. (1990) reported that the occipito-nasal length was more difficult to be measured than BPD and had the same accuracy for predicting fetal age. Gonzalez et al. (1998) found a high correlation (r = 0.95) between the fetal occipito-nasal length and the gestational age from Days 38 to 91 of gestation.

Regarding the diameter of the fetal orbit, Gonzalez et al. (1998) reported that the ovine fetal orbit increased in diameter from 2 mm at Day 36 to 17 mm at Day 90 of gestation and it gave a high correlation (r = 0.92) with the fetal age.

## D-Thoracic diameter

Ultrasonographic measurements of the ovine fetal thoracic diameter showed high

correlation with the fetal age from Days 49 to 109 (Sergeev et al. 1990) and from Days 23 to 90 of gestation (Gonzalez et al. 1998).

# E-Fetal heart rate

By using 7.5 MHz transrectal ultrasonography, the rhythmic pulsations within the ovine embryonic vesicle were first detected on Day18 or 19 after mating (Schrick and Inskeep 1993), while by using 5 MHz transrectal ultrasonography, they were first observed from Days 21-23 after mating (Garcia et al. 1993). Aium1amai et al. (1992) measured the ovine fetal heart rate during the second half of pregnancy by using transabdominal ultrasonography and reported that the fetal heart rate reached a plateau at 7 weeks before lambing ( $167 \pm 1.5$  bpm) then decreased at 3 weeks before lambing ( $139.0 \pm 15.7$  bpm) and reached  $117.0 \pm 9.2$  bpm at birth. In addition, a significant correlation was found between fetal heart rate and gestational age.

#### F-Placentome size

Placentomes could be detected by transrectal ultrasonography (5 MHz) on Day 30 (Buckrell et al. 1986) and on Day 32 of gestation (Doize et al. 1997). At this period the placentomes appeared as echogenic areas on the surface of endometrium. On Day 42, the ovine placentomes presented cup-shaped forms and reached the maximum size by Day 74 (Doize et al. 1997). There was a poor correlation between placentome size and ovine gestational age due to great variation in the size of placentome in the same observations (Doize et al. 1997; Gonzalez et al. 1998). In contrast, Kelly et al. (1987) found a significant quadratic relationship between ultrasonographic cotyledon diameter and square root transformation of day of pregnancy.

## G-Other fetal structures

There was a high correlation (r = 0.96) between the width of three ovine fetal coccygeal vertebrae and gestational age. At the same time, somewhat lower correlation was found for umbilical cord diameter (r = 0.72) and fetal femur length (r = 0.78) (Gonzalez et al. 1998).

### 5.4. Determination of fetal sex

Depending on the location of the genital tubercle of the ovine fetus, the accuracy of the transrectal ultrasonography (5 MHz) for detecting male and female fetuses was 100% and 76%, respectively from Days 60 to 69 of gestation (Coubrough and Castell 1998).

## Conclusions

Early detection of pregnancy and determination of the fetal numbers have economical benefits to sheep producers. The method used for pregnancy diagnosis should be simple, accurate, rapid, inexpensive, practical and safe for both operators and animals. Accurate pregnancy diagnosis can be achieved by progesterone and oPAG or oPSPB assays, however, their accuracy for differentiating single and multiple fetuses would not be regarded as sufficiently high to be of practical value and they are expensive. Rectal abdominal palpation is a simple, cheap and quick method, however its accuracy for determining multiple pregnancies is low and it may cause abortion or rectal perforation. Doppler technique requires great skill to achieve high accuracy for prediction of fetal numbers. Radiography and transabdominal B-mode ultrasonography accurately diagnose both pregnancy and fetal numbers, but the second technique is cheaper than the first one and has the advantages of being safe and able to detect the fetal viability. The optimum time for using transabdominal or transrectal ultrasonography in sheep ranges from 25 to 100 days of gestation.

## Diagnostika březosti u ovcí: přehled nejdůležitějších praktických metod

Pro stanovení březosti u ovcí se využívají různé praktické metody. Březost i počet plodů lze spolehlivě radiologicky vyšetřit po 70 dnu březosti. Rektální abdominální technika detekuje březost s přesností 66-100% od 49. do 109. dne březosti, s nízkou přesností stanovení(17-57%) počtu plodů. Progesteronové metody jsou vysoce citlivé (88-100%) s nízkou specifitou (60-72%), od 16. do 18. dne. Stanovení estron sulfátu přesně detekuje březí ovce od 30. do 35. dne březosti. Metoda bovinního specifického proteinu březosti B (PSPB) s přesností (100%) detekuje březost již od 26. dne. Spolehlivost těchto metod - progesteronové, estron sulfátové a PSPB metody technikou "A-mode" a Dopplerovou lze březost přesně detekovat v druhé polovině. Počet plodů nemůže být stanoven technikou A mode, zatímco za použití Dopplerovy je třeba získat další zkušenosti abychom mohli dosáhnout vyšší přesnosti. Transrektální ultrasonografický postup "B-mode" identifikuje počet embryí již 13. den po zabřeznutí, ale citlivost techniky pro březost je velmi nízká (12%), menší než 25. den po zabřeznutí. U transabdominální B-mode ultrasonografie bylo dosaženo vysoké spolehlivosti pro diagnostiku březosti (94-100%) a stanovení počtu plodů (92-99%) od 29. do 106. dne. Tato metoda diagnostiky březosti a stanovení počtu plodů u ovcí je v současné době nejpřesnější a praktická.

### References

AIUMLAMAI, S., FREDRIKSSON, G., NILSFORS, L. 1992: Real-time ultrasonography for determining the gestational age of ewes. Vet. Rec. 131: 560-562

AMEZCUA-MORENO, M. D. R. 1988: Pregnancy diagnosis in ewes by means of the determination of blood progesterone concentration on Day18 after insemination using enzyme immunoassay. Vet. Mexico **19**: 386 (Abstract)

- BORJESSON, D. L., BOYCE, W. M., GARDNER, I. A., DEFORGE, J., LASLEY, B. 1996: Pregnancy detection in Bighorn sheep (*Ovis canadensis*) using a fecal-based enzyme immunoassay. J. Wildl. Dis. **32**: 67-74
- BRETZLAFF, K., EDWARDS, J., FORREST, D., NUTI, L. 1993: Ultrasonographic determination of pregnancy in small ruminants. Vet. Med. 88: 12-24
- BUCKRELL, B. C., BONNETT, B. N., JOHNSON, W. H. 1986: The use of real-time ultrasound rectally for early pregnancy diagnosis in sheep. Theriogenology 25: 665-673
- BUTLER, J. E., HAMILTON, W. C., SASSER, R. G., RUDER, C. A., HASS, G. M., WILLIAMS, R. R. 1982: Detection and partial characterization of two bovine pregnancy-specific proteins. Biol. Reprod. 26: 925-933
- CHAN, J. S. D., ROBERTSON, H. A., FRIESEN, H. G. 1978: Maternal and fetal concentration of ovine placental lactogen measured by RIA. Endocrin. **102**: 1606-1613
- CHAUHAN, F. S., SANDABE, U. K., OYEDIPE, E. O. 1991: Predicting number of fetus(es) in small ruminants. Indian Vet. J. 68: 751-754
- CHAUHAN, F. S., WAZIRI, M. A. 1991: Evaluation of rectal-abdominal palpation technique and hormonal diagnosis of pregnancy in small ruminants. Indian J. Anim. Reprod. **12**: 63-67
- COUBROUGH, C. A., CASTELL, M. C. 1998: Fetal sex determination by ultrasonically locating the genital tubercle in ewes. Theriogenology **50**: 263-267
- DAVEY, C. G. 1986: An evaluation of pregnancy testing in sheep using a real-time ultrasound scanner. Aust. Vet. J. 63: 347-348
- DEAS, D.W. 1977: Pregnancy diagnosis in the ewe by an ultrasonic rectal probe. Vet. Rec. 101: 113-115
- DOIZE, F., VAILLANCOURT, D., CARABIN, H., BELANGER, D. 1997: Determination of gestational age in sheep and goats using transrectal ultrasonographic measurement of placentomes. Theriogenology 48: 449-460
- ECKBLAD, W. P., SASSER, R. G., RUDER C. A., PANLASIGUI, P., KUCZYNSKI, T. 1985: Localization of pregnancy-specific protein B (PSPB) in bovine placental cells using glucose oxidase-anti-glucose oxidase immunohistochemical stain. J. Anim. Sci. **61** (Suppl.): 149-150
- FOLCH, J., BENITEZ, W., ALABART, J. L., BECKERS, J.F. 1993: Determination de la concentracion plasmatica de PAG (pregnancy- assosciated glycoprotein) en cabras Blanca Celtibérica y su utilizacion como diagnostico de gestacion. ITEA 12: 364-366 (in Spanish).
- FORD, E. J. H., CLARK, J. W., GALLUP, A. L. 1963: The detection of fetal numbers in sheep by means of Xrays. Vet. Rec. **75**: 958-960
- FORD, E. J. H. 1983: Pregnancy toxemia. In: MARTIN, W. B.: Diseases of sheep. Blackwell Scientific Publications, London, pp. 147-151
- FOWLER, D. G., WILKINS, J. F. 1984: Diagnosis of pregnancy and number of the fetuses in sheep by real-time ultrasonic imaging. 1. Effect of number of fetuses, stage of gestation, operator and breed of ewe on accuracy of diagnosis. Livest. Prod. Sci. **11**: 437-450

FUKUI, Y., KIMURA, T., ONO, H. 1984: Multiple pregnancy diagnosis in sheep using an ultrasonic Doppler method. Vet. Rec. 114: 145

- FUKUI, Y., KOBAYASHI, M., TSUBAKI, M., TETSUKA, K., SHIMODA, K., ONO, H. 1986: Comparison of two ultrasonic methods for multiple pregnancy diagnosis in sheep and indicators of multiple pregnant ewes in the blood. Anim. Reprod. Sci. 11: 25-33
- GAJEWSKI, Z., BECKERS, J. F., MELO DE SOUSA, N., THUN, R., SULON, J., FAUNDEZ 1999: Determination of pregnancy associated glycoprotein concentrations in sheep: A retrospective study. Advances in Cell Biology 26 (suppl. 12): 89-96 (in Polish)
- GARCIA, A., NEARY, M. K., KELLY, G. R., PIERSON, R. A.1993: Accuracy of ultrasonography in early pregnancy diagnosis in the ewe. Theriogenology 39: 847-861
- GÉARHART, M. A., WINGFIELD, W. E., KNIGHT, A. P., SMITH, J. A., DARGATZ, D. A., BOON, J. A., STOKES C.A. 1988: Real-time ultrasonography for determining pregnancy status and viable fetal numbers in ewes. Theriogenology 30: 323-337
- GONZALEZ, B. A., SANTIAGO, M. J., LOPEZ, S. A. 1998: Estimation of fetal development in Manchega dairy ewes by transrectal ultrasonographic measurements. Small Rumin. Res. 27: 243-250
- GONZALEZ, F. SULON, J. GARBAYO, J. M., BATISTA, M., CABRERA, F., CALERO, P., GRACIA, A., BECKERS, J. F. 1999: Early pregnancy diagnosis in goats by determination of pregnancy-associated glycoprotein concentrations in plasma samples. Theriogenology **52**: 717-725
- GRACE, N. D., BEACH, A. D., QUINLIVAN, T. D., WARD, B. 1989: Multiple pregnancy diagnosis of using real time ultrasonic body scanner and video-fluoroscopy systems. Proc. N. Z. Soc. Anim. Prod. 49: 107-111
- GVOZDIC, D., IVKOV, V. 1994: Early pregnancy diagnosis in ewes. Acta Vet. (Beograd) 44: 215-219.
- HAIBEL, G. K., PERKINS, N. R. 1989: Real-time ultrasonic biparietal diameter of second trimester Suffolk and Finn sheep fetuses and prediction of gestational age. Theriogenology 32: 863-869
- HULET, C. V. 1972: A rectal-abdominal palpation technique for diagnosing pregnancy in the ewe. J. Anim. Sci. 35: 814-818

HULET, C. V. 1973: Determining fetal numbers in pregnant ewes. J. Anim. Sci. 36: 325-330

ILLERA, J. C., GRACIA, O., SILVAN, G., ILLERA, M. J., MUNRO, C. J., ILLERA, M. 2000. Assessment of fetal number and fetal viability throughout pregnancy in Manchega ewe. 14th Inter. Cong. Anim. Reprod., Stockholm, 2:181

- ISHWAR, A. K. 1995: Pregnancy diagnosis in sheep and goat: A review. Small Rumin. Res. **17**: 37-44 KALKAN C., CETIN, H., KAYGUSUZOGLU, E., YILMAZ, B., CIFTCI, M., YILDIZ H., YILDIZ, A., DEVECI, H., APAYDIN A. M., OCAL, H. 1996: An investigation on plasma progesterone levels during pregnancy and parturition in the Ivesi sheep. Acta Vet. Hung. 44: 335-340.
- KELLY, R. W., NEWNHAM, J. P., JOHNSON, T., SPEIJERS, E. J. 1987: An ultrasound technique to measure placental growth in ewes. Aust. J. Agric. Res. 38: 757-764
- KELLY, R. W., NEWNHAM, J. P. 1989: Estimation of gestational age in Merino ewes by ultrasound measurement of fetal head size. Aust. J. Agri. Res. 40: 1293-1299
- KUTTY, C. I., SUDARSANAN, V. 1996: Bimanual examination of reproductive organs of nanny goats. Proc. 8th Kerala Sci. Cong. 113-114
- KUTTY, C. I. 1999: Gynecological examination and pregnancy diagnosis in small ruminants using bimanual palpation technique: A review. Theriogenology 51: 1555-1564
- LINDAHL, I. L. 1971: Pregnancy diagnosis in the ewe by intrarectal Doppler. J. Anim. Sci. 32: 922-925
- LOGUE, N. D., HALL, J. T., MCROBERTS, S., WATERHOUSE, H. 1987: Real-time ultrasonic scanning in sheep: The results of first year of its application on farms in South-west Scotland. Vet. Rec. 121: 146-149
- MADEL, A. J. 1983: Detection of pregnancy in ewe lambs by A-mode ultrasound. Vet. Rec. **112**:11-12 McPHEE, I. M., TIBERGHIEN, M. P. 1987: Assessment of pregnancy in sheep by analysis of plasma progesterone using an amplified enzyme immunoassay technique. Vet. Rec. 121: 63-65
- MEREDITH, M. J., MADANI, M. O. K. 1980: The detection of pregnancy in sheep by A-mode ultrasound. Br. Vet. J. 136: 325-330
- RANILLA, M. J., SULON, J., CARRO, M. D., MANTECON, A. R., BECKERS, J. F. 1994: Plasmatic profiles of pregnancy-associated glycoprotein and progesterone levels during gestation in Churra and Merino sheep. Theriogenology 42: 537-545
- RANILLA, M. J., SULON, J., MANTECON, A. R., BECKERS, J. F., CARRO, A. D. 1997: Plasma pregnancyassociated glycoprotein and progesterone concentrations in pregnant Assaf ewes carrying single and twin lambs. Small Rumin. Res. 24: 125-131
- ROBERTSON, H. A., CHAN, J. S. D., RIESEN, H. G. 1980: The use of a pregnancy-specific antigen, chorionic somatomammotrohin, as an indicator of pregnancy in sheep. J. Reprod. Fert. 58: 279-281
- RUDER, C. A., STELLFLUG, J. N., DAHMEN, J. J., SASSER, R. G. 1988: Detection of pregnancy in sheep by radioimmunoassay of sera for pregnancy-specific protein B. Theriogenology **29**: 905-912 RUSSEL, A. J. F., GODDARD, P. J. 1995: Small ruminant reproductive ultrasonography. In: GODDARD, P.
- G.:Veterinary Ultrasonography. CAB International, Wallingford, Oxon OX108DE, UK, pp. 257-274 SANDABE, U. K., CHAUHAN, F. S., CHAUDHRI, S. U. R., WIZIRI, M. A. 1994: Studies on predicting number
- of foetus(es) in small ruminants. Pakistan Vet. J. 14: 97-100

SCHNEIDER, F. A., HALLFORD, D. M. 1996: Use of a rapid progesterone radioimmunoassay to predict pregnancy and fetal numbers in ewes. Sheep and Goat Res. J. 12: 33-38

- SCHRICK, F. N., INSKEEP, E. K. 1993: Determination of early pregnancy in ewes utilizing transrectal ultrasonography. Theriogenology **40**: 295-306 SERGEEV, L., KLEEMANN, D. O., WALKER, S. K., SMITH, D. H., GROSSER, T. I., MANN, T., SEAMARK,
- R. F. 1990: Real-time ultrasound imaging for predicting ovine fetal age. Theriogenology 34: 593-601
- SUSMEL, P., PIASENTIER, E. 1992: Assessment of pregnancy in Bergamasca ewes by analysis of plasma progesterone. Small Rumin. Res. 8: 325-332SZENCI, O., BECKERS, J. F. HUMBLOT, P., SULON, J., SASSER, G., TAVERNE, M. A. M., VARGA, J.,
- BALTUSEN, R., SCHEKK, GY. 1998: Comparison of ultrasonography, bovine pregnancy-specific protein B, and
- bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. Theriogenology **50**: 77-88 TAVERNE, M. A. M., LAVOIR, M. C., VAN OORD, R., VAN DER WEYDEN, G. C. 1985: Accuracy of pregnancy diagnosis and prediction of fetal numbers in sheep with linear-array real- time ultrasound scanning. The Vet. Quart. 7: 256-263
- TRAPP, M. J., SLYTER, A. L. 1983: Pregnancy diagnosis in the ewe. J. Anim. Sci. 57: 1-5
- TSANG, C. P. W. 1978: Plasma levels of estrone sulphate, free estrogens and progesterone in the pregnant ewe throughout gestation. Theriogenology 10: 97-110
- TURNER, C. B., HINDSON, J. C. 1975: An assessment of a method of manual pregnancy diagnosis in the ewe. Vet. Rec. 96: 56-58
- TYRRELL, R.N., PLANT, J. W. 1979: Rectal damage in ewes following pregnancy diagnosis by rectal-abdominal palpation. J. Anim. Sci. 48: 348-350
- VECCHIO, R. P. D., SUTHERLAND, W. D., SASSER, R. G. 1995: Effect of pregnancy-specific protein B on luteal cell progesterone, prostaglandin, and oxytocin production during two stages of the bovine estrus cycle. J. Anim. Sci. 73: 2662-2668
- WALLACE, J. M., AITKEN, R. P., CHEYNE, M. A., HUMBLOT P. 1997: Pregnancy-specific protein B and progesterone concentrations in relation to nutritional regimen, placental mass and pregnancy outcome in growing adolescent ewes carrying singleton fetuses. J. Reprod. Fert. 109: 53-58
- WÄNI, N. A., WANI, G. M., MÚFTI, A. M., KHAN, M. Z. 1998: Ultrasonic pregnancy diagnosis in gaddi goats. Small Rumin. Res. 29: 239-240
- WATT, B. R., ANDRESON, G. A., CAMPELL, I. P. 1984: A Comparison of six methods used for detecting pregnancy in sheep. Aust. Vet. J. 61: 377-382
- WEST, D. M. 1986: Pregnancy diagnosis in the ewe. In: MORROW, D. A.: Current Therapy in Theriogenology. W. B. SAUNDERS, Philadelphia pp. 850-852
- WHITE, I. R., RUSSEL, A. J. F., FOWLER, D. G. 1984: Real-time ultrasonic scanning in the diagnosis of pregnancy and the determination of fetal numbers in sheep. Vet. Rec. **115**: 140-143 WILLARD, J. M., RUDER, C. A., SASSER, R. G., STELLFLUG, J., 1987: Ovine pregnancy-specific protein
- B concentration in the sera of early pregnant and peripartum ewes. J. Anim. Sci. 65 (suppl.): 514
- WILLARD, J. M., WHITE, D. R., WESSON, C. A. R., STELLFLUG, J., SASSER, R. G. 1995: Detection of fetal
- twins in sheep using radioimmunoassay for pregnancy-specific protein B. J. Anim. Sci. **73**: 960-966 WORSFOLD, A. I., CHAMINGS, R. J., BOOTH, J. M. 1986: Measurement of estrone sulphate in sheep plasma as a possible indicator of pregnancy and the number of viable fetuses present. Br. Vet. J. 142: 195-197
- XIE, S., LOW, B. G., NAGEL, R. G., KRAMER, K. K, ANTHONY, R. V., ZOLI, A. P., BECKERS, J. F., ROBERTS, R. M. 1991: Identification of the major pregnancy specific antigens of cattle and sheep as inactive members of aspartic proteinase family. Proc. Nat. Acad. Sci. (USA) 88: 10247-10251
- XIE, S., GREEN, J., BANGA, B., BECKERS, J. F., VALDEZ, K. E., HAKAMI, L. ROBERTS, R. M. 1997: Multiple pregnancy-associated glycoproteins are secreted by Day 100 ovine placental tissue. Biol. Reprod. 57: 1384-1393
- ZARKAWI, M. 1997: Monitoring the reproductive performance in Awassi ewes using progesterone radioimmunoassay. Small Rumin. Res. 26: 291-294
- ZARKAWI, M., AL-MERSETANI, M. R., WARDEH, M. F. 1999: Induction of synchronized estrus and early pregnancy diagnosis in Syrian Awassi ewes outside the breeding season. Small Rumin. Res. 33: 99-102
- ZÓLI, A. P., BECKERS, J. F., ECTORS, F. 1995: Isolation and partial characterization of a pregnancy- associated glycoprotein in the ewe Ann. Méd.Vét., 139:177-184. (in French)
- ZOLI, A. P., GUILBAULT, L. A., DELAHAUT, P., ORTIZ, W. B., BECKERS, J. F. 1992; Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: Its application for pregnancy diagnosis. Biol. Reprod. 46:83-92