

Transrectal ultrasonography in diagnosing the ovulation rate in sheep

Piotr Ślósarz, Agnieszka Frankowska, Maciej Miś

Department of Sheep and Goat Breeding,
The August Cieszkowski Agricultural University of Poznań,
Złotniki, Słoneczna 1, 62-002 Suchy Las, Poland

(Received July 29, 2003; accepted September 2, 2003)

On the day of ovulation and next on day 7 after ovulation the ovaries of 20 sheep were examined using the transrectal ultrasound method. To verify the results, similar examination was made with the laparoscopic method. Depending on the stage of the sexual cycle (day 1 vs day 7) the number of Graafian follicles ($\varnothing \geq 5$ mm) was determined as well as the total number of *corpora lutea* found in both ovaries. Out of the total number of 53 Graafian follicles found laparoscopically, 42 (79,2%) were detected with the ultrasound method. The detectability of *corpora lutea* with the ultrasound method was found similar (79,5%). A considerable improvement in the accuracy of ultrasonic measuring was observed when the determinations were repeated next year. This reflects the effect of the experience gathered by the person taking the measurements.

KEY WORDS: ovulation rate / sheep / ultrasonography

Ovulation rate determined on the basis of the number of *corpora lutea* in a given oestrous cycle is an important trait in the selection of high-prolificacy sheep. Till now the trait has routinely been measured – also in Poland – using the laparoscopic method. However, the method does not meet the current obligatory standards for the animals welfare protection [Haresign *et al.* 1995].

The improved ultrasound technique have made it possible to use the transrectal examinations aiming at monitoring the growth of Graafian follicles, as well as the development of the embryo in the early period of pregnancy [Garcia *et al.* 1993, Ravindra *et al.* 1994, Souza *et al.* 1997, Ślósarz *et al.* 1999, Bartlewski *et al.* 2000, Zięba *et al.* 2001]. Relatively fewer are studies concerning the application of the ultrasound technique to

assess the morphology and number of *corpora lutea* [Kaulfuss 1996, Anderson *et al.* 1997, Dickie *et al.* 1998]. The aim of the investigations presented here was to compare the ultrasonical transrectal detectability of *corpora lutea* with that obtained conventionally with laparoscopic method in sheep with an average prolificacy potential.

Material and methods

The investigations were conducted in two consecutive years (replication I and II, 9 and 11 randomly selected ewes, respectively) on 2-3 years old animals of a dairy line (5/8 East Friesian Milk Sheep and 3/8 Polish Merino). In order to facilitate the examinations in precisely determined stages of the sexual cycle the ewes were subjected to oestrus synchronization with the implantation of intravaginal sponges containing 45 mg of Cronolone over 14 days (Chrono-Gest® method – INTERVET International B.V.). During the removal of the intravaginal sponges a stimulating dose of serogonadotropin (500 units) was injected intramuscularly. A total of 28 sheep were subjected to oestrus synchronization, out of which ovulation did not take place on the expected date in eight ewes, which were excluded from further studies (Tab. 1). Ovaries were examined ultrasonically on the day of ovulation and then on day 7 after ovulation (day 1 and 7, respectively). Each time the results were verified laparoscopically. Depending on the stage of the sexual cycle (day 1 vs day 7), the number of Graafian follicles ($\varnothing \geq 5$ mm) and the total number of *corpora lutea* in both ovaries was determined and recorded.

For rectal examinations the PieMedical SCANNER 100LC ultrasound device was used with a 8 MHz linear probe (axial and lateral resolution 1.0×1.2 mm). Ultrasound images were recorded using a VHS recorder for further analysis. Ultrasound examinations were conducted *via* the rectum when a sheep was put horizontally on the back. Such a position resulted in the uterus falling – resulting from its own weight – directly on the ultrasound probe inserted into the rectum. The small distance between the surface of the ultrasound probe and the uterus made it possible to conduct examinations in a high range of sound waves (8 MHz) and thus to obtain clear ultrasound images. The ultrasound probe was covered with gel and inserted into the rectum approx. 10 cm deep, and first the right and then the left ovary was found (Fig. 1).

Immediately after the ultrasound examinations the laparoscopic procedure was performed to verify the results. This was done with a Wolf endoscope ($\varnothing 7$ mm) under general anaesthesia (2% xylazinum) according to Boyd and Ducker [1973]. The results were recorded on the VHS tape with a Wolf-CCD 5501 endocamera. Both ultrasound and endoscopic images collected were used to verify and document the results of the examinations (Fig. 1-4).

The total number of Graafian follicles and *corpora lutea* observed in both ovaries were recorded on the day of ovulation and then on day 7 after ovulation. Moreover, the per cent detectability of both ovarian structures was calculated, assuming the results obtained laparoscopically as 100.

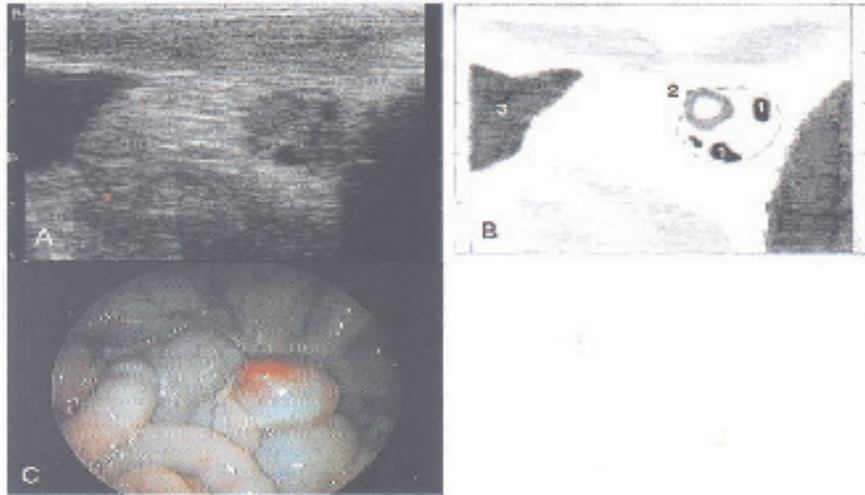


Fig. 1. A, B: **1** – ovary marked with a dotted line with two Graafian follicles, **2** – single *corpus luteum*, **3** – urinary bladder. C: right – ovary with a formed mushroom-like *corpus luteum*, left – twisted horns of the uterus. Photo by Piotr Ślósarz.

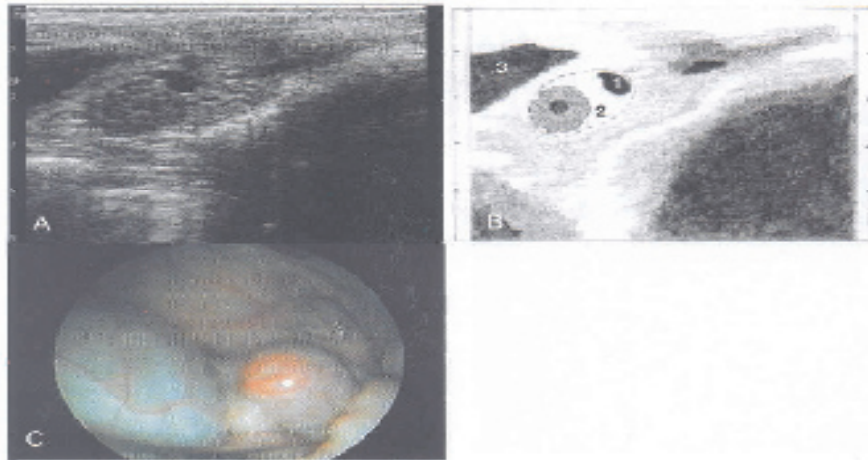


Fig. 2. A, B: **1** – ovary marked with a dotted line with the Graafian follicle, **2** – *corpus luteum* with the central non-echogenic lacuna, **3** – urinary bladder. C: right – ovary with a well-formed corpus luteum, left – urinary bladder. Photo by Piotr Ślósarz.

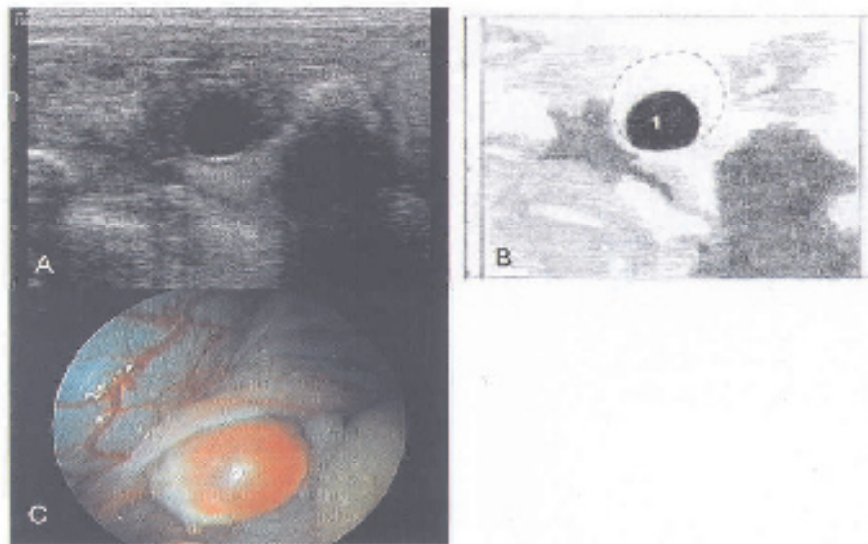


Fig. 3. A, B: 1 – large Graafian follicle in the ovary marked with a dotted line. C: ovary with a pre-ovulating Graafian follicle. Photo by Piotr Ślósarz.

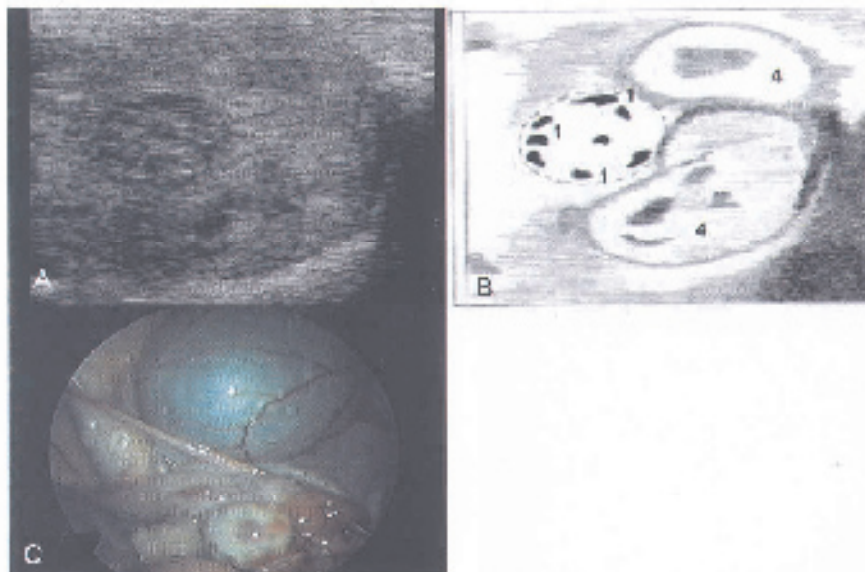


Fig. 4. A, B: 1 – ovary marked with a dotted line with small Graafian follicles, 4 – horns of the uterus. C: bottom – ovary with small follicles, upper – urinary bladder. Photo by Piotr Ślósarz.

Results and discussion

The mean ovulation rate was 2.2 (Tab. 1) appearing very similar to that characteristic of the breed as reported by Steppa and Ślósarz [1999]. It may indicate the appropriate selection of animals for this study and no negative effects appearing of oestrus synchronization on the ovaries activity. Emphasized should be the imbalance in the distribution of *corpora lutea* – the right vs left ovary. Similar imbalance was observed earlier in sheep during spontaneous oestrus [Ślósarz and Stanisz 1997, Steppa and Ślósarz 1999]. The differences in the activity of both ovaries in sheep have also been mentioned e.g. by Dickie *et al.* [1998] who, however, did not explain the biological cause of this phenomenon.

Table 1. Ovulation rate in ewes

Replication (number of ewes)	Mean age of ewes (years)	Number of <i>corpora lutea</i>		Mean ovulation rate
		left ovary	right ovary	
I (9)	2.1	7	13	2.22
II (11)	2.4	10	14	2.18
Mean	2.2	0.85	1.35	2.20

Table 2. The detectability of Graafian follicles and *corpora lutea* with the transrectal ultrasonography

Replication (number of ewes)	Graafian follicles number (%)		<i>Corpora lutea</i> number (%)	
	laparoscopy	ultrasonography	laparoscopy	ultrasonography
I (9)	22 (100)	17 (77.3)	20 (100)	15 (75.0)
II (11)	31 (100)	25 (80.6)	24 (100)	20 (83.3)
Mean		79.2 %		79.5 %

The detectability with laparoscopy was assumed as 100%

Table 2 presents the total number of Graafian follicles and *corpora lutea* observed in ewes in both replications. Relatively low ultrasound detectability of Graafian follicles (about 80%) resulted probably from liquid filling the follicles, clearly seen as dark non-echogenic areas of a regular oval shape (Fig. 3 and 4). It may partially be explained by the fact that the examination was performed during oestrus, when the considerable volume of liquid in the uterus made observations difficult.

The detectability of *corpora lutea* amounting to almost 80% (Tab. 2) seems satisfactory at this stage of investigations, especially as the results appeared better in the second replication (rise of ultrasound detectability from 75.0 to 83.3%). A similar increase in the accuracy of the detection of the *corpora lutea* (from 75 in the first, to over 90% in the third year of the study) was reported by Kaulfuss [1996]. Moreover,

Anderson *et al.* [1997] and Dickie *et al.* [1998] reported the detectability of *corpora lutea* close to 90%. Also in other animal species ultrasonic detection of all *corpora lutea* is rather difficult as the luteal tissue gives an echo similar to that of the stroma of the ovary [Martinat-Botte *et al.* 2000]. This phenomenon may partially explain different detectabilities of *corpora lutea* in the present study, depending on their number. In ewes with two *corpora lutea* on the ovary, their ultrasonic detectability was 100%. Wrong diagnoses (the *corpus luteum* not detected at all) occurred in case of only one *corpus luteum* present or when their number was ≥ 3 .

Two morphologically different forms of *corpora lutea* were observed. One of them was visible in the ultrasound image as a uniform echogenic area (Fig. 1), while the other had a centrally located non-echogenic spot, indicating the presence of liquid surrounded by the luteal tissue (Fig. 2). Such two forms, not different functionally, were also observed by Kaulfuss [1996] and Martinat-Botte *et al.* [2000].

Increased ultrasonic detectability of *corpora lutea* from 75% in year 1 to 83% in year 2 indicates the effect of the rising experience of the person performing the measurements. The results obtained show the feasibility to replace the laparoscopic method with a non-invasive ultrasound technique to assess the ovulation rate in sheep. However, further improvement in the measurement accuracy of ovulation rate is necessary.

REFERENCES

1. ANDERSON J.M.L., DICKIE A.M., PATERSON C., BOYD J.S., HARVEY M.J.A., WATERHOUSE A., 1997 – An evaluation of techniques for determining the number of corpora lutea or embryos in the ewe. Proceedings of 48th EAAP, Vienna, pp. 315.
2. BARTLEWSKI P.M., BEARD A.P., RAWLINGS N.C., 2000 – Ultrasonographic study of ovarian function during early pregnancy and after parturition in the ewe. *Theriogenology* 53, 673-689.
3. BOYD J.S., DUCKER M.J., 1973 – A method of examining the cyclic changes occurring in the sheep ovary using endoscopy. *Veterinary Record* 93, 40-43.
4. DICKIE A.M., PATERSON C., ANDERSON J.L.M., BOYD J.S., 1998 – Determination of corpora lutea numbers in Booroola-Texel ewes using transrectal ultrasound. *Theriogenology* 51, 1209-1224.
5. 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.
6. HARESIGN W., WILLIAMS R.J., KHALID M., RODWAY R., 1995 – Heart rate responses and plasma cortisol and β -endorphin concentrations in ewes subjected to laparoscopy and its associated handling procedures. *Animal Science* 61, 77-83.
7. KAULFUSS K-H., 1996 – Ultrasonography in ovine gynaecology. University of Halle-Wittenberg – the booklet.
8. MARTINAT-BOTTE F., RENAUD G., MADEC F., COSTIOU P., TERQUI M., 2000 – Ultrasonography and reproduction in swine. Principles and practical applications. INRA, Paris pp. 40-48.
9. RAVINDRA J.P., RAWLINGS N.C., EVANS A.C.O., ADAMS G.P., 1994 – Ultrasonographic study of ovarian follicular dynamics in ewes during the oestrous cycle. *Journal of Reproduction and Fertility* 101, 501-509.
10. SOUZA C.J.H., CAMPBELL B.K., BAIRD D.T., 1997 – Follicular dynamics and ovarian steroid secretion in sheep during the follicular and early luteal phases of the estrous cycle. *Biology of Reproduction* 56, 483-488.

11. STEPPA R., ŚLÓSZARZ P., 1999 – Plenność owiec linii matecznej 05 w zależności od typu transferyny i hemoglobiny (Prolificacy of the sheep of maternal line 05, depending on the type of transferrin and haemoglobin). In Polish with English summary. *Zeszyty Naukowe Przeglądu Hodowlanego* 43, 323-329.
12. ŚLÓSZARZ P., STANISZ M., 1997 – Stopień owulacji jako wskaźnik plenności owiec syntetycznej linii matecznej. Ovulation rate as the indicator of litter size of the synthetic dam line ewes (in Polish with English summary). *Prace Komisji Nauk Rolniczych i Leśnych PTPN* 83, 201-206.
13. ŚLÓSZARZ P., STEPPA R., GADEK A., 1999 – Próba wykorzystania ultrasonografii transrektalnej do wczesnej diagnostyki ciąży u owiec (The application of ultrasound technique for early pregnancy diagnosis in sheep). In Polish with English summary. *Medycyna Weterynaryjna* 55, 686-688.
14. ZIĘBA D.A., MURAWSKI M., WIERZCHOŚ E., 2001 – Pattern of follicular development during the oestrous cycle of prolific Olkuska sheep. *Archiv für Tierzucht* 44 (S), 203-212.

Piotr Śłószarz, Agnieszka Frankowska, Maciej Miś

Zastosowanie ultrasonografii *trans rectum* do pomiaru stopnia owulacji owiec

Streszczenie

W dniu owulacji i w siódmym dniu po owulacji badano metodą ultrasonograficzną (*trans rectum*) jajniki 20 dorosłych owiec, a następnie, dla weryfikacji wyników, badania powtórzono metodą laparoskopii. W obu fazach cyklu płciowego określono liczbę pęcherzyków Graafa ($\varnothing \geq 5$ mm) oraz liczbę ciałek żółtych występujących na obu jajnikach łącznie. Z ogólnej liczby 53 pęcherzyków jajnikowych wykrytych laparoskopowo, 42 (79,2%) zidentyfikowano metodą ultrasonograficzną. W przypadku ciałek żółtych, wykrywalność metodą ultrasonografii była podobna (79,5%). W powtórzeniu II (rok 2) stwierdzono wyraźną poprawę wyników ultrasonografii, co świadczy o rosnącym doświadczeniu osoby wykonującej pomiary.

