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The ovulation rate, plasma progesterone and estradiol concentration, and litter size of a local ewe breed kept in a barn vs. those kept under an overhead shelter

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Abstract. A herd of sheep (40 heads) was divided into two equal groups: group 1 was kept under an overhead shelter and group 2 in a warm barn. The effect of maintenance on ewe's reproductive performance, corpora lutea and corpora lutea of pregnancy rate, as well as the concentration of plasma progesterone and estradiol in peripheral blood was investigated. At the first laparoscopy fewer corpora lutea than during the following one were observed ($P \leq 0.01$). In sheep kept under the overhead shelter, a greater number of corpora lutea were noted. More corpora lutea of pregnancy were observed in group 1 than in the group from the barn. On the first day after mating the average level of progesterone was 190 pg mL^{-1}, and after 2 weeks it increased to more than 300 pg mL^{-1} ($P \leq 0.01$). The positive correlation between the number of corpora lutea of pregnancy and progesterone concentration ($P \leq 0.01$) and the negative correlation ($P \leq 0.01$) with number of corpora lutea of pregnancy, as well as between the litter size and estradiol concentration, were observed. Harsh environmental conditions did not cause a significant decrease of the reproduction performance of ewes kept under the overhead shed.

1 Introduction

Local sheep breeds are capable of sustaining extensive farming conditions (Dwyder and Lawrence, 2005). In Poland one of these breeds is Żelaźnieńska sheep, a cross of local Łowicz ewes with Leicester and Polish Merino rams. The population of this breed has been included in a conservation breeding program, which attained 1496 heads in 2015 among 23 flocks of central Poland (NRIAP, 2016). Żelaźnieńska sheep are well adapted to the poor soil conditions, in which rye and potatoes can be grown. This animal is bred for the dual purposes of meat and wool. Since 1955, it has been selected for reproductive performance. The body weights of ewes and rams are 65 and 95 kg, respectively. The coat is white; the performance of uniform wool is 6.0 kg for ewes and 9.0 kg for rams. Female and males reach sexual maturity at the age of 7 months. The average age at first parturition is 24 months. The average lifetime fecundity expressed as the number of lambs weaned per ewe per year reaches 1.5 (Kuźnicka et al., 2005; Radzik-Rant et al., 2012; Kuźnicka and Rant, 2013). However, the observations so far have been carried out in warm barn conditions.

The new trend in livestock production in Poland and in other European Union countries is to move from intensive to extensive management systems attributed with the maintenance of animals in semi-open sheds (Dwyer and Lawrence, 2005). But the harsh environmental conditions may influence the reproductive performance of the flock. The care of breeding ewes before mating as well as during pregnancy and the environmental conditions are important factors influencing the reproductive performance (Lassoued et al., 2004). The litter size of a ewe is affected by the ovulation level, fertilization percentage, the process of implantation, and then the survival of embryos (Fthenakis et al., 2012).

In this context, the objective of the presented study was to investigate whether the maintenance of ewes under overhead shelter affects their reproductive performance.

2 Material and methods

The investigation was carried out in three successive years at the Sheep Experimental Station located in central Poland with a mean annual temperature of 7.9 °C and annual mean precipitation of 528 mm. The mean maximum temperature during 10 years in spring, summer, autumn and winter reached 25, 31, 24 and 10 °C, respectively, and the mean minimum temperature during these periods of the year dropped to −4, 7, −4 and −14 °C, respectively (IMGW, 2016).

2.1 Animals

During the experiment, the yearly average flock size was 88 ewes. The reproductive performance was estimated based on 262 breeding records. The analyzed ewes were between 2 and 8 years of age and descended from single and multiple litters. Most of mothers (64 %) nursed twin lambs, 29 % had only one lamb, and 7 % were barren or not nursing offspring. The investigated animals (40 heads each year) were divided into two groups: experimental (1) and control (2). In both groups there were ewes with a similar reproductive performance and age. In each group 10 ewes had a higher and another 10 had a lower fertility and prolificacy than the flock average. The average age of ewes from the overhead shelter was 3 years and 10 months while for the barn 3 years and 9 months. For the whole flock, average ewe age was 3 years 10 months.

2.2 Maintenance

The animals were fed standard diets recommended by INRA (1988). The ewes were fed with farm-produced fodder (grass hay, corn grain, ground rapeseed, wheat bran, red carrot), and in the summer feeding period both groups grazed 8 h day^{-1} on separate paddocks on the same pasture.

During the entire period of experiment, the treated group 1 was kept under the overhead shelter, constructed of three wooden walls, with a wire netting and open front on the southern side, and an uninsulated tin roof. The control group 2 was kept in the barn made of bricks with a tin ridge roof, equipped with a usable loft and gravity ventilation, with temperature never dropping below 12 °C at 75 % relative humidity when air temperature under the overhead shelter during winter dropped to −10 °C. Both groups were kept on deep litter. All ewes were clipped to a coat length of about 5 mm one month before the lambing season.

2.3 Laparoscopy procedures

The mating season was held at typical term for that breed (September/October), implying parturition at the turn of February and March. During three successive reproduction seasons, the evaluation of ovulation rate was made by laparoscopy in two consecutive cycles of estrus. The first laparoscopy took place at the beginning of reproduction season, in September, and the following one at the following estrus cycle after 20 days. Each year observation was carried out on the same ewes. Sheep were fasted for 24 h before being placed on a table, and they were given anesthetic (0.1 mL 1 kg^{-1} of body weight) and Atropine (0.3 mL $head^{-1}$) to help prevent aspiration of saliva. Introduction of a laparoscope into a body cavity plus a small amount of air and then the manipulator enabled the retrieval of the ovaries. After the observation, the wound created by the introduction of the endoscope was closed with Michel's buckle, and liquid penicillin was protectively administered (2 mL $head^{-1}$). After the observation of the second estrus cycle the vaginal sponges were introduced (40 mg cronolone) to synchronize the estrus. The sponges were removed after 2 weeks, and ewes were inserted into harems (3 to 5 ewes 1 ram^{-1}). The small number of ewes in harems was due to avoid mating in consanguinity. Thus, in each group five rams were used. Each year on the 10th day after mating, using a laparoscope, the number of corpora lutea of pregnancy was checked as well.

2.4 Plasma progesterone and estradiol immunoassays

On the 1st, 6th and 14th day after mating, every 2 h, blood was collected from the ewes to determine the progesterone and estradiol concentration. Blood samples were immediately placed on ice until centrifuged at 1500 G for 10 min to separate plasma. Plasma was then collected and stored at −20 °C until analysis. The analysis of progesterone concentration was performed by direct radioimmunoassay (Kokot and Stupnicki, 1979; Romeu et al., 1995) using a gamma counter – COBRA II from Canberra – Packard using I^{125}-labeled hormone. Kits for the determination of progesterone (progesterone – RIA, DSL 399 ACTIVE) and of estradiol Spectra (Orion Diagnostic) were used. The sensitivity of the set of progesterone was less than 0.12 ng mL^{-1} while that of estradiol was 1.36 pg mL^{-1}.

2.5 Statistical analysis

The distribution of corpora lutea and corpora lutea of pregnancy depending on the maintenance system and the observation time was tested using non-parametric chi-square test. The data for progesterone and estradiol concentration were analyzed statistically by the analysis of variance and via Tukey's test of the SPSS 21.0 package software. The equation used was

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (A_i \times B_j) + (A_i \times C_k) + (B_j \times C_k) + e_{ijkl}, \quad (1)$$

where Y_{ijkl} is the dependent variable; μ the general mean; A_i the effect of maintenance system (i = overhead shelter or barn); B_j the effect of period (j = 1st, 6th or 14th day after

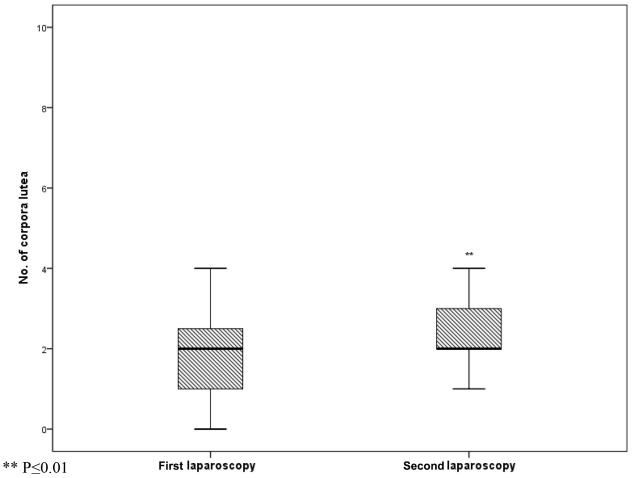


Figure 1. The distribution of corpora lutea in two successive estrus cycles.

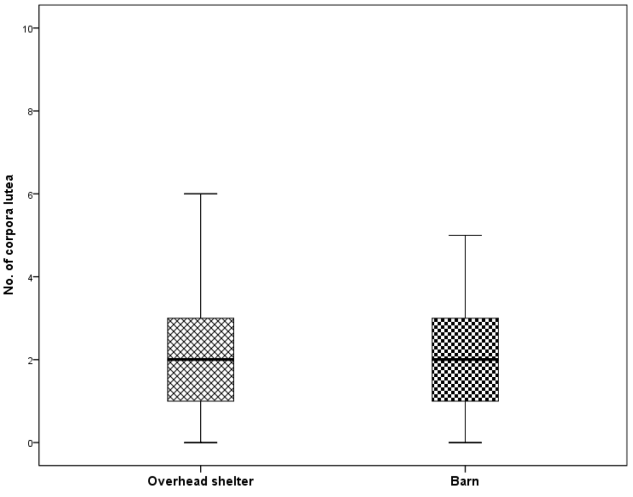


Figure 2. The distribution of corpora lutea in ewes kept in the overhead shelter and in the barn.

mating); C_k the year of experiment; $(A_i \times B_j)$ the the interaction between maintenance system and period; $(A_i \times C_k)$ the interaction between maintenance system and year of experiment; $(B_j \times C_k)$ the interaction period and year of experiment; and e_{ijkl} the random error.

The data for reproductive performance was analyzed using the following equation:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (A_i \times B_j) + (A_i \times C_k) + e_{ijkl}, \quad (2)$$

where Y_{ijkl} is the dependent variable; μ the general mean; A_i the effect of maintenance system (i = overhead shelter or barn); B_j the effect of ewe's age ($j = 2$, 3, 4, 5, 6, 7, 8 years); C_k year of experiment; $(A_i \times B_j)$ the interaction between maintenance system and ewe's age; $(A_i \times C_k)$ the interaction between maintenance system and year of experiment, and e_{ijkl} the random error.

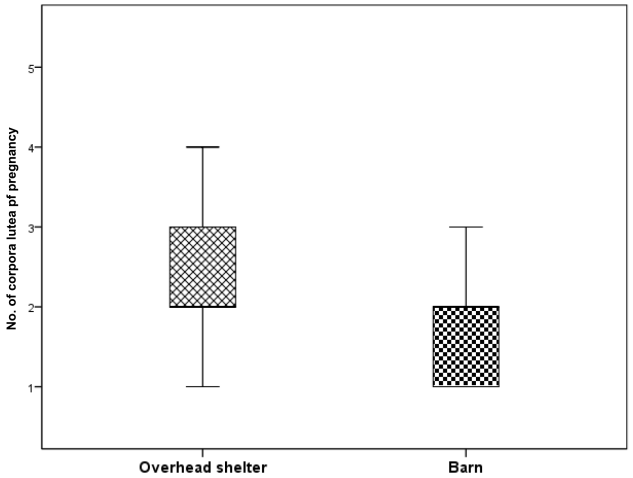


Figure 3. The distribution of corpora lutea of pregnancy in ewes kept in the overhead shelter and in the barn.

3 Results

The observation of corpora lutea number on the first and second estrus cycle showed that there were fewer corpora lutea at the first laparoscopy than at the following one (Fig. 1). This difference was proved to be highly significant. In sheep kept under the overhead shelter, a greater number of corpora lutea were noted than in those staying in the barn, but its significance has not been confirmed (Fig. 2). Although there was no significant effect between the investigated groups on the number of corpora lutea of pregnancy, more corpora lutea of pregnancy were observed in group 1 than in the group from the barn (Fig. 3). In 77 % of ewes kept under the overhead shelter more than two corpora lutea of pregnancy were noted, while in group 2 only in 62 % of mothers were multiple pregnancies detected.

There were no significant differences in the progesterone and estradiol level in the peripheral blood of ewes between the years of the experiment. But the differences were observed after mating. During that period the level of estradiol significantly decreased, while progesterone significantly increased (Fig. 4). On the 1st day after mating no differences in the concentration of progesterone and estradiol between groups were observed. However, on the 6th day after mating the ewes kept under overhead shelter secreted significantly more and on 14th day after mating highly significantly more progesterone than those from the barn (Fig. 5). Although concentration of estradiol in peripheral blood of group 1 decreased more in comparison with group 2, the difference was not statistically confirmed.

In the current experiment a positive correlation between the number of corpora lutea of pregnancy and progesterone concentration was found ($P \leq 0.01$). Negative correlations ($P \leq 0.01$) with number of corpora lutea of pregnancy as

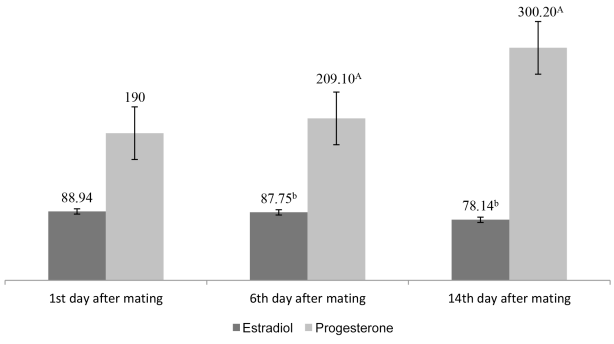
Table 1. The correlation of progesterone and estradiol secretion with the number of corpora lutea of pregnancy and litter size.

	Corpora lutea of pregnancy	Progesterone	Estradiol	Litter size
Corpora lutea of pregnancy	1	0.148**	−0.289**	−0.192**
Progesterone	0.148**	1	−0.034	−0.197**
Estradiol	−0.289**	−0.034	1	−0.014
Litter size	−0.192**	−0.197**	−0.014	1

** $P \leq 0.01$.

Table 2. The distribution of the number of ewes in the age groups and average litter size of the ewes by group and age.

Ewe's age (years)	Overhead shelter		Barn	
	No. of ewes	Litter size	No. of ewes	Litter size
2	10	1.52	11	1.65
3	16	1.50	17	1.74
4	19	1.89	18	1.85
5	9	1.67	8	1.71
6	2	1.80	2	1.80
7	2	2.25	2	2.17
8	2	1.67	2	1.50



Mean values with the same superscripts were significantly different: small letters P≤ 0.05; capital letters P≤ 0.01

Figure 4. The concentration of progesterone and estradiol (pg mL^{-1}) in peripheral blood of ewes.

well as between the litter size and estradiol concentration were observed (Table 1).

In both groups the average litter size, calculated as the ratio of the number of lambs born to the number of ewes, exceeded 1.7. Although not conformed statistically, the highest prolificacy was observed in ewes lambing for the third time at the age of 4 years and the sixth time at the age of 7 years (1.87 and 2.21 respectively). The distributions of the number of ewes in the age groups and average litter size by group and age of ewes kept under the overhead shelter and barn were similar (Table 2).

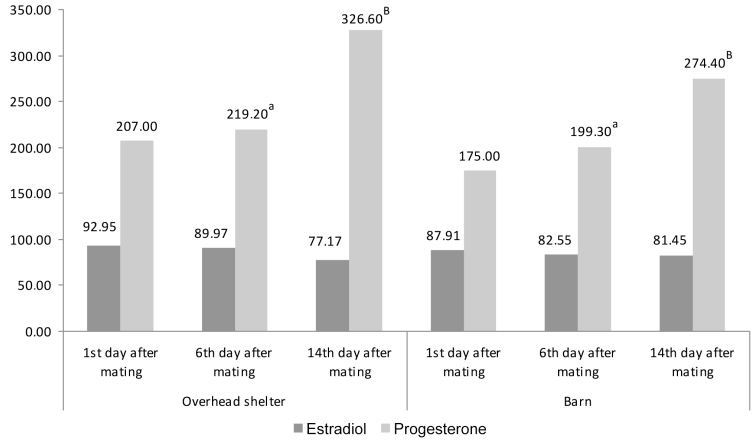
The year of observation, the management system and the interaction group × the ewe's age showed no statistical differences.

4 Discussion

The mating season for Żelaźnieńska sheep takes place in the autumn. The fewer corpora lutea at the first laparoscopy than at the next one (Fig. 1) were the effect of a shortening day length and changing duration of the nocturnal melatonin secretion. Melatonin can be considered as a pro-gonadotropic stimulus increasing the ovarian activity (Abecia et al., 2012).

The date of blood collection proved to have a highly significant impact on the concentration of hormones. The average level of progesterone on the first day after mating increased after 2 weeks of over 200 pg mL^{-1} (Fig. 4). Probably it was associated with placental development and preparation of the uterus for embryo implantation (Roy et al., 2012). The level of progesterone in the blood can be an indicator of the ovulation rate in sheep because the content of this hormone in ewes with a single ovulation is lower than the case of multiple ovulations (Ranilla et al., 1997). The concentration of progesterone also can be an indicator of pregnancy. According to Ganaie et al. (2009) the mean plasma progesterone concentration increased from 1.41 on the 6th day to 4.0 ng mL^{-1} on the 16th day in pregnant ewes, while the progesterone level dropped to less than 1.0 ng mL^{-1} in case of ewes that returned to estrus. In the current experiment at the same time significantly decreasing the level of estradiol confirmed the pregnancy of ewes. This was in accordance with the findings of other authors (Ganaie et al., 2009; Roy et al., 2012). The highly significantly more progesterone and less estradiol secretion on the 14th day after mating in peripheral blood of sheep kept under overhead shelter (Fig. 5) could be due to a greater number of corpora lutea of pregnancy in this group (Fig. 3), which confirms the highly significant correlation between the number of corpora lutea of pregnancy and the concentration of progesterone (Table 1). Ranilla et al. (1997) have reported a higher concentration of progesterone in the case of multiple pregnancies.

The analysis of litter size distribution indicates that the best reproductive potential was shown by ewes at the age of 4 years. Admittedly, the 7-year-old ewes reached the highest prolificacy, but their number was low and resulted from



Mean values with the same superscripts were significantly different: small letters P≤ 0.05; capital letters P≤ 0.01

Figure 5. The concentration of progesterone and estradiol (pg mL^{-1}) in peripheral blood of ewes kept in the overhead shelter and in the barn at first day after mating as well as sixth and fourteenth days after mating.

the fact that ewes with the highest reproductive performance were left longer in the herd. These dependences were observed in both groups (Table 2).

In young females the smaller litter size and notably fewer number of corpora lutea and corpora lutea of pregnancy were probably affected by lower progesterone endocrine (Roy et al., 2012).

The lack of differences in prolificacy between the control and experimental groups confirmed the high adaptability of ewes to harsh environmental conditions. Dwyer and Lawrence (2005) indicated that local breeds are very well adapted to be maintained in extensive production systems. The lack of difference of prolificacy between the two investigated groups in spite of more quantity of corpora lutea of pregnancy noted in ewes from overhead shelter (Fig. 3) may be influenced by greater embryo mortality as a result of the harsh environmental conditions. But according to Laburn et al. (2002) thermoregulatory strategies used by the pregnant ewe for thermoregulation during heat or cold exposure appear to protect the fetus from changes in its thermal environment. The findings of other authors confirm the effect of mother's environment on embryo death (Fthenakis et al., 2012). Most of the losses of embryos occur between 3 and 26 days after fertilization (Davies-Morel and Beck, 2003); according to Fthenakis et al. (2012) embryo survival rates decline as ovulation numbers increase. Mortality of embryos during early pregnancy is probably affected by the differences of hormone secretion in ewes at different ages (Davies-Morel and Beck, 1996).

The lack of difference between the reproduction performances of ewes from the two groups indicated a good adaptability to harsh environmental conditions. The low temperature did not translate into a decrease of ewe's prolificacy. The local sheep breeds may be housed in semi-open sheds with no negative impact on the reproduction characteristics. This may encourage breeders to develop this branch of livestock production avoiding the high expenses of construction and amortization of massive, warm barns.

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