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Seasonal variations in the morphometric analysis of the ovary and uterus and in progesterone and 17β-oestradiol production in the brown hare (*Lepus europaeus*)

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ABSTRACT

Seasonal variations in the morphometry of the ovary and uterus, and in progesterone and 17β-oestradiol production in 37 brown hares (*Lepus europaeus*), 10 in spring, 7 in summer, 14 in autumn, and 6 in winter, are reported.

Evaluation of the relative volume of primary and growing follicles, and stroma in the hare ovary revealed a stable level of primary follicles during the year. The highest relative volume of growing follicles was found in the autumn, with a similar value in winter. As the relative volume of growing follicles increased, the amount of stroma decreased. The lowest relative volume of stroma was found in the autumn (90.1%) and the highest in the spring (97.3%). No significant differences were found in the relative volumes of follicles and stroma in the ovary. The diameter of primary follicles ranged from 30.8 to 35.9 μm during the year, with no significant seasonal difference. The diameter of growing follicles ranged from 139 to 222 μm.

The highest relative volume of endometrium in the uterus occurred in the autumn and summer. This difference was significant in comparison with spring ($P<0.05$). The height of the endometrium was highest in the summer and lowest in the winter. Detailed analysis of the endometrium showed that the highest relative volume of the surface epithelium was in the spring (6.5%), the highest relative volume of glandular epithelium in the winter (22.4%), and the highest relative volume of stroma in the autumn (85.8%). The highest surface epithelium was found in the spring. The average diameter of uterine glands was highest in the spring (63.5%), but the differences between seasons were not significant.

Analysis of progesterone and 17β-oestradol in hare blood showed that the highest levels of progesterone occurred in the spring (22.91 ng/ml). Significant differences were found in the progesterone concentration between spring and autumn ($P<0.001$). For 17β-oestradiol, the highest concentrations occurred in the winter and the lowest in the summer.

The results of this study clearly demonstrate that the highest follicular growth in the brown hare occurs in the autumn, which corresponds with the highest relative volume of uterine glandular epithelium in winter as well as with the highest diameter of uterine glands in the spring. We suggest that in our conditions, the reproductive activity in the female brown hare starts in the autumn.

KEY WORDS: hare (*Lepus europaeus*), ovary, uterus, progesterone, 17β-oestradiol

INTRODUCTION

Much attention is being paid to applied research on the brown hare, which had been the most plentiful game in Slovakia (Hell and Slamečka, 1999) in connection with the critical decline in its population during the last ten years (in the spring of 1989, the standard stock was 65.5 heads per 100 ha^{-1}, in 1992, only 58.5 heads per 100 ha^{-1}; in the autumn of 1989, the standard stock was 114.8 heads per 100 ha^{-1}, in 1992 only 81.0 heads per 100 ha^{-1}). Attention should also be paid to fundamental research, supllemented with descriptions of microscopic structure, to solve this problem in practical terms.

Although there are many reports describing the quantitative structure of reproductive organs in farm animals (Jantošovičová et al., 1996; Kolodziejyski and Danko, 1995; Danko, 1997) there are few observations on the morphometry and secretory activity of sexual organs in the hare. Enlargement of the uterus (Calliol et al., 1989a) and increases in the levels of progesterone (Calliol and Martinet, 1976; Calliol et al., 1991) and oestradiol (Caillol et al., 1989a,b, 1991; Semizorová et al., 1990) have been documented. These authors studied mostly cage-bred brown hares, not animals from the wild. Knowledge of the natural reproductive changes that take place in the hare in the wild is interesting from the point of view of the venatical management of the species.

The purpose of this study was to identify seasonal variations in the microscopic structure of the ovary and uterus and in the blood concentrations of progesterone and 17β-oestradiol in the brown hare (*Lepus europaeus*).

MATERIAL AND METHODS

The samples were taken from hares (*Lepus europaeus*), which were caught in the surroundings of the town of Trnava, West Slovakia. This is a hunting area with optimum soil and climatic conditions for this game, crop plant production, howe-

ver, the ecology of the landscape is seriously affected by intensive, large-scale industrial production and its biodiversity is therefore markedly reduced. The hunting ground is situated on the table, which is in fact the northern prominence of the West Slovakian (Danube) Lowland.

Over a period of one year, 37 female hares were analysed. In the spring we collected the samples in March, April and May (10 animals), in the summer, in June, July and August (7 animals), in autumn, in September, October and November (14 animals), and in winter, in December, January and February (6 animals). The weight of animals was the highest in the spring (4.4±0.50 kg), lower in winter (4.10±0.33 kg) and autumn (3.84±0.75), and lowest in summer (3.23±1.70 kg), but the differences were not significant. In the spring the ovaries weighed 3.61±0.5 g, in winter 3.42±0.75 g, in summer 3.03±0.66 g and the least in autumn 1.99±1.02 g. The weight of uterus was the highest in the summer (113.5±20.3 g), lower in spring (29.3±5.0 g), winter (25.7± 6.3 g) and autumn (9.1±3.5 g). During the year, 5 females were pregnant (3 in summer, 1 in spring and 1 in winter).

Determination of the age (adult, juvenile) of the shot hares was based on the study of dry eye lens weights. This method is much more reliable that palpation examination of the Stroh's character, which does not enable identification of young from the first litters in December. The critical value of the eye lens weight, differentiating the yearlings and adult individuals was approximately 280 mg, and it ranged from 270 to 290 mg (Slamečka et al., 1997).

Immediately after killing the animals, samples of ovaries and uterus were fixed in 10% formol. After fixation the samples were dehydrated in a graded series of ethanol (70, 80, 90 and 100%), saturated in benzene, benzene-paraffin and embedded into paraffin. Blocks of samples were then sectioned on a microtome into 10 μm thick sections, which were stained with haematoxylin and eosin (Vacek et al., 1974). From microphotographs (Docuval, Carl Zeiss Jena) based on micromorphological criteria (Weibel et al., 1966; Uhrín, 1992; Massányi and Uhrín, 1996a) the quantitative values of ovarian and uterine structures were evaluated with respect to each sample.

Ovarian tissue was investigated in terms of its qualitative microscopical structure, the relative volume (%) of primary and growing follicles and stroma, and the diameter (μm) of primary and growing follicles. The qualitative microscopical structure of the uterus was determined, so too was the relative volume (%) of the endometrium, myometrium, as well as of the surface epithelium, glandular epithelium and stroma in the endometrium. The height (μm) of the surface epithelium and the diameter (μm) of the uterine glands were determined and the data were analysed for seasonal effects.

The levels of progesterone and 17β-oestradiol in the blood of hares were assessed by RIA (radioimmunoassay) using sets (Institute for Radioecology and Use of Nuclear Technique, Košice). The sensitivity of RIA to progesterone was

2 pM/ml, the antibody against progesterone had 58.6% cross-reaction with 11 α-hydroprogesterone and lower than 0.01% with cortisol, testosterone, estradiol and estrone. The sensitivity of RIA to estradiol was 2.5 pM/ml, the antibody had a cross-reaction with estrone 25%, with estradiol 1.84% and lower than 0.001% with progesterone, testosterone and cortisol.

To compare these treatment means the analysis of variance as well as Student's t-test and Scheffe's test were applied (SAS, 1989) and EXCEL (1998).

RESULTS

The ovary

The ovaries are small, flattened ovoid organs, laying in the right and left lateral pelvic cavities. The surface of the hare ovary is covered by a single layer of epithelium. A substantial basement membrane (*tunica albuginea*) separates the surface cells from the underlying ovarian tissue divided to the inner medulla and outer cortex, which consist of follicles and stroma (Figures 1, 2 and 3).

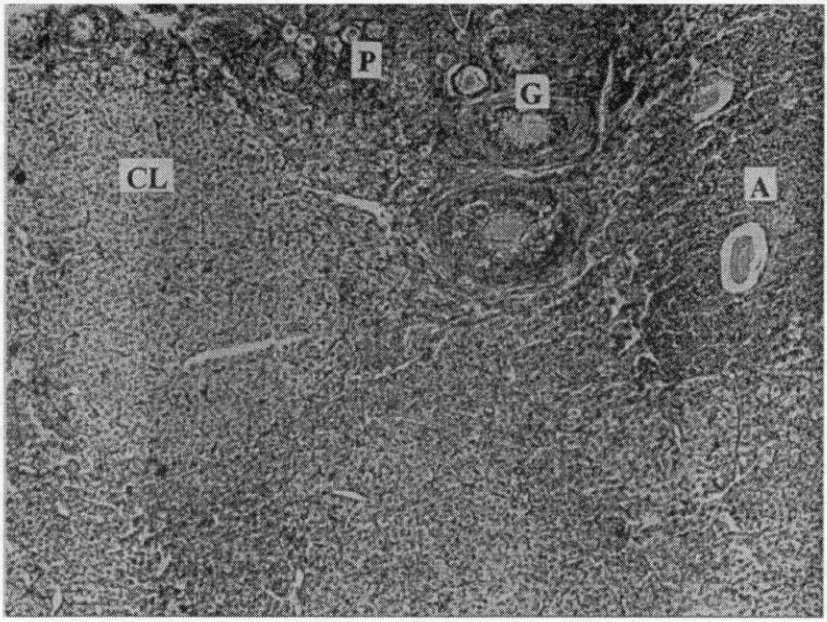

Figure 1. Ovarian structure in brown hare in autumn. Ovarian cortex consist of primary (P), growing follicles (G) and *corpus luteum* (CL). There also some follicle undergoing to degeneration – atretic follicle (A) (H-E, 200x)

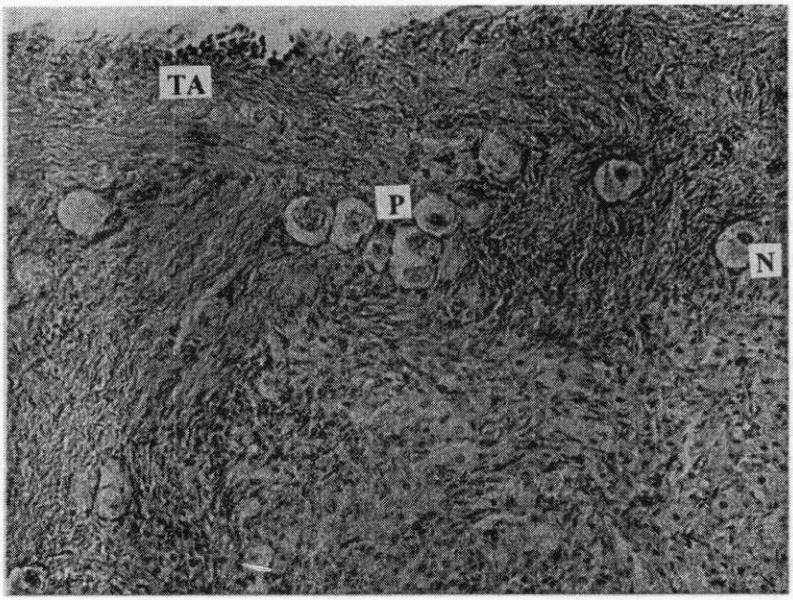

Figure 2. Detail of primary follicles (P) which contain oocytes with well formed nucleus (N). A layer of connective tissue (*tunica albuginea*, TA) covers the ovary (H-E, 450x)

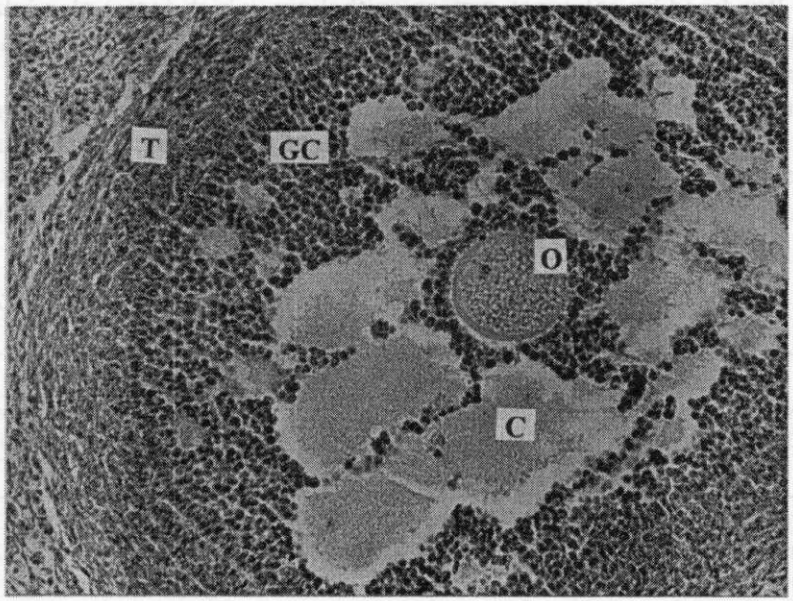

Figure 3. Growing follicle with formed follicular cavity (C). In the follicle granulosa cells (GC) and oocyte (O) are present. Connective tissue around the follicle forms theca folliculi (T) (H-E, 450x)

An evaluation of the relative volume of primary follicles, growing follicles and stroma in the hare ovary (Table 1), indicated that the relative volume of primary follicles is very stable throughout the year, ranging from 0.53 to 0.86%. The highest relative volume of growing follicles occurred in the autumn (9.2%) with a similar value in the winter (7.9%). The difference between the autumn when the hare reproduction season starts and spring was 7.0%. On the other hand, with the increase in the relative volume of growing follicles the relative volume of stroma decreased. The lowest relative volume of stroma was found in the autumn and the highest in the spring (97.3%). In the evaluation of the relative volume of follicles and stroma, no significant differences were found, but a tendency towards increased folliculogenesis in the autumn and winter was evident (Figure 6).

The diameter of primary follicles ranged from 30.8 to 35.9 μm, with no significant seasonal differences. The diameter of growing follicles ranged from 139 to 222 μm. Since we did not study growing follicles further (follicles with less than 2 layers of granulosa cells; follicles with more than 2 layers of granulosa cells; follicles with antral formation; antral follicles), the standard deviation is high and no significant differences were found.

The uterus

The uterus is a muscular organ and receives the right and left fallopian tubes. It is lined by columnar surface epithelium, which forms glandular epithelium (Figures 4 and 5). The hare has a *uterus duplex*, i.e. the uterus has two bodies and two cervices.

Data reporting the relative volume of endometrium and myometrium are given in Table 2. The highest relative volume of the endometrium occurred in the autumn and summer (38.3%) and the lowest in the spring 23.9% ($P<0.05$). Although the highest values for the relative volume of the endometrium oc-

TABLE 1

Hare ovarian structures in relation to season

Season (n=37)	Relative volume, %						Diameter, μm			
	primary follicles		growing follicles		stroma		primary follicles		growing follicles	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.	x	s.d.
Spring (n=10)	0.53	0.21	2.2	1.97	97.3	1.98	35.9	6.28	139	152.06
Summer (n=7)	0.71	0.31	3.9	2.88	95.4	5.62	32.8	7.39	222	210.28
Autumn (n=14)	0.71	0.45	9.2	5.33	90.1	5.63	30.8	5.04	175	159.52
Winter (n=6)	0.86	0.52	7.9	6.85	91.3	6.42	30.8	4.11	216	193.97

x – mean, s.d. – standard deviation, n – number of animals

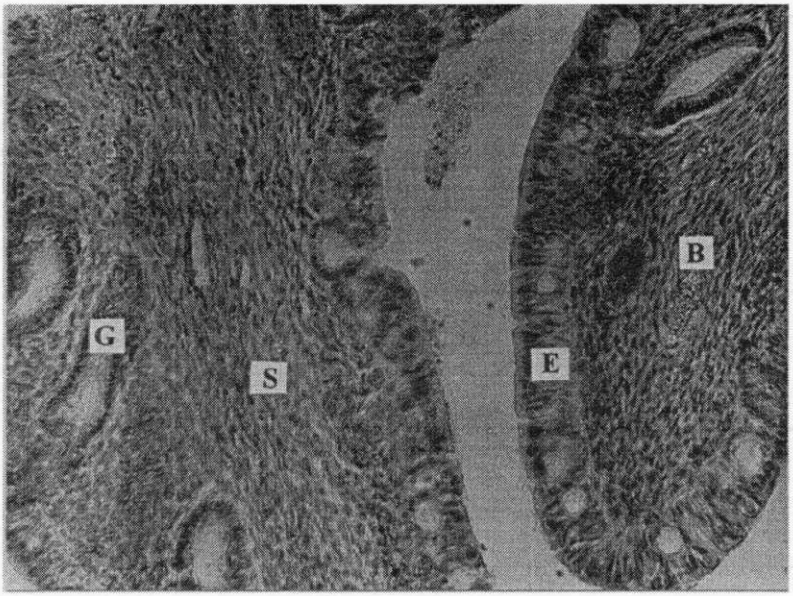

Figure 4. The uterus is lined by columnar surface epithelium (E), which forms glandular epithelium (G). In endometrium the highest relative volume forms connective tissue - stroma (S), were blood cappilaries (B) are present (H-E, 450x)

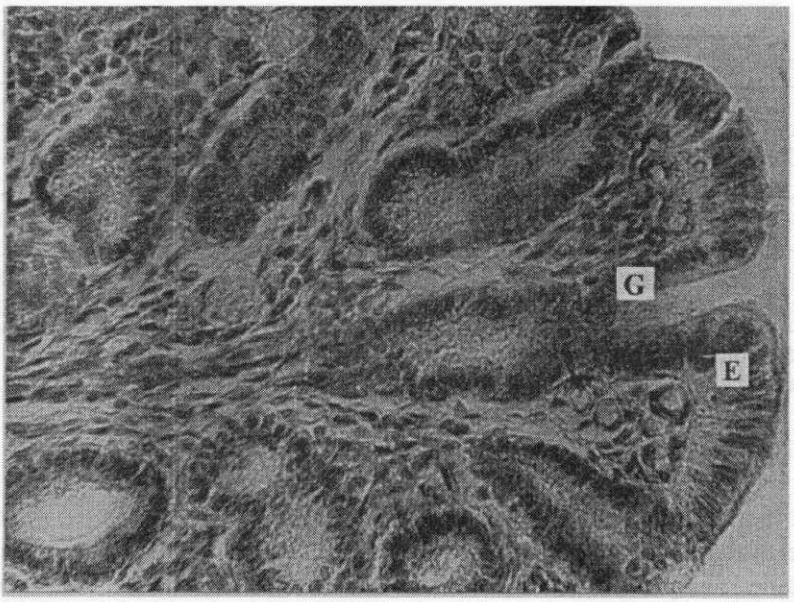

Figure 5. Detail of the endometrium in hare with columnar surface epithelium (E) forming uterine glands (G) (H-E, 500x)

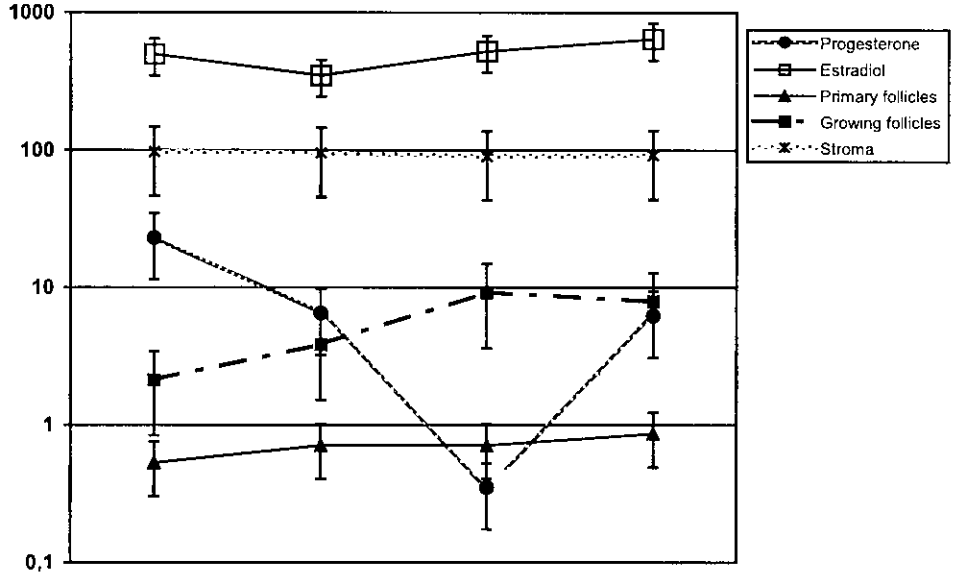

Figure 6. Relations of the levels of progesterone and 17-β oestradiol to the ovarian structure. Progesterone, ng/ml; 17β-oestradiol, pg/ml; the relative volume of primary follicles, growing follicles and stroma; %, with standard deviations (s.d.)

TABLE 2

Hare uterine structures in relation to season

Season (n=37)	Relative volume, %				Heigh, μm			
	endometrium		myometrium		endometrium		myometrium	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
Spring (n=10)	23.9*	3.83	76.1	3.83	265	112.64	1077	226.83
Summer (n=7)	36.9	8.61	63.1	8.61	430	201.46	772	206.72
Autumn (n=14)	38.3	9.21	61.7	9.21	359	185.95	649	224.17
Winter (n=6)	27.1	3.97	72.9	3.97	222	92.60	757	325.86

* $P<0.05$ (spring autumn, summer)

x – mean; s.d. standard deviation, n – number of animals

curred in autumn and the lowest in spring, the seasonal difference in this parameter was not significant.

Detailed analysis of the endometrium showed that the relative volume of surface epithelium ranged from 3.5 to 6.5%, of glandular epithelium 10.0-22.4%, and of stroma 74.1-85.8% (Table 3). The highest relative volume of surface epithelium

TABLE 3

Hare uterine endometrium structures in relation to season

Season (n=37)	Relative volume, %						Height, μm surface epithelium		Diameter, μm uterine glands	
	surface epithelium		glandular epithelium		stroma					
	x	s.d.	x	s.d.	x	s.d.	x	s.d.	x	s.d.
Spring (n=10)	6.5	2.96	16.6	5.67	76.9	6.06	28.4	7.89	63.5	32.31
Summer (n=7)	5.6	3.33	14.9	4.75	79.5	2.67	22.45	8.63	44.5	19.91
Autumn (n=14)	4.2	2.58	10.0	2.72	85.8	2.99	29.3	9.50	37.1	9.76
Winter (n=6)	3.5	0.81	22.4	4.01	74.1	3.83	35.8	9.89	38.9	16.89

was in the spring, the highest relative volume of glandular epithelium was in the winter and the highest relative volume of stroma was in the autumn. These data suggest that the highest activity of the endometrium is in the winter and spring. The maximum height of surface epithelium was also found in the spring, but the seasonal differences were not significant. In the evaluation of the diameter of uterine glands responsible for secretory activity of endometrium, the highest average value was observed in the spring (63.5%), but again the differences were not significant.

Steroidogenesis

Seasonal variations in the morphometric analysis of the ovary and uterus were also confirmed by analysis of steroidogenesis. The highest level of progesterone in blood (Table 4) was found in the spring (22.91 ng/ml). Significant differences were found in the progesterone concentration between spring and autumn ($P<0.001$). The highest concentration of 17β-oestradiol occurred in the winter and the lowest in the summer, which corresponds with the morphometric values for the growing follicles found in the ovary.

TABLE 4

Hare progesterone and 17β-oestradiol blood levels in relation to season

Season	Progesterone, ng/ml		17β-oestradiol, pg/ml	
	x	s	x	s
Spring (n=10)	22.91	10.23	499.37	127.35
Summer (n=7)	6.51	4.55	350.22	110.25
Autumn (n=14)	0.35*	0.22	525.72	158.25
Winter (n=6)	6.23	5.23	645.27	210.36

* $P<0.001$ (autumn – spring)

DISCUSSION

Generally, there is very little data describing reproduction in the brown hare, especially data reporting microscopic structure.

In this study we report seasonal variations in the morphometry of the ovary and uterus. In the ovary we found the highest activity in autumn and winter, as shown by the highest relative volume of growing follicles. Changes were also observed in the uterus where the highest relative volume of glandular epithelium was found. The highest diameter of uterine glands occurred in the spring. Our results are supported by the analysis of steroids in the blood, where the highest concentration of progesterone was in the spring and the highest level of 17β-oestradiol in the winter.

It has been reported that in the brown hare, fertile matings take place from the beginning of December to September (Caillol et al., 1990). The different climatic condition in our region in comparison with those where these results were obtained (France) must be taken into account. The basal concentrations of LH remain undetectable until the end of January, rise from February to a maximum in July. On the other hand, Semizorová et al. (1990) reported that the progesterone level in the brown hare is low before pregnancy (0.41 ng/ml). During pregnancy it gradually increases, culminates on day 31-40 of pregnancy (41.9 ng/ml) and decreases immediately before parturition (2.8 ng/ml). Our results also correspond with the results reported by Slamečka et al. (1997) where they found the highest ovarian weight in the spring (March, April, May).

Microscopic analysis of female reproductive organs is mainly reported in farm animals (Kliment and Žitný, 1989; Pivko, 1995; Krajničáková et al., 1999). In comparison with rabbits, our morphometric analysis of the hare ovary shows a lower relative volume of growing follicles. In our earlier study of the rabbit ovary (Massányi and Uhrín, 1996a), 3.0% of its volume comprised primary follicles, 30.7% growing follicles and 66.3% formed stroma. The diameter of primary follicles in hares was 30.8-35.9 μm and in rabbits 29.6±4.9 μm (Massányi and Uhrín, 1996b). In the uterus, luminal epithelium formed 5.9%, glandular epithelium 5.8% and stroma 88.3% of the total volume of endometrium.

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STRESZCZENIE

Sezonowe zmiany w morfometrycznej ocenie jajników i macicy oraz produkcji progesteronu i 17β- estradiolu u zająca (*Lepus europaeus*)

Badano morfometryczne zmiany jajników i macicy oraz produkcję progesteronu i 17β-estrodiolu u 37 zajęcy: wiosną u 10 osobników, latem u 7, jesienią u 14 i zimą u 6.

Względna objętość pierwotnych pęcherzyków jajnikowych była stała w ciągu roku, rosnących pęcherzyków była największa jesienią i zimą. Wraz ze zwiększającą się względną objętością rosnących pęcherzyków obniżała się ilość zrębu jajnika. Najmniejszą względną objętość zrębu stwierdzono jesienią (90,1%), największą wiosną (97,3%). Nie stwierdzono istotnych różnic we względnej objętości pęcherzyków i zrębu w jajnikach. Średnica pierwotnych pęcherzyków wynosiła 30,8-35,9 μm, a rosnących pęcherzyków 139-222 μm.

Względna objętość błony śluzowej macicy była największa jesienią i zimą i istotnie większa ($P<0,5$) niż wiosną, natomiast jej wysokość była największa latem, a najmniejsza zimą. Największą względną objętość nabłonka błony śluzowej stwierdzono wiosną (6,5%), nabłonka gruczołowego zimą (22,4%), a zrębu jesienią (85,8%). Powierzchnia nabłonka błony śluzowej była największa wiosną. Średnica gruczołów macicznych była większa wiosną (63,5%) niż w pozostałych porach roku, lecz różnice nie były istotne.

Najwyższe stężenie progesteronu w krwi stwierdzono wiosną (22,91 ng/ml) i różniło się istotnie ($P<0,01$) od wartości otrzymywanych jesienią. Stężenie 17β-estradiolu było największe w zimie, najniższe latem.

Na podstawie otrzymanych wyników można sądzić, że w warunkach Słowacji aktywność płciowa (reprodukcyjna) samic zająca zaczyna się jesienią.