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OVARIAN ACTIVITY AND THE SIZE OF OOCYTES IN OVARIAN FOLLICLES IN SEXUALLY MATURE GILTS IN INDIVIDUAL SEASONS

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ABSTRACT

The aim of this study was to evaluate ovarian activity and the size of oocytes in ovarian follicles in sexually mature Landrace-Yorkshire gilts in relation to the individual seasons of the year. The study was carried out on 240 gilts slaughtered at an abattoir during the four yearly seasons. The size and weight of the ovaries, the number of follicles and corpora lutea (CL) according to individual size categories were evaluated. The oocytes were aspirated from follicles and their sizes were measured. Our evaluation of the size of the ovaries showed that they were the largest in autumn, when their mean length reached 25.8 ± 3.4 mm, while in winter their mean length was 24.2 ± 2.9 mm. The smallest weight of the ovaries was determined in autumn (mean 5.7 ± 1.4 g) and the highest in spring (mean 6.2 ± 2.2 g). The largest number of follicles in the ovaries of the gilts was recorded during the autumn months, with a predominance of follicles up to 3 mm (mean number 17.9 ± 7.5). The largest number of corpora lutea was observed in spring (mean number 12.1 ± 2.6) and the smallest in winter (mean number 6.1 ± 1.1). The oocytes from follicles of up to 3 mm size, were the smallest in spring (mean size $16.99 \times 10^3 \pm 3.42 \times 10^3$ μm^2) and the largest in winter (mean size $18.90 \times 10^3 \pm 2.99 \times 10^3$ μm^2). In total, the largest oocytes were aspirated from 4—6 mm follicles in autumn (mean size $19.60 \times 10^3 \pm 5.37 \times 10^3 \pm$ μm^2). The values recorded indicated that the seasons affected the ovarian activity and the growth of oocytes in gilts.

Key words: follicles; oocytes; season, sexually mature gilts

INTRODUCTION

The successful reproductive performance of gilts and sows are based upon the active functioning of their ovaries, characterised by intensive folliculogenesis. Numerous growing and ripening ovarian follicles ensure conditions for favourable conception abilities of gilts and sows and are an optimum source of oocytes for the use in embryo transfers.

Folliculogenesis is the process of the development of follicles that are the basic structures of mammalian ovaries. It is one of the most important phases of development of the female reproductive organs that commences already during their foetal life [12]. Extensive studies have shown that after reaching sexual maturity, folliculogenesis involves ripening and ovulation of minimally 14—20 follicles during each subsequent oestrous cycle. However, every population of these follicles is morphologically and biochemically heterogenic. The differences in the number of granulosa cells, activation of lutropin receptors (LH) receptors or concentration of steroids in the follicular fluid are recorded even in follicles of the same size.

It is evident that the quality of follicles affects the quality of the oocytes [11]. The pre-ovulation development of follicles and oocytes can thus affect positively or negatively the formation, development and survival of embryos.

The aim of this study was to analyse ovarian activity in sexually mature gilts and to evaluate the size of oocytes according to individual size categories of follicles in the individual seasons of the year.

MATERIALS AND METHODS

Ovarian activity was evaluated on ovaries from 240 Landrace-Yorkshire gilts, 9—10 months old with weights of 150—160 kg. The ovaries were obtained after slaughtering the animals at the abattoir. The ovaries were evaluated for their size and weight and the number of follicles and corpora lutea (CL), according to individual size categories. In follicles the size categories were up to 3 mm, 4—6 mm, 7—9 mm, 10—11 mm and 12 mm and more, in corpora lutea up to 5 mm, 6—9 mm, 10—11 mm and 12 mm and more. Using an insulin syringe BD Micro-Fine Plus 0.5 ml (BD Medical, Franklin Lakes, USA), the follicular fluid with oocytes were aspirated and applied to Petri dishes (Gama group, a. s., České Budejovice, Czech Republic) and filled up to one third with sodium chloride 0.9 % infusion solution (KO, Košice, Slovak Republic). The Petri dishes were marked with the size category of follicles from which the oocytes were aspirated. The identification of the oocytes in the dishes were carried out under a stereoscopic microscope STM 723 (Kapaoptic, Kvant, Bratislava, Slovak Republic) at 20—40-fold magnification (Fig. 1). Using an adjusted Monoject tuberculin syringe 1 cc (Covidien, Mansfield, England) with a piece of IUI pipette, we transferred the oocytes to the Petri dishes containing clear sodium chloride 0.9 % infusion solution and after calibration of the microscope we photographed them using a camera 1,3 MP Moticam 1SP (Motic China Group Co., Ltd., HongKong, China). The size of oocytes (Fig. 2) were measured by a "Software Motic Images Plus 2.0" (Motic China Group Co., Ltd., HongKong, China).

The statistical evaluations of the results were carried out by the software "Microsoft Office Excel 2013" (Microsoft Corporation, Redmond, USA) and the significance of differences of two sets of data were determined by the Student t-test.

RESULTS AND DISCUSSION

The functionality of the reproductive apparatus of the sows is affected by a number of factors. One of them is the season of the year. It has been demonstrated that the sea-

Fig. 1. Search for oocytes

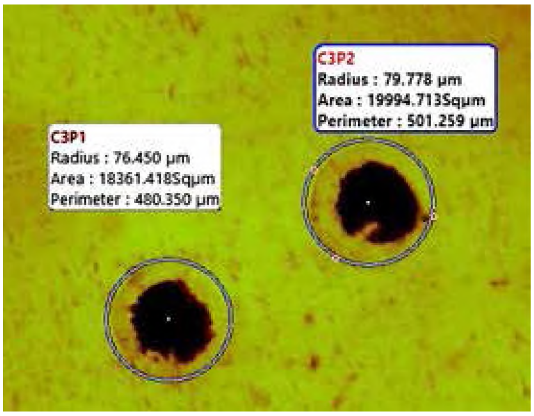

Fig. 2. Oocyte measurement

sonal conception abilities of gilts and sows change and that may cause disturbances of the oestrous cycle and embryonal mortalities may occur. The reasons for these disorders are alterations of synthesis and release of gonadotropins from the hypophysis [10], particularly insufficient production of lutropin, which affects the activity of the luteal cells of the corpora lutea and causes a decreased production of progesterone. The low production of progesterone by the ovaries of gilts results in the reduced developmental competence of the oocytes [2]. Yoshizawa et al. [13] stated that an optimum concentration of progesterone during folliculogenesis in gilts is necessary for the oocyte to reach its full developmental potential. Gupta, Uhm, Lee [6] and Bertoldo et al. [3] reported that oocytes obtained from the corpora lutea containing ovarian follicles of gilts and sows exhibited higher developmental potential than oocytes obtained from follicles that did not possess CL. It was recorded that the concentration of progesterone varies with the seasons and its marked decrease is associated with a reduced conception rate or increased embryonal and foetal mortality rates in gilts and sows. Our evaluation of ovarian size (Fig. 3) revealed that the smallest ovaries were observed in winter (mean size 24.2 ± 2.9 mm) and the largest in autumn (mean size 25.8 ± 3.4 mm). The ovaries of gilts reached the highest weight (Fig. 4), in spring (mean weight 6.2 ± 2.2 g) and the lowest one in autumn (mean weight 5.7 ± 1.4 g).

The above findings correspond with the largest number of ovarian follicles in gilts (mean number 24.8 ± 7.7) in autumn (Fig. 5) that resulted in enlarged volume of ovaries, and the highest number of *corpora lutea* (mean number 12.1 ± 2.6) in spring (Fig. 6) that affected the weight of the gilts' ovaries. In autumn, the predominant size of the follicles was up to 3 mm (mean number from 13.7 ± 6.3 to 17.9 ± 7.5). The ovarian follicles of size 4—9 mm were found most frequently in autumn and winter (mean number 6.9 ± 2.0 and 6.9 ± 1.8, resp.).

It has been assumed that the quality of oocytes and their fertilization ability as well as maturation competence are affected by the size of the follicles from which they originated [1]. Oocytes are present in ovarian follicles in all stages of folliculogenesis and can be obtained already from follicles 2—5 mm in size [8]. However, there is an assumption that with the increasing size of the follicle, the environment that affects the quality of the oocyte and its fertilization ability improves as its maturation competence improves. With the growth of follicles, the number of layers and counts of columnar cells surrounding the oocytes increase [7]. Oocytes obtained from larger follicles (3—8 mm) exhibit increased intensity of meiotic maturation and higher cytoplasmic activity than oocytes obtained from small follicles (up to 3 mm). It was reported that higher proportion of oocytes collected from larger follicles developed to metaphase II compared to oocytes from small follicles [7, 9]. During

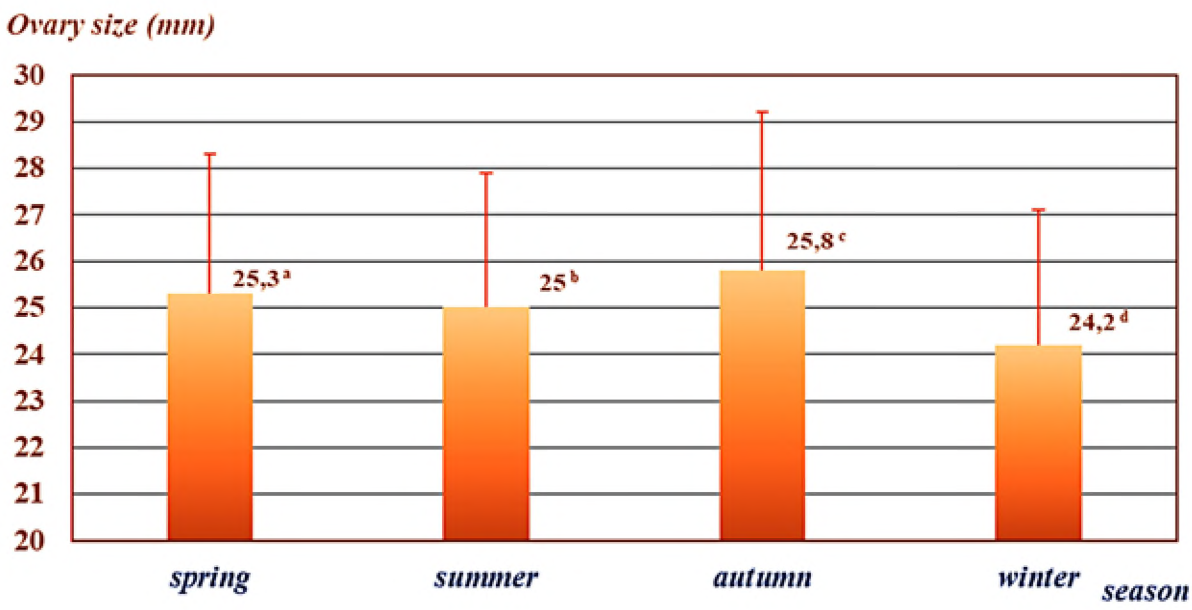

Fig. 3. The mean size of the ovaries of gilts during the seasons
t-test: b : c, d = P < 0.05; a : d = P < 0.01

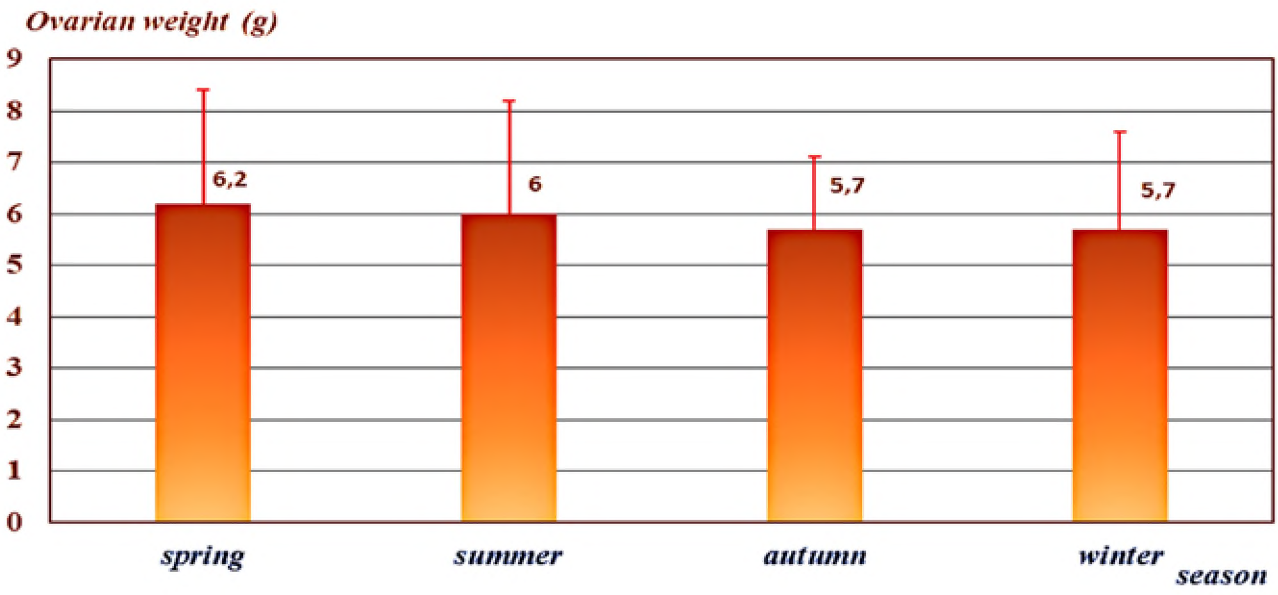

Fig. 4. The mean weight of the ovaries of gilts during the seasons
t-test: P > 0.05

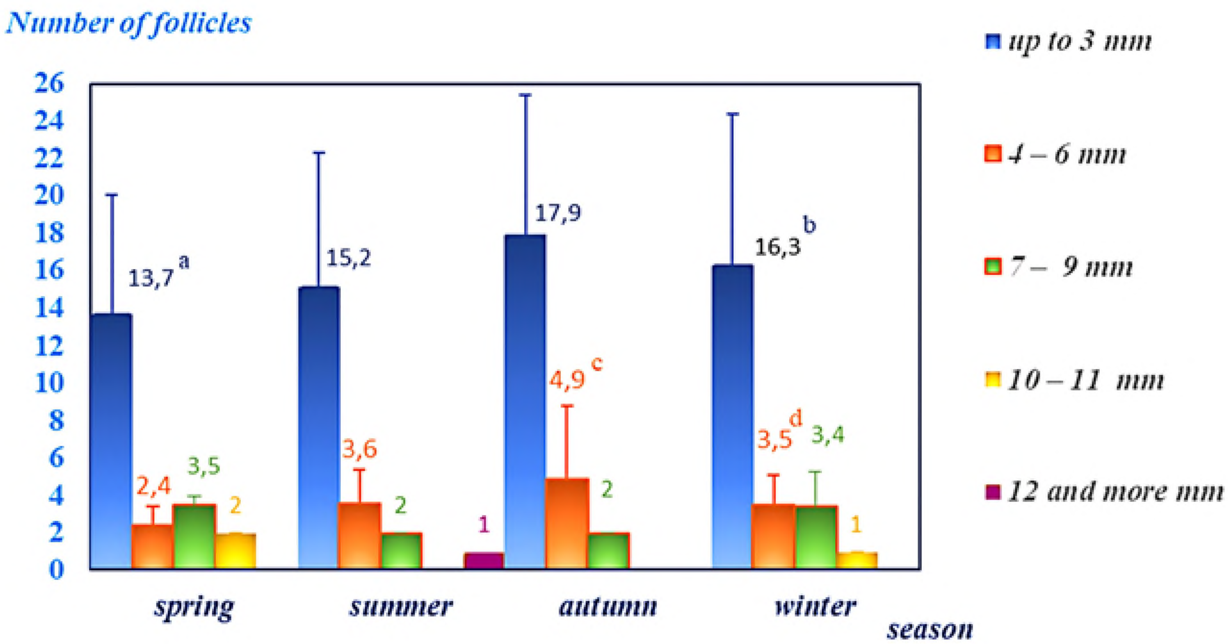

Fig. 5. The mean number of follicles per ovary in gilts during the seasons
t-test: c : d = P < 0.05; a : b = P < 0.01

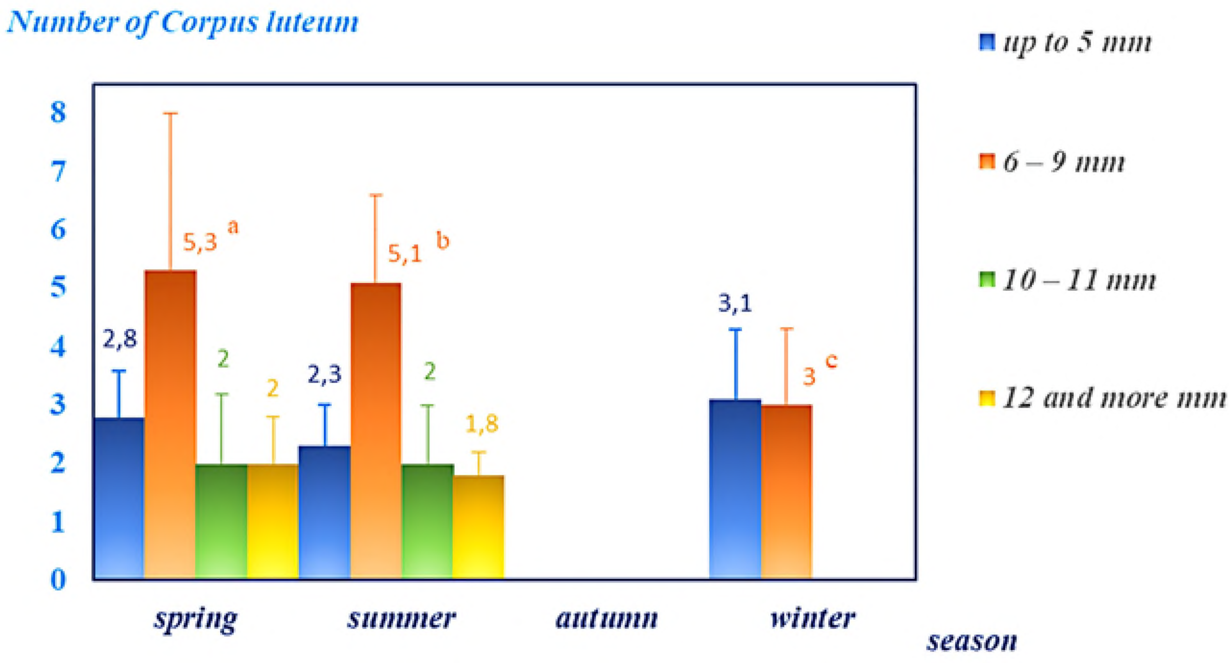

Fig. 6. The mean number of Corpus luteum per ovary in gilts during the seasons
t-test: c : a, b = P < 0.05

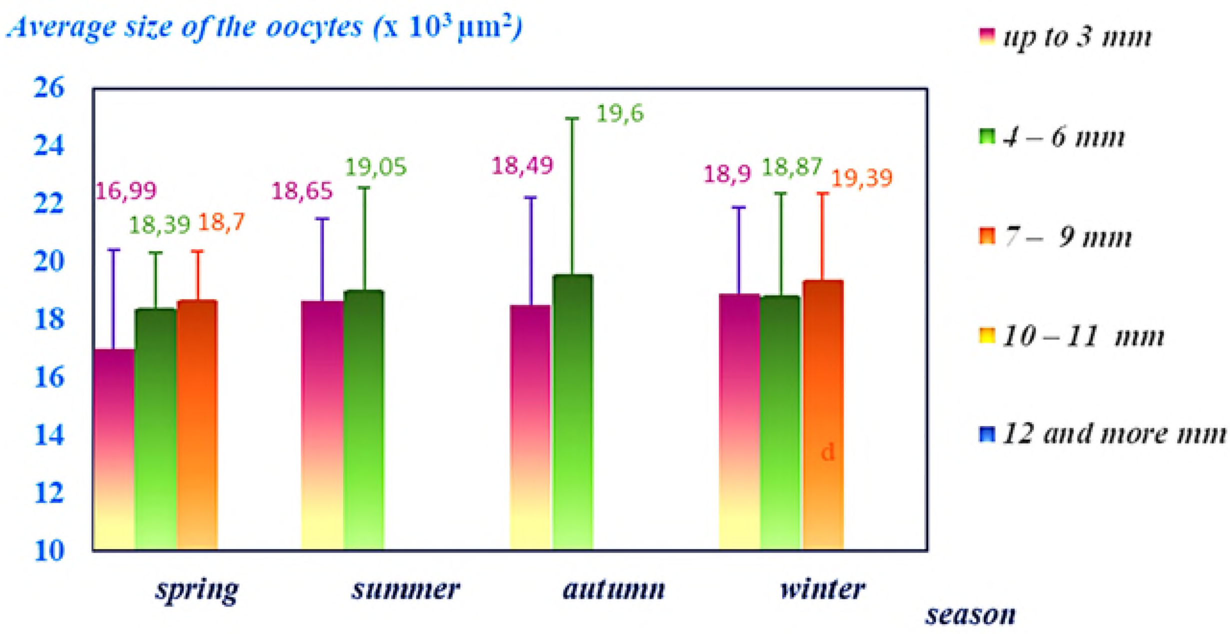

Fig. 7. The average size of the oocytes of gilts during the seasons
t-test: P > 0.05

in vitro cultivation, such oocytes exhibit a higher ability to develop to the blastocyst stage than oocytes collected from smaller follicles. The concentrations of steroid hormones, particularly progesterone (P4) and 17-beta estradiol (E2) increases in the follicular fluid of growing follicles which is positively reflected in terms of an increased developmental competence of oocytes [7]. Differences in the concentrations of these steroid hormones were recorded also between ovarian follicles of pre-pubertal and sexually mature gilts [5]. The concentration of these hormones was substantially lower in follicles of pre-pubertal gilts compared to follicles of the same size collected from sexually mature gilts. Similarly, oocytes obtained from follicles of pre-pubertal gilts at *in vitro* fertilization showed markedly a higher degree of polyspermy than oocytes from follicles of sexually mature gilts. This indicates insufficient cytoplasmic maturation in pre-pubertal gilts and the fact that the age of gilts also affects the developmental competence of oocytes. Griffin et al. [4] observed that the developmental competence of oocytes during *in vitro* fertilization correlated with the size of oocytes; the larger the oocyte, the higher the probability of its good developmental competence. The size of oocytes is again in correlation with the size of the follicles. Only sufficiently large follicles contain the optimum number of cells necessary for the adequate developmental competence of oocytes, numerous receptors for gonadotropic hormones and optimum concentrations of steroids in the follicular fluid. When evaluating the size of oocytes collected from follicles of gilts of size up to 3 mm, we detected different sizes of oocytes during individual seasons (Fig. 7). The smallest were oocytes collected from follicles up to 3 mm size in spring (mean size $16.99 \times 10^3 \pm 3.42 \times 10^3$ pm^2) and the largest were those collected in winter (mean size $18.90 \times 10^3 \pm 2.99 \times 10^3$ pm^2). In total, the largest were oocytes collected from 4—6 mm follicles in autumn (mean size $19.60 \times 10^3 \pm 5.37 \times 10^3$ pm^2). The results obtained in this study confirmed the influence of seasons on ovarian activity and oocyte growth in gilts and sows.

CONCLUSIONS

The fertilization abilities and developmental competences of oocytes are affected by a number of factors including the age of gilts, their nutritional status during mating, the seasons and the growth of the follicles. Without acceptation and optimisation of the influence of these factors on folliculogenesis and thus also on the development and maturation of oocytes, the breeders will be unable to ensure the required reproductive parameters in pig farming and the experimental workers will not reach the expected results of *in vitro* fertilization in embryo transfer programmes developed for pigs.

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