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Quality of oocytes in prepubertal and pubertal swine

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Abstract: In vitro fertilization is a common method of fertilization in the case of both humans and animals. Although this method has developed very rapidly, there are still many unanswered questions about the optimal characteristics that oocytes should have for performing this procedure. The object of this study was to describe the correlation between the diameter and size of swine oocytes in relation to pubescence. The research was conducted on ovaries of piglets (n = 65) and adult swine (n = 69). Each oocyte was examined with and without the zona pellucida. The analysis showed that the average size of the oocyte from antral follicles is 134.59 ± 11.87 μm and the size of the oocyte is significantly larger for swine in the reproductive period than for piglets entering the prepubertal period. The differences were observed for oocytes both with and without the zona pellucida, as well as in the zona pellucida itself.

Key words: Oocyte, quality, in vitro fertilization, pubescence, swine

1. Introduction

Nowadays, in vitro fertilization (IVF) methods are widely used in both humans and animals. Despite the knowledge gained over the years, information regarding the selection criteria for oocyte quality is still lacking. The oocyte is a special type of cell that develops in the body. It is the longest-living cell in the body, and its activity is associated with the transmission of genetic material from one generation to the next (1). Evaluation of the collected oocytes' quality is one of the most important steps in the procedure of IVF. In recent years, research about methods of IVF has been mainly focused on pigs. Studies by Alwarez et al. (2) showed that the quality of oocytes increases with the size. However, the size of the follicles is also useful in evaluating the quality of immature oocytes, with follicle size shown to be closely related to the quality of oocytes (3). It is widely accepted that follicle size affects cytoplasmic and nuclear maturity and the developmental potential of oocytes. In fact, follicular cell assistance to the oocyte is essential for acquiring in vitro developmental competence. It is generally accepted that cumulus cells support oocyte maturation to the metaphase II stage and are involved in the cytoplasmic maturation needed for postfertilization developmental capability (4). Usually, oocytes with a multilayered cumulus are used for in vitro maturation protocols, but it is better to use some other selection criteria for the cumulus–oocyte complex (2). It has been reported that nuclear status of immature oocytes in cattle is related to morphological characteristics of the cumulus–oocyte complex and to its maturational competence (2). The present research study focused on swine oocytes, as little is known about this species in relation to the size of follicles and oocytes and the correlation between these factors and pubescence. The work described herein determines the relationships between the diameter and maturation phase of the porcine oocyte, follicle size, and stage of sexual maturity. In this research, the aim was to present some aspects of the quality of oocytes, which should be considered when ova are used for IVF.

2. Materials and methods

This study was performed on the ovaries of gilts (n = 65) and sows (n = 69) obtained from the meat processing factory AGRYF S.A. in Szczecin, Poland.

Immediately after the animals were slaughtered, the ovaries were placed in physiological saline and transported to the laboratory at an ambient room temperature of about

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25 °C. The oocytes were collected by aspiration and were transferred into 0.1% trypsin solution before measurement. The oocytes were washed repeatedly to remove the cumulus cells. The sizes of the oocytes (Figures 1–4) were measured by using a micrometer eyepiece mounted on a microscope (OK 15KM; 150 magnification×) (5). For each oocyte, we performed 2 measurements: with and without the zona pellucida (Figure 1). On the basis of these measurements, we also calculated the thickness of the zona pellucida by subtracting the oocyte measurement as well the perivitelline space (PVS) from the measurement with zona pellucida.

In our study, we present the relationship between the diameter of porcine oocytes and sexual maturity. In this part of the study, 198 oocytes from gilts and 276 oocytes from sows were examined. In each group, a similar number of same-sized follicles was taken into consideration.

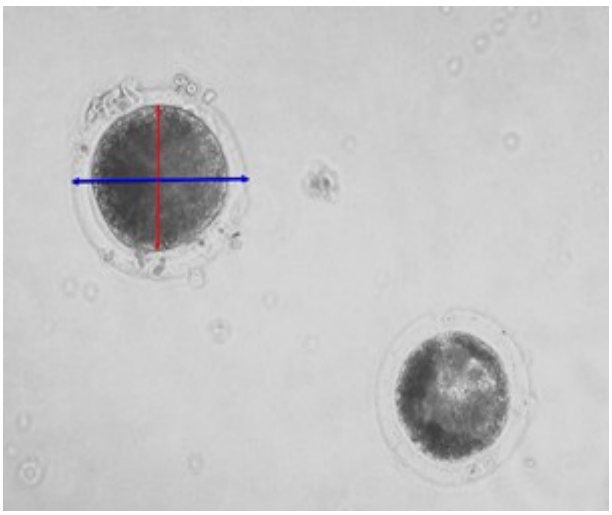

Figure 1. Porcine oocyte measurements. The blue and red lines indicate the point of measurement for the diameter of the oocyte with and without the zona pellucida, respectively.

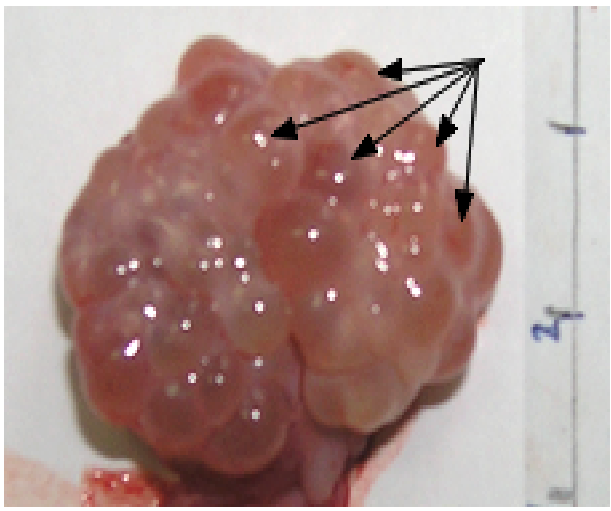

Figure 2. The porcine ovary in follicular phase (the arrows show an ovarian follicle).

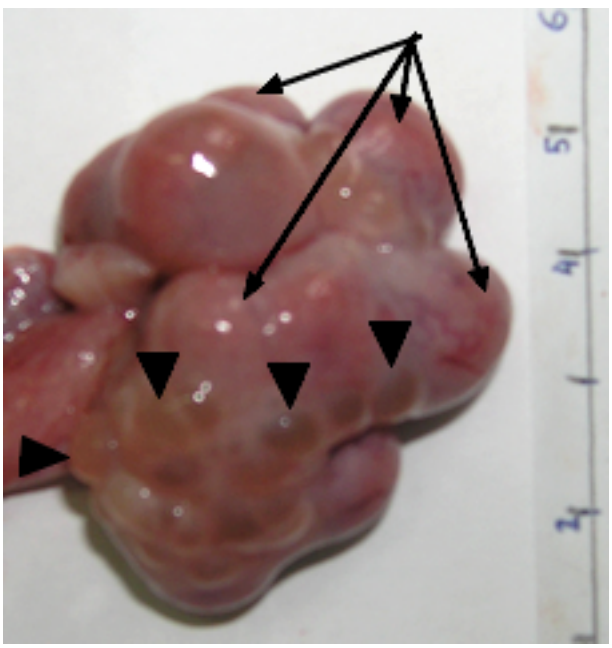

Figure 3. The porcine ovary in luteal phase (the arrows show the corpus luteum and the black triangle an ovarian follicle).

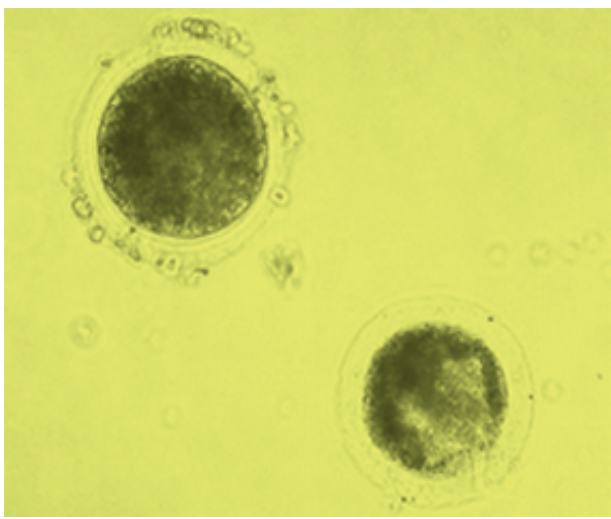

Figure 4. Oocytes obtained from the porcine antral follicle. Note that various sizes can be seen.

2.1. Determining the relationship between the diameter of the porcine oocyte and follicle size

In this part of the work, only oocytes obtained from gilt ovaries were analyzed in order to eliminate the possible effect of age on the size of oocytes. Follicles were grouped based on the following criteria: small follicles (up to 4 mm, n = 89), medium follicles (5–7 mm, n = 82), and large follicles (8–10 mm, n = 28).

2.2. Determining the relationship between the diameter of porcine oocytes and ovarian cycle phase

To assess the relationship between the diameter of oocytes and the ovarian cycle phase, only sow ovaries were considered, as gilt ovaries were insufficient for the presence of corpus luteum. Twenty-four ovaries were in the luteal phase and 43 ovaries in the follicular phase. A total of 276 oocytes were examined, from which 148 oocytes were acquired from ovaries in the follicular phase and 128 from ovaries in the luteal phase.

2.3. Statistical analysis

The results were given as median values ± standard deviations. Statistical significance between groups was determined by the Duncan test. Correlation between the size of the follicle and the oocyte diameter was determined using Pearson's factor. The calculations were made using the statistical package STATISTICA (version 7.1, StatSoft, Poland).

3. Results

The current study demonstrated that the size of porcine oocytes from antral follicles was an average of 134.59 ± 11.87 µm in diameter, with a range from 100 to 156 µm (Figure 4).

There was a significant difference in size between the oocytes of gilts and sows. The formation of morphometric traits depending on the age of the animals is shown in Table 1. The diameters of younger female oocytes (in the prepubertal period) were significantly smaller than those of sow oocytes ($P < 0.01$). These differences were observed among the whole oocyte, oocyte diameter without the zona pellucida, and the thickness of the zona pellucida. These results indicate that there is a correlation between the age of animals (sexual maturity) and the size of oocytes.

Furthermore, a difference was noted in the thickness of the zona pellucida, which may be the most likely cause of disturbances in the process of fertilization. There was a significant difference between medium follicles and large follicles ($P < 0.05$) but no significant difference between small follicles and medium/large follicles.

Table 2 shows the relationship between the sizes of oocytes obtained from sow ovaries in different phases of the ovarian cycle. We found that oocytes obtained in the luteal phase were significantly larger than oocytes in the follicular phase ($P < 0.01$). These differences were related to the oocyte diameter and the thickness of the zona pellucida, and they indicate a connection between the size of porcine oocytes and ovarian cycle phase. Our study demonstrates a significant difference ($P < 0.01$) between the size of the gilt oocytes and sow oocytes and the stage of the ovarian cycle phase.

Another criterion in this work was to examine the size of the follicle (Table 3). In this part of the work, we assessed only the oocytes that were derived from gilts. The

Table 1. Morphometric characteristics of porcine oocytes depending on the age of the animals.

	Number of follicles, N	Oocyte diameter with zona pellucida (µm)	Oocyte diameter without zona pellucida (µm)	Thickness of zona pellucida (µm)
Gilts	262	124.35 ± 12.33 ^{A}	100.80 ± 10.47 ^{A}	25.32 ± 8.62 ^{A}
Adult swine	276	139.24 ± 9.88 ^{B}	110.43 ± 7.13 ^{B}	28.79 ± 5.37 ^{B}

A, B: the averages in a column marked with different letters show significant difference at $P < 0.01$.

Table 2. Morphometric characteristics of sow oocytes depending on the ovarian cycle phase.

Ovarian phase	Number of follicles, N	Oocyte diameter with zona pellucida (µm)	Oocyte diameter without zona pellucida (µm)	Thickness of zona pellucida (µm)
Follicular phase	148	136.62 ± 8.41^{A}	108.71 ± 5.35 ^{A}	27.87 ± 4.94 ^{A}
Luteal phase	128	142.28 ± 10.59 ^{B}	112.43 ± 8.33 ^{B}	29.85 ± 5.68 ^{B}

A, B: the averages in a column marked with different letters show significant difference at $P < 0.01$.

Table 3. Morphometric characteristics of gilt oocytes depending on follicle size.

Diameter of the follicle	Number of follicles, N	Oocyte diameter with zona pellucida (μm)	Oocyte diameter without zona pellucida (μm)	Thickness of zona pellucida (μm)
Small (2–4 mm)	89	121.44 ± 11.55 ^{Bb}	99.11 ± 8.84	25.91 ± 9.43
Medium (5–7 mm)	82	127.07 ± 12.05 ^{a}	103,21 ± 10.45	23.92 ± 6.80 ^{a}
Large (8–10 mm)	28	131.44 ± 12.05 ^{A}	101.58 ±15.44	29. 33 ± 10.81 ^{b}

A, B: the averages in a column marked with different letters show significant difference at $P < 0.01$.
a, b: the averages in a column marked with different letters show significant difference at $P < 0.05$.

oocytes obtained from the small follicles were significantly smaller than oocytes from the medium and large follicles ($P < 0.01$ and $P < 0.05$, respectively).

There was a significant difference related to the thickness of the zona pellucida. Oocytes in the large follicles had significantly thicker ($P < 0.05$) zona pellucida compared to the medium-sized ones.

The results indicate the relationship between the size of the oocyte and the follicle size.

Our results indicate a positive correlation ($r = 0.15$, $P < 0.05$) between oocyte and follicle size, indicating that porcine oocytes increase in size not only in the early stages of folliculogenesis, but also during the development of antral follicles.

4. Discussion

The size of porcine oocytes from large antral follicles observed in this study was found to be about 131 μm, similar to sizes reported by other authors (6,7). In comparison to the other domestic animal species, this value is somewhat smaller than those of other species. For example, the diameter of oocytes of ruminants (cows, sheep, goats) is approximately 150 μm; human oocytes are smaller, with a diameter of about 120 μm (6,7). Taking together these data, the size of the oocytes might be species-dependent.

Our study also demonstrates a difference between oocyte size in gilts that have never been pregnant and sows. In addition, oocytes obtained from prepubertal gilts compared to oocytes collected from sows had lower developmental competence and fewer of them reached the stage of metaphase II (7). These data confirmed the observations of other authors who pointed out that the varying quality of oocytes may depend upon the sexual maturity of the animals (8–11). Our data may support the ideas of Bagg et al. (12), who suggested that the best quality and most suitable oocytes for in vitro fertilization are from sows which have had at least one estrous cycle. According to these authors, an oocyte could not achieve developmental competence immediately after puberty. Moreover, Sherrer et al. (11) and Gandolfi et al. (13) reported that oocytes obtained from mature females as compared to immature females are characterized by greater developmental capacity, and as a result of fertilization, more good quality embryos are achieved.

The quality of embryos is significantly affected by the processes of synthesis and storage of protein and mRNA during maturation of oocytes (14).

What is more, Peters et al. (9) and Ikeda et al. (10) showed that the lower effectiveness of extracorporeal embryos obtained from prepubertal gilts may be due to greater sensitivity to environmental factors and lower cytoplasmic content in oocytes compared with those derived from older gilts. In line with this work, our results show that gilts' oocytes were smaller than sows', which may indirectly indicate a reduced volume of cytoplasm and smaller quantity of the cellular components necessary for embryonic development.

It has been suggested that oocytes from older animals have a lower polyspermic fertilization rate than gilt oocytes (8). It should also be mentioned that the frequency and accuracy of the polyspermy occurrence in the case of porcine oocytes is the main factor limiting the effectiveness of porcine embryo collection (15). The results obtained in this study and those above are an important consideration due to the fact that during the complex production of porcine embryos, oocytes from sexually immature females are most commonly used (15). According to researchers, one of the methods that improve the quality of gilt oocytes is to use nutrition enriched with steroids and growth factors (such as epidermal growth factor) (15). It is also important to consider the possibility of harvesting the whole follicles, especially the preantral and early antral follicles (5,16). Perhaps this line of research will result in obtaining better quality porcine oocytes, and may thereby increase the efficiency of in vitro fertilization in this species. The results of the research presented by Hiaro et al. (17) indicate that oocytes 'grown' in isolated early antral follicles increase their diameter from 15 to 20 μm.

Current results obtained from determining the size of oocytes obtained from sow ovaries in different phases of

the ovarian cycle are consistent with the results of Bagg et al. (12), who noticed that larger oocytes were obtained in the luteal phase than the oocytes obtained in the follicular phase. However, the authors mentioned that the ovarian cycle phase did not affect the quality or the meiotic and developmental competence of received oocytes. The lack of correlation between the developmental capacity of the oocytes and the phase of ovarian cycle has also been shown among other species (18–20). For example, Chian et al. (18) indicated the lack of differences in the strengthening of atresia changes in oocytes between the follicular and luteal phases. However, Manjunath et al. (21) suggested that oocyte quality depends on the morphological and functional state of the ovary.

In contrast to the results obtained during this study, the authors mentioned above indicated that the size of oocytes is not related to the ovarian cycle phase. Most likely, these discrepancies result from differences among species and the number of developing ovarian follicles in a single cycle. Some other authors also indicated the existence of differences in the oocyte kinetics of growth between species (22).

In this current study, a positive correlation between the size of the oocyte and the follicle was confirmed and is consistent with the observations of other researchers (22–24). We think that this criterion should be included in the evaluation of oocyte quality before the procedure of IVF. Similar indications were raised by other researchers (22–24). It is known that the main sources of porcine oocytes are not only large follicles but also follicles ranging from 2 to 5 mm (25). In culture, the oocytes should increase their capacity to achieve their developmental competency.

In conclusion, in the current study, the morphometric parameters of pre- and postpubescent porcine females were characterized, and differences in the oocyte diameter between gilts and sows were observed. These data could indicate that the size of these cells depend upon the female's stage of sexual maturity. In the present study, differences in the size of the oocytes obtained at different phases of the porcine ovarian cycle were demonstrated. Moreover, these results could indicate a correlation between the oocyte size and the morphofunctional stage of the ovary in swine. Furthermore, a positive correlation between oocyte and follicle size was confirmed. This could confirm that porcine oocytes arise in all stages of folliculogenesis; these parameters should be taken into consideration while estimating the quality of oocytes following maturation and preceding the IVF procedure, in order to improve pregnancy outcome in swine.

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