Comparative Biometrical and Follicular Study in Pig Ovary

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Abstract

Porcine ovaries are a biological material frequently used in studies that analyze follicular development processes, oocyte maturation in close connection with the stages of the estrus cycle. Ovarian follicles and their degree of development represent the point of departure in obtaining good quality oocytes, capable of being used in in vitro fertilization processes. The present study aims to identify the possible correlations between some biometric parameters and the degree of development of the follicles in the ovaries of pigs harvested after slaughtering the animals. The study was carried out on a number of 36 ovaries from 2 slaughters that were carried out in different stages of the estrus cycle, grouped into two experimental groups. In the laboratory, 3 biometric parameters weight (g), length (mm) and width (mm) were determined, respectively they were identified, photographed, numerically quantified and analyzed to establish the type of ovarian follicle. Based on the determinations made, we can conclude that the number of follicles and their type is changed in the ovaries of the two experimental groups, these two parameters are closely related to the stage of the estrous cycle and do not cause significant changes in the biometric parameters studied - weight, length, width.

Keywords: preantral follicle, antral follicle, estrus,

1. Introduction

Assisted reproduction techniques in farm animals are mostly based on an ovarian overstimulation that results in obtaining a large number of immature oocytes that must be matured in vitro if they are to be used later in the in vitro fertilization process. The in vitro maturation process of oocytes is a complex one, being influenced by several factors, the most important being the size of the harvested ovarian follicles. The oocytes that have been extracted from the preantral follicles have the ability to enter meiosis-type cell division, but are characterized by a limited capacity to develop up to the blastocyst stage. The metabolic activity of oocytes influences their maturation capacity, all the transformations that take place at the level of the cytoplasm and nucleus of the oocyte, responsible for ensuring in vitro an efficient maturation and obtaining superior quality oocytes capable of fertilization and further development [1].

Follicular development within the estrus cycle takes place and presents 3 distinct characteristics: a. recruitment, selection and dominance; b. the intervention of gonadotropins - follicle-stimulating hormone (FSH) with a role in follicular recruitment and luteinizing hormone (LH) with a role in choosing dominant follicles; c. the number of waves / estrus cycle and the number of follicles / estrus cycle. Studies have been carried out on follicular development in farm animals, hormonal discharge waves being observed in pigs only during the prepubertal period [2].

Follicular development in pigs involves two of the above characteristics namely - recruitment and selection. In pigs in the prepubertal and pubertal phase, but also in sows, a number of approximately 50 follicles, whose size is 1-6 mm, can be seen on the surface of the ovary. Pigs have a higher number of follicles/ovaries compared to

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other species of domestic animals. In the case of the luteal phase, between 30-90 ovarian follicles of small size 1-2 mm, respectively 30-50 ovarian follicles of medium size 2-7 mm can be identified in each ovary [3,4].

In pigs during the follicular phase, the number of small and medium-sized follicles decreases in both ovaries, reaching a number of approximately 20 follicles / ovary, most of them being ovulatory follicles whose sizes reach 7-10 mm in diameter before ovulation occurs [5,6].

Studies assessing the number and size of follicles at different stages of reproduction in pigs have been performed using different collection methods from the reproductive tract, such as surgery and ultrasound. The most modern method of evaluating the number and size of follicles is the use of ultrasound for evaluation in live pigs, allowing the establishment of repeated measures and links with endocrinology, estrus, ovulation and fertility. The ability to numerically evaluate and accurately measure follicles >1 mm in size varies depending on the quality of the equipment used, the frequency of the transducer, but also the distance and level of interference to the follicles [7]. The oldest method of evaluating the number and size of follicles, but also the type of follicles in pigs, involves the slaughter of the animal and the excision of the ovaries. The method allows, after the analysis of some ovarian biometric parameters, to identify and count the ovarian follicles, respectively to isolate the oocyte-cumulus complexes for the purpose of in vitro cultivation to ensure the maturation of the oocytes for the purpose of their subsequent use in the in vitro fertilization process.

In the present study, the aim is to identify the possible differences between 3 biometric parameters: weight, length and length, respectively the stage of the estrus cycle in which the ovaries are located at sampling. Also, the type of follicles present in the ovaries is monitored depending on the period of the estrus cycle in which the animals were slaughtered.

The aim of the work is to determine some biometric parameters at the level of pig oocytes and to identify the types of ovarian follicles, but also to identify possible correlations between the biometric parameters and the stage of follicular development.

2. Materials and methods

2.1. Harvesting the ovaries

The ovaries were harvested from sows on the slaughter and transection line in a slaughterhouse specialized in animal slaughter. Slaughtered sows presented an average weight of ±110 kg, age of approximately 5.5 months and ovaries in different stages of the estrous cycle. After cutting the animal and highlighting the female genital apparatus, the ovaries were excised with the help of scissors. After excision, the ovaries were placed in a sterile container with sterile 9% saline at a temperature of 30°C.

In total, 36 sow ovaries were sampled and brought to the laboratory for the tests. The sampled ovaries were divided into 2 batches: Batch A - 18 ovaries and Batch B - 18 ovaries

2.2. Examination of the ovaries

The ovaries were brought to the laboratory and subjected to biometric determinations for 3 parameters: weight (G), length (L) and width (l). Ovaries were weighed with an analytical balance (Kern model ALJ 220-4NM, Denver Instrument Gmbh, Göttingen, Germany). The other 2 biometric parameters of the ovaries, length and width, were measured using slide calipers.

2.3. Identification of ovarian follicles

After the determination of the biometric parameters, the ovaries were subjected to the examination and identification of ovarian follicles, the type of follicles and the presence of oocyte-cumulus complexes (COC). Microscopic examination and photography were carried out with an inverted microscope - Olympus CX41 microscope equipped with a camera.

2.4. Statistical analysis

The values of the biometric parameters and the maturation rate of the ovaries were statistically analyzed with the non-parametric Mann-Whitney test. Differences were considered to be significant at $p<0.05$.

3. Results and discussion

The results obtained following the biometric determinations at the level of the ovaries in the 2 experimental groups, respectively the identification of the type of follicles and the oocyte-cumulus complexes were centralized and can be found in Tables 1 and Table 2.

The biometric parameters of the ovaries from batch A recorded the following values:
- The weight of the ovaries between 6.19 g and 16.0 g, with an average value / lot of 10.92±2.91 grams,
- The length of the ovaries between 20.11 mm and 44.5 mm, with an average value / lot of 32.73±6.86 mm,
- The width of the ovaries between 15.25 mm and 28.85 mm, with an average value / batch of 24.57±4.20 mm.

The ovaries taken from the sows from Batch A (the first experimental batch) were in the luteal phase of the estrous cycle, which led to the identification of preantral follicles. In this phase, no cumulus oocyte complexes were identified. The number of preantral follicles varied in the analyzed ovaries from 3-9/ovary, with an average value/lot of 5.88±1.96.

Table 1. Biometric parameters, the number and type of follicles identified in the ovaries of group A

No	Weight (g)	Length (mm)	Width (mm)	Preantral follicles
1	7.88	28.14	22.88	3
2	10.09	39.36	26.05	5
3	12.33	39.02	26.1	7
4	6.32	20.11	15.25	4
5	9.45	38.77	26.15	6
6	9.35	37.89	25.97	5
7	9.51	30.56	26.35	6
8	11.91	34.98	28.12	4
9	6.4	30.23	23.34	3
10	16	44.5	28.85	9
11	12.92	38.94	28.68	7
12	12.78	30.56	27.88	8
13	12.86	28.68	28.62	8
14	12.6	25.24	19.4	5
15	11.96	28.46	26.68	7
16	13.54	36.23	24.86	8
17	14.49	37.19	21.55	8
18	6.19	20.32	15.56	3
Medie	*10.92*	*32.73*	*24.57*	*5.88*
DVST	*2.91*	*6.86*	*4.20*	*1.96*

The biometric parameters of the ovaries from batch B recorded the following values:
- the weight of the ovaries between 7.13 g and 15.51 g, with an average value / batch of 11.43±2.63 grams,
- the length of the ovaries between 24.10 mm and 45.29 mm, with an average value / batch of 33.75±6.94 mm,
- the width of the ovaries between 17.62 mm and 37.17 mm, with an average value / batch of 24.80±6.32 mm.

The ovaries taken from the sows from Batch B (the second experimental batch) were in the follicular and luteal phase of the estrous cycle. In these two phases of the estrus cycle, cumulus oocyte complexes were identified in the analyzed ovaries. The number of cumulus oocyte complexes was a maximum of 12/ovary in the ovaries in the luteal phase of the estrous cycle, respectively the number of cumulus oocyte complexes from the ovaries in the follicular phase was a maximum of 25/ovary.

Table 2. Biometric parameters, the number and type of follicles identified in the ovaries of group B

No	Weight (g)	Length (mm)	Width (mm)	COC	Phases estrus cycle (F /L)
1	9.5	29.83	20.71	1	L
2	8.14	34.24	20.38	0	L
3	9.79	25.84	20.17	7	F
4	12.93	26.64	18.61	15	F
5	14.23	45.29	31.9	7	L
6	12.32	24.1	17.62	22	F
7	8.83	31.21	20.62	25	F
8	7.13	24.51	20.31	18	F
9	12.62	25.93	20.51	12	F
10	14.77	41.73	33.11	12	L
11	12.03	36.88	20.84	3	L
12	16.6	43.41	37.17	4	L
13	15.51	43.67	22.69	1	L
14	10.82	39.07	28.4	8	L
15	10.12	37.49	30.98	12	L
16	10.39	33.03	34.97	2	L
17	9.2	32.33	27.43	6	L
18	10.95	32.4	20	5	L
Medie	*11.43*	*33.75*	*24.80*	*8.88*	-
DVST	*2.63*	*6.944*	*6.32*	*7.36*	-

(Cumulus oocyte complexes - COC, Follicular - F, Luteal - L)

Statistically analyzing the values of the biometric parameters at the level of the ovaries in the two experimental groups and considering the significance threshold of $p<0.05$, we find that there are no significant differences for any of the 3

parameters studied: for weight, a value of p= 0.68, for length the value determined for p was p=0.75, and for width p had the value of p=0.70.

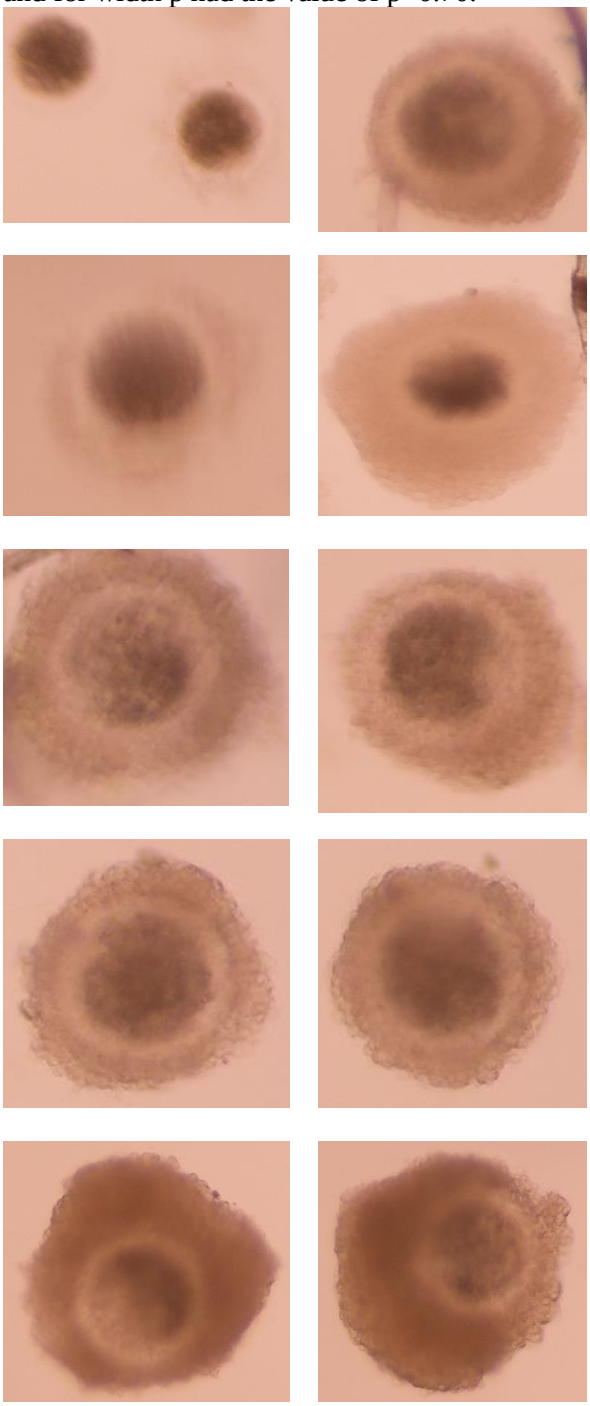

Figure 1. Types of ovocites in the analyzed pig ovaries

Porcine ovarian tissue has been used extensively to perform numerous studies of follicular development, oocyte maturation and granulosa cell luteinization. In most studies, the ovarian tissue used in the studies very frequently comes from slaughterhouses from pigs with uncertain physiological status, the stage of the estrous cycle in which the ovaries are located is not known.

Pigs represent an opportune study animal to study the genesis of follicles for a polytocous species, but also to examine the interrelationships in the follicles and the types of cells present in them. In pigs, follicles from the proliferation group are recruited during days 14-16 of the estrous cycle. The growth of follicles selected to ovulate is associated with rapid atresia of smaller follicles. There are numerous aspects in the morphological and biochemical development processes of the dominant follicles from the early follicular phase, this aspect suggests that the follicles are recruited in very different stages of development or that their recruitment continues in the follicular phase as well. It is known that there is an important connection between the follicular diameter and the volume of the follicular fluid, and a comparison of these two characteristics highlights a gradual increase in the volume of the follicular tissue as a proportion of the total volume of the follicle, of the ovary. The growth of follicles from 2 mm to 4 mm is due to and associated with the proportional increase in the number of granulosa cells, so the number of granulosa cells cannot be used as an indicator of atresia in porcine follicles [8].

Using sonography, a first study was carried out on follicular dynamics from the beginning of estrus until ovulation in pigs. Thus, in young and adult pigs, the follicles identified and observed at the beginning of the scans, which corresponds to the end of the luteal phase, were 3 mm to 5 mm in size. After the luteal phase, it is found that small and medium follicles disappear rapidly, while follicles of large dimensions of 6.5 mm were identified and recorded a numerical increase near the time of ovulation. The selection of follicles in young pigs is a complex process and is only performed when the corpus luteum is present [5]. Previous studies show that the presence of the corpus luteum produces changes in blood flow and influences the growth of follicles, in addition, the population of follicles registers significant variations both before and after the formation of the corpus luteum, but also before and after luteolysis [9].

At the level of the studies carried out regarding the evolution of the follicular population in pig ovaries, certain stages of their morphological and numerical development were identified. Thus, from the onset of estrus to the moment of

ovulation, a decrease in the number of ovarian follicles was observed, a fact mainly due to a reduction in the number of follicles with sizes from 6 mm to 9 mm. Some 6-7 mm follicles grew to 8-9 mm, which caused the number of medium-sized follicles to decrease and the number of large-sized follicles to increase. The decrease in the number of follicles of 6-7 mm occurs in the first 24 hours from the onset of estrus, and the increase in the number of follicles of 8-9 mm occurred in the interval 24 - 32 hours from the onset of estrus. Other 6-7 mm follicles were atretic but reduced in size and became the recruitment pool of 3-5 mm follicles [5].

In the studies carried out, it was established that in the case of adult pigs, from the onset of estrus to the moment of ovulation, there are changes in the periods of the estrous cycle: ovulation occurs approximately 35 hours after the onset of estrus and ends approximately 39 hours later. Also, the sizes of the preovulatory follicles record values of 7.0±1.0 mm, so that at the time of ovulation the size of the follicles reaches 9.3 mm [10,11].

The process of obtaining good quality oocytes, in order to be used in the in vitro fertilization process, is influenced by numerous factors, the most important being the way in which the follicular development is carried out. The process requires knowledge of: the origin of the oocyte (oocytes obtained from large follicles are more capable of fertilization compared to those obtained from small follicles), the state of health of the follicle - the dominance of the follicle and atresia influence the development capacity of the oocyte, if it had place or not hormonal stimulation to ensure the development of the follicle and implicitly the increase in the quality of the oocytes, the links established between the oocyte and the cells of the cumulus that ensure the development of a mature and good quality oocyte [1,12].

4. Conclusions

The biometric parameters studied do not record statistically significant differences at the level of the ovaries from the two experimental groups, for the biometric parameters: weight, length, width. Ovarian follicles identified in the analyzed ovaries were preantral and antral with cumulus oocyte complexes. The type of ovarian follicles identified does not correlate with the determined biometric parameters. The type of ovarian follicle identified is closely related to the period of the estrous cycle, the pigs from the 2 experimental groups were slaughtered in different periods of the estrous cycle.

References

1. Krisher, R. L., The effect of oocyte quality on development. J. Anim. Sci., 2004, 82, E14-23. DOI: 10.2527/2004.8213.
2. Schwarz, T., Murawski, M., Wierzchoś, A., Bartlewski, P., An ultrasonographic study of ovarian antral follicular dynamics in prepubertal gilts during the expected activation of the hypothalamo-pituitary-ovarian axis, J. Reprod. Dev., 2013, 59, 409-414.
3. Evans, A. C. O. Characteristics of ovarian follicle development in domestic animals, Reprod. Dom. Anim., 2003, 38, 240-246.
4. Guthrie, H.D., Grimes, R.W., Cooper, B.S., Hammond JM. Follicular atresia in pigs: Measurement and physiology, J. Anim. Sci., 1995, 73, 2834-2844.
5. Williams, S.I., de la Sota, R.L., Follicular dynamics and ovulation time in gilts and post-weaning sows, Can. Vet. J., 2017, 58, 65-69.
6. Dalin, A.M., Nanda, T., Hultén, F., Einarsson, S., Ovarian activity at naturally attained oestrus in the sow. An ultrasonographic and LH study, Acta Vet. Scand., 1995, 36, 377-382.
7. Knox, R.V., Follicle development in pigs: State of the art, Mol. Reprod. Dev., 2023, 90, 480-490. DOI: 10.1002/mrd.23576
8. Foxcroft, G. R., Hunter, M. G., Basic physiology of follicular maturation in the pig, Journals of Reproduction & Fertility, 1985, 33, 1-19.
9. Murphy, B.D., Gevry, N., Ruiz-Cortes, T., Côté, F., Downey, B.R., Sirois, J., Formation and early development of the corpus luteum in pigs, Reprod., 2001, 58, 47-63 .
10. Soede, N.M., Bouwman, E.G., Lanjendijk, P., van der Laan, I., Canora, A., Kemp, B., Follicular development during luteal phase and altrenogest treatment in pigs, Reprod. Dom. Anim., 2007, 42, 329-332.
11. Soede, N.M., Langendijk, P., Kemp, B., Reproductive cycles in pigs, Anim. Reprod. Sci., 2011, 124, 251-258.
12. Sirard, M.A., Richard, F., Blondin, P., Robert, C., Contribution of the oocyte to embryo quality, Theriogenology, 2006, 65, 126-136.