VETERINARSKI ARHIV 89 (3), 309-315, 2019 DOI: 10.24099/vet.arhiv.0231

Determination of optimal follicular size for obtaining the most quality oocytes for *in vitro* fertilization

Renalda Juodžentytė[1]*, Vytuolis Žilaitis[1], and Giedrius Palubinskas[2]

[1]*Large Animals Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuanian*

[2]*The Centre for Veterinary Continuing Education and Consulting, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuanian*

JUODŽENTYTĖ, R., V. ŽILAITIS, G PALUBINSKAS: Determination of optimal follicular size for obtaining the most quality oocytes for *in vitro* fertilization. Vet. arhiv 89, 309-315, 2019.

ABSTRACT

The aim of this study was to determinate optimal follicular size for collection of high quality oocytes, and the subsequent capacity of the oocyte to mature and be fertilized and to develop *in vitro*. The ovaries of dairy cows were cut out immediately after slaughter and transported within one hour. The follicles were separated and divided into three groups according to diameter, *i.e.* small (3-5 mm), medium-size (6-9 mm), large (10-20 mm). Quality grading (A, B, C, D) of the oocytes was performed on the basis of cumulus cell development and homogeneity of cytoplasm. A total of 284 COCs were aspirated from 123 ovaries. Among 143 COCs, 236 were grades A and B and 48 were grades C and D. The maturation rate of the oocytes from medium size follicles A and B grade was 56.01% better than C and D grade. Only A and B graded oocytes were fertilized *in vitro* with 1×10^6 sperm/mL using Fert-TL, with 0.3% BSA, 22 µg/mL sodium pyruvate, 10 µg/mL heparin. The cleavage rate in the small follicle oocytes was significantly lower (35.89%) than in the medium-size follicle oocytes (56.41%), and a similar trend was observed in the morula development rates, independent of the oocyte grade. Good quality oocytes received from medium size follicles, and oocytes with more than three complete layers of cumulus cells (Grades A and B) have better competence for *in vitro* maturation and cleavage.

Key words: *in vitro* fertilization; oocytes; cumulus cells; maturation; cleavage

Introduction

In the last decade, *in vitro* fertilization procedure (IVF) has become the technique of choice for bovine embryo production.

In animal embryo production, the diameter of the follicles and oocytes, the presence of cumulus cells, the homogeneity of the cytoplasm, and maternal age all affect the

*Corresponding author:

Renalda Juodžentytė, Lithuanian University of Health Sciences, Veterinary Academy, Large Animals Clinic, Kaunas, Lithuanian, Phone: +37 062 327 622; E-mail: renalda.juodzentyte@lsmuni.lt

ISSN 0372-5480
Printed in Croatia

maturation of oocytes. ROMAGUERA et al. (2010) demonstrated that oocytes which generated from small follicles (<3 mm) in prepubertal goats produce significantly lower blastocyst rates following fertilization compared to oocytes from follicles with a diameter greater than 3 mm (ROMAGUERA et al., 2010)

In *in vitro* production of cattle and sheep embryos, it has been reported that oocytes aspirated from prepubescent animals have poor cytoplasmic maturation, abnormalities of their ultrastructure, alterations in metabolic activity, and lower embryo development rates compared to adult counterparts (SUCCU et al., 2007).

In cattle, the oocyte first acquires competence to develop *in vitro* into blastocysts at a follicular size of 2-3 mm. When follicles were pooled according to size, it was shown that large follicles (>10 mm diameter) contain oocytes with a higher potential to become embryos. Some studies have described the fate of individual oocytes according to the exact follicular size and confirmed the increased competence with larger follicle size, *i.e.* bovine oocyte complexes (COCs) isolated from ovaries carrying follicles of 2-5 mm in diameter showed lower rates of maturation and blastocyst formation than those from ovaries carrying follicles of >10 mm in diameter. This indicates that large follicles (>6 mm diameter) provide the oocyte with a microenvironment which improves its quality. Dramatic changes in oocyte nuclei, especially nucleoli, are known to occur as the bovine follicle grows from 1-20 mm. Such changes may have a crucial effect on the developmental potential of the oocytes. However, other reports suggest that follicular size may not be the only important criterion, since some bovine oocytes originating from large follicles failed to produce embryos, while some oocytes from medium-size follicles already have this capacity (AYMAN et al., 2016). The development of follicles is associated with processes occurring in the ovaries. The main indicator of the quality of an oocyte is its ability to be fertilized.

Objective. The aim of this study was to determine the optimal follicular size for collection of high quality oocytes and the oocyte's subsequent capacity to mature, be fertilized and develop *in vitro*.

Materials and methods

The research was conducted in accordance with the provisions of the Law of the Republic of Lithuania No. 1-2271 on Protection, Keeping and Use of Animals, dated 03/10/2012 (Valstybės Žinios (Official Gazette) No. 122-6126 dated 20/10/2012) and of the by-laws, Education and training purposes of animals used in storage, maintenance and conditions of use No. B1-866, dated 31/10/2012 (Valstybės žinios (Official Gazette) No. 130-6595 dated 10/11/2012).

The ovaries of Holstein cow breed from Lithuanian, of 2nd - 4th lactation were cut out immediately after slaughter and transported to the laboratory within one hour. The

diameter of the various follicles present in each ovary was measured with ruler. The follicles collected were separated and divided into three different groups according to their diameter, *i.e.* small (3-5 mm), medium-size (6-9 mm) and large (10-20 mm). The oocytes were aspirated from each follicle group separately, using a sterile syringe and a 22 G needle.

Quality grading of the oocytes was performed on the basis of cumulus cell development and the homogeneity of the cytoplasm according to CHAUBAL et al. (2006), as follows: Grade A: those with more than 3 layers of cumulus cells surrounding the oocyte and uniform cytoplasm; Grade B: those with less than 3 layers of cumulus cells surrounding the oocyte and uniform cytoplasm; Grade C: those with 1 layer of cumulus cells or no cumulus cells surrounding the oocyte; Grade D: denuded oocytes. All oocytes (COCs) were washed five times in TCM-199 medium (Minitub, Germany) supplemented with 10% BSA (bovine albumin serum), 0.5 μg/mLFSH, 5 μg/mLLH. The groups of COCs were matured in 400 μL of TCM-199 medium (Minitub), covered with mineral oil, in four well plates (Minitub, Germany) for 24 hours at 38.5 °C, and in an atmosphere of 5% CO_2. After maturation (Day 0), the matured COCs were washed and assessed.

Frozen bull semen was thawed at 37 °C for 40 seconds. Sperm capacitation was performed *in vitro* in 2 mL of Sperm-TL medium (Minitub, Germany) supplemented with 0.6% BSA, 22 μg/mL sodium pyruvate, 50 μg/mL gentamycin and centrifuged twice at 160 and 108 g for 10 minutes. Only grades A and B COCs were used for fertilization. Fertilization was performed *in vitro* with Fert-TL (Minitub, Germany), supplemented with 0.3% BSA, 22 μg/mL sodium pyruvate, and 10 μg/mL heparin. Fert-TL medium was added to the fertilization wells (Minitub, Germany) and the spermatozoa concentration was adjusted to 1×10^6 sperm/mL. It was co-incubated for 24 hours at 38.5 °C in an atmosphere of 5% CO_2 in the air, with maximum humidity.

After fertilization (Day 1), the cumulus cells were removed from the presumptive zygotes by manual pipetting and transferred into 100 μL of SOF medium (Minitub, Germany), supplemented with 8 mg/mL BSA and 22 μg/mL sodium pyruvate, and incubated for 24 hours at 38.5 °C, in an atmosphere of 5% CO_2 in the air, with maximum humidity.

The embryonic cleavage was evaluated after 48 hours (cleavage rate), and morula (>16 - cell stage) formation was identified after 120 hours (5 days).

Results

A total of 284 COCs were aspirated from 123 ovaries. Among the 284 COCs, 236 were grades A and B, and 48 were grades C and D. In the small follicle group, the percentage of COCs collected was 37.89%. In the large follicle group, the percentage of COCs collected

was 13.17%. A higher percentage of oocytes was collected from medium-size follicles (48.94%) than from the other follicle groups (P<0.05) (Fig. 1).

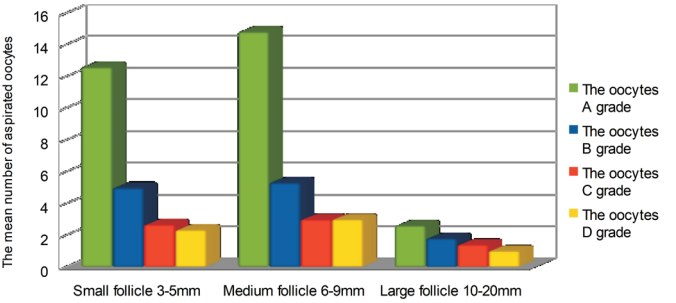

Fig. 1. The mean number of oocytes from different follicle groups a:b, b:c (P<0.05)

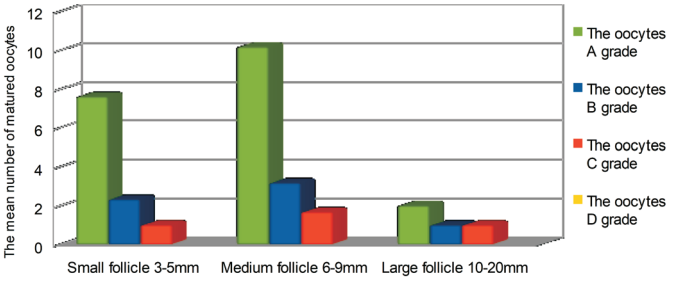

Fig. 2. The mean number of matured oocytes from different follicle groups a:b, b:c (P<0.05)

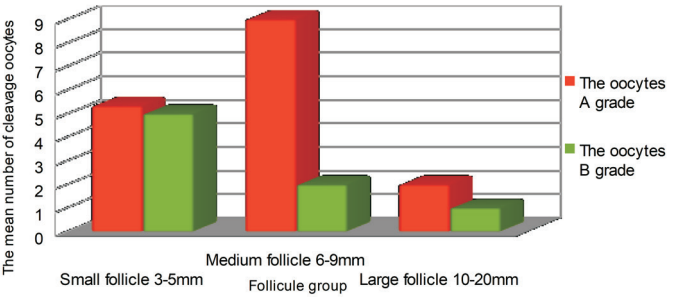

Fig. 3. The mean cleavage embryos from different follicles (P>0.05)

In the small follicle group, the percentage of matured COCs was 44.82% (Grades A and B). In the medium-size follicle group, the percentage of matured COCs was 61.15% (Grades A and B). In the large follicle group, the percentage of matured COCs was 37.82% (Grades A and B). In all follicle groups, the percentage of matured COCs was 19.31% (Grades C and D). A significant difference (23.23%) was detected between the medium-size group and the two others (small and large) ($P<0.05$). The mean of maturated oocytes from small, medium-size and large follicles is presented in Fig. 2.

The cleavage percentage in small and large size follicle oocytes was significantly lower (35.89%) than in the medium-size follicle oocytes (56.41%), and a similar trend was observed in the morula development rates independent of the grade. However, no significant difference in the mean of fertilized oocytes by follicle size groups was established, and it was statistically insignificant ($p>0.05$) (Fig. 3).

Discussion

Many factors effect *in vitro* maturation of cattle oocytes. These factors are either the selection of the proper maturation medium, the quality of the oocytes, or the hormones added (DE WIT et al., 2000). Such changes may have a crucial effect on the developmental potential of the oocytes. It is known that a very stable form of RNA accumulates in the oocyte and that it is translated during maturation, fertilization and early embryonic development. This RNA accumulation may be influenced by the nature of the follicle growth. However, other reports suggest that follicular size may not be the only important criterion, since some bovine oocytes originating from large follicles fail to produce embryos, while some oocytes from medium-size follicles had this capacity. KAKKASSERY et al. (2010) reported that from the 288 aspirated COCs, 212 were grades A and B, and 76 were grades C and D. Our results showed that from the 284 aspirated COCs, 236 were grades A and B and 48 were grades C and D.

LONERGAN et al. (1994) reported that more oocytes with many layers of cumulus cells were obtained from follicles with >6 mm diameter (70.2%) compared to 2-6 mm diameter follicles (46.8%) in bovines, which was also found in the present study in terms of yield of usable and normal quality oocytes.

Furthermore, according to KAKKASSERY et al. (2010), the maturation rate of oocytes collected from all follicle groups, based on cumulus cell expansion by oocyte grade, was as follows: grade A - 83.08%, grade B - 68.29%, and grade C - 44.74%. IWATA et al. (2004) established a similar tendency in the maturation rate of small and medium-size follicle oocytes (87 and 88%, respectively) in cattle (IWATA et al., 2004).

Our results showed higher maturation rate in oocytes from medium-size follicles (61.15%) as compared with the small or large follicle oocytes (18.30%).

According to LEQUARRE et al. (2005), the recorded oocyte cleavage rates were 38.63 and 58.69% in small and medium-size follicle oocytes, respectively, and were significantly different from each other.

The results showed a similar cleavage rate in oocytes from medium-size follicles (56.41%) as compared with the small or large follicle oocytes (35.89%).

The higher number of good quality and usable oocytes recovered from the medium-size follicles compared to small follicles might be attributed to the development of oocytes in medium-size follicles.

Conclusion

The highest number of good quality (A and B) oocytes were aspirated from medium size follicles group. The oocytes with multiple layers of cumulus cells (A and B grade) matured better, at a rate of 56.01%, with cleavage of 56.41%, than denuded oocytes or the oocytes with less than three layers of cumulus cells (C and D). The results show that although the oocytes from small follicles showed lower percentages of development, they may enable an increase in the total number of good quality and transferable embryos. The conclusion is that the number of oocytes that can develop depends on the size of the follicles from which they were collected. Oocytes with more than three complete layers of cumulus cells (Grade A and B) have better competence for *in vitro* maturation and cleavage.

References

AYMAN, H., A. EL-AZIZ, E. M. USAMA, Z. K. SHERIEF, A. S. AHMED (2016): Factors influencing *in vitro* production of bovine embryos. Rev. Asian J. Anim. Vet. Adv. 11, 737-756.

CHAUBAL, S. A., J. A. MOLINA, C. L. OHLRICHS, L. B. FERRE, D. C. FABER, P. E. BOLS, J. W. RIESEN, X. TIAN, X. YANG (2006): Comparison of different transvaginal ovum pick-up protocols to optimise oocyte retrieval and embryo production over a 10-week period in cows. Theriogenology 65, 1631-1648.

DOI: 10.1016/j.theriogenology.2005.07.020

DE WIT, A. A., Y. A. WURTH, T. A. KRUIP (2000): Effect of ovarian phase and follicle quality on morphology and developmental capacity of the bovine cumulus-oocyte complex. J. Anim. Sci. 78, 1277-1283.

DOI: 10.2527/2000.7851277x

IWATA, H., S. HASHIMOTO, M. OHOTA, K. KIMURA, K. SHIBANO, M. MIYAKE (2004): Effect of follicle size and electrolytes and glucose in maturation medium on nuclear maturation and developmental competence of bovine oocytes. Reproduction 127, 159-164.

DOI: 10.1530/rep.1.00084

KAKKASSERY, M. P., V. VIJAYAKUMARAN, T. SREEKUMARAN (2010): Effect of cumulus oocyte complex morphology on in vitro maturation of bovine oocytes. J. Vet. Anim. Sci. 41, 12-17.

LEQUARRE, A. S., C. VIGNERON, R. FABRICE, P. HOLM, I. DONNAY, R. DALBIES-TRAN, H. CALLESEN, P. MERMILLOD (2005): Influence of antral follicle size on oocyte characteristics and embryo development in the bovine. Theriogenology 63, 841-859.

DOI: 10.1016/j.theriogenology.2004.05.015

LONERGAN, P. P., D. MONAGHAM, M. RIZOS, P. BOLAND, L. GORDON (1994): Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization and culture *in vitro*. Mol. Reprod. Dev. 37, 48-53.

DOI: 10.1002/mrd.1080370107

ROMAGUERA, R., R. MORATO, A. R. JIMENEZ-MACEDO, M. CATALA, M. ROURA, M. T. PARAMIO, M. J. PALOMO, T. MOGAS, D. IZQUIERDO (2010): Oocyte secreted factors improve embryo developmental competence of COCs from small follicles in prepubertal goats. Theriogenelogy 74, 1050-1059.

DOI: 10.1016/j.theriogenology.2010.04.035

SUCCU, S., G. G. LEONI, F. BERLINGUER, M. MADEDDU, D. BEBBERE, F. MOSSA, L. BOGLIOLO, S. LEDDA, S. NAITANA (2007): Effect of vitrification solutions and cooling upon *in vitro* matured prepubertal ovine oocytes. Theriogenology 68, 107-114.

DOI: 10.1016/j.theriogenology.2007.04.035

Received: 28 January 2018
Accepted: 1 April 2019

JUODŽENTYTĖ, R., V. ŽILAITIS, G PALUBINSKAS: Određivanje optimalne veličine folikula za dobivanje najkvalitetnijih oocita za *in vitro* oplodnju. Vet. arhiv 89, 309-315, 2019.

SAŽETAK

Cilj ovoga istraživanja bio je odrediti optimalnu veličinu folikula kako bi se prikupile oocite visoke kvalitete s obzirom na visoki kapacitete za sazrijevanje, oplodnju i razvoj *in vitro*. Jajnici krava mliječne pasmine izrezani su odmah nakon klanja i prevezeni unutar sat vremena. Folikuli su izdvojeni i podijeljeni u tri skupine s obzirom na njihov promjer: u skupinu malih folikula (3 - 5 mm), skupinu folikula srednje veličine (6 - 9 mm) i skupinu velikih folikula (10 - 20 mm). Stupnjevanje kvalitete oocita (A, B, C, D) učinjeno je na temelju razvoja kumulusnih stanica i homogenosti citoplazme. Aspirirane su ukupno 284 kumulusne oocite iz 123 jajnika. Od ukupno 284 kumulusne oocite njih 236 bilo je A i B-stupnja, a 48 ih je bilo C i D-stupnja. Sazrijevanje oocita iz skupine folikula srednje veličine A i B-stupnja bilo je 56,01 % bolje od onih stupnja C i D. Samo su oocite A i B-stupnja oplođene in vitro s 1×10^6 sperme po mililitru uz upotrebu Fert-TL-a, s 0,3 % Bovine Serum Albumin-a, 22 µg/mL natrijeva piruvata i 10 µg/mL heparina. Brazdanje oocita dobivenih iz folikula male veličine bilo je znakovito niže (35,89 %) nego u skupini folikula srednje veličine (56,41 %), a slično je uočeno i u razvoju morula, neovisno o kvaliteti oocita. Zaključeno je da oocite dobre kvalitete dobivene u skupini folikula srednje veličine, oocite s više od tri cjelovita sloja kumulusnih stanica (stupnjevi A i B) imaju veće šanse za sazrijevanje in vitro i brazdanje.

Ključne riječi: *in vitro* oplodnja; oocite; kumulusne stanice; sazrijevanje; brazdanje