

OCCURRENCE OF MORPHOLOGICALLY CHANGED OOCYTES IN OVARIAN FOLLICLES OF COWS IN THE COURSE OF SEXUAL CYCLE

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Abstract

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The basic morphological features of oocytes in the course of the sexual cycle were studied in cows. The oocytes were collected from ovaries of slaughtered cows of the Czech Pied, Black Lowland Pied breeds and their crosses. The animals were 3 to 9 years old.

The oocytes were recovered from small (3–5 mm), medium size (7–9 mm) and large (10–15 mm) Graafian follicles. In the course of the cycle, the oocyte size, thickness of the zona pellucida, presence of cumulus oophorus cells and the number of degenerated oocytes were followed. The average size of the oocyte was 162.8 μm , the zona pellucida was 20.38 μm thick.

About 60 % oocytes recovered from all follicles had the cumulus oophorus preserved. The largest number of morphologically unchanged oocytes with intact cumulus oophorus was found in medium size follicles in the last third of the cycle (89.41 %). On the other hand, morphologically changed oocytes were most frequent in all sizes of follicles in the middle phase of the cycle.

These findings are in agreement with better results when using biotechnology methods in cows treated during the middle or more within the second half of the sexual cycle.

Oocyte, Graafian follicle, size, zona pellucida, cumulus oophorus.

Introduction of large-scale technologies into cattle husbandry requires the use of modern biotechnology methods of reproduction. There exists, however, a number of hitherto unresolved problems requiring an intensive research into the physiology of all phases of the sexual cycle. Already in 1960 Rajakovski called attention to the fact that the quality of oocytes in the Graafian follicles is dependent on the phase of the sexual cycle of the dams. Chowdry et al. (1968) gives average numbers of 23.7 % non-atretic and 76.3 % atretic tertiary follicles on ovaries of cows and heifers in the course of their cycles. According to Kudláč et al. (1977) in sexually mature cows 46 \pm 4.8 per cent of follicles 1–10 mm in size are atretic. Maraček et al. (1977) found 26.7 per cent of non-atretic follicles in the follicular phase of the cycle, and 21.9 per cent of non-atretic and 78.1 per cent of atretic follicles during the luteal phase of the cycle in cows.

Motlik (1973) emphasized the effect of cumular cells upon the development and maturation of oocytes. Pivko et al. (1981) conformed a close functional relationship between the oocyte and the cells of the cumulus oophorus. Kudláč et al. (1977) and Hahn (1984) have called attention to the fact that in the synchronized estrus in cattle by prostaglandins or with stimulated superovulation in donors for embryo transfer programmes the results are affected by the phase of the cycle and by the functional status of the ovaries.

The aim of the present work was to investigate the basic morphological features of oocytes in the course of the ovarian cycle in the cow.

Table 1

Follicle sizes, numbers and sizes of recovered oocytes and thickness of the zona pellucida

Follicle size in mm	Number of examined follicles	Oocytes recovered		Oocyte size in μm	Thickness of zona pellucida in μm	No. of examinations
		No.	%			
Small 3—5	971	432	44,5	\bar{x} 157,7 119,9—174,4	\bar{x} 18,6 13,2—21,8	36
Medium 7—9	834	433	51,9	\bar{x} 164,6 152,6—185,3	\bar{x} 20,6 13,2—27,2	34
Large 10—15	419	251	59,9	\bar{x} 166,2 132,4—185,3	\bar{x} 22,0 13,2—27,2	33
Total	2 224	1 116	50,2			

Table 2

Quality of oocytes recovered from small follicles

Phase of cycle (day)	No. of oocytes examined	With cumulus oophorus No. (%)	Without cumulus oophorus No. (%)	Oocytes with cumulus oophorus morphologically	
				unchanged No. (%)	degenerated No. (%)
1.—8.	95	45 (47,4)	50 (52,6)	24 (53,3)	21 (46,7)
9.—14.	141	86 (61,0)	55 (39,0)	51 (59,3)	35 (40,7)
15.—20.	112	66 (58,9)	46 (41,0)	49 (74,2)	17 (25,8)
Total	348	197 (56,6)	151 (43,4)	124 (62,9)	73 (37,0)

Table 3

Quality of oocytes from medium size follicles

Phase of cycle (day)	No. of oocytes examined	With cumulus oophorus No. (%)	Without cumulus oophorus No. (%)	Oocytes with cumulus oophorus morphologically	
				unchanged No. (%)	degenerated No. (%)
1.—8.	117	62 (53,0)	55 (47,0)	36 (58,0)	26 (41,9)
9.—14.	114	77 (67,5)	37 (32,5)	39 (50,6)	38 (49,3)
15.—20.	126	85 (67,5)	41 (32,5)	76 (89,4)	9 (10,6)
Total	357	224 (62,7)	133 (37,2)	151 (67,4)	73 (32,6)

Materials and Methods

Ovaries for oocyte collection were collected from slaughtered cows at a municipal slaughterhouse. A total of 429 ovaries from 218 cows were investigated. The animals were 3 to 9 years old and of the Czech Pied and Black Lowland Pied breeds and their crosses. Investigated were only ovaries from cows in which a physiological course of the sexual cycle could be assumed. The oocytes were investigated within 2 hours of slaughter.

The ovaries were divided according to the status of the corpus luteum and maturation of the follicles into groups corresponding to days 1 to 8, 9 to 14, and 15 to 20 of the cycle. Oocytes were collected from large (10–15 mm), medium (7–9 mm) and small (3–5 mm) follicles. The oocytes with the follicular fluid were transferred to a watch glass with a double volume of the medium (phosphate buffered physiological saline after Dulbec with 5 % bovine fetal serum). Oocytes were viewed using a stereomicroscope (magnification 6.3×1.6) and transferred into clean medium. Their quality was evaluated according to the presence of cumulus oophorus cells and intact zona pellucida. After mechanical removal of the cumulus oophorus cells the intactness of the zona pellucida and evaluation of the oocyte proper was carried out at a magnification 8×4 or 8×10 . The oocytes were measured with an ocular micrometer at a magnification of 15×10 .

The oocytes collected from the small, medium and large follicles were further divided into a group of oocytes with intact cumulus oophorus and a group with no cumulus oophorus.

Oocytes with intact zona pellucida, finely granular and evenly distributed deutoplasma were considered morphologically normal, and those with ruptured zona pellucida, unevenly distributed deutoplasma, vacuoles present in deutoplasma and various clusters of deutoplasma in the form of granules of various sizes were evaluated as morphologically changed.

Results and Discussion

The numbers of collected oocytes and their size groups are given in Table 1. The greatest recovery rates were from the large follicles (59.9 %). Examination of 407 follicles showed that the recovery rates increased with increasing amount of the follicular fluid which in turn was the largest in the group of large oocytes.

The follicular fluid amounted 0.04 to 0.08 ml in small follicles, 0.18 to 0.44 ml in medium and 0.5 to 1.66 ml in large follicles.

After mechanical removal of the cumulus cells the oocytes recovered from the three size groups of follicles were measured and the results are given in Table 1. According to Kudláč et al. (1977) the size of bovine oocytes is 130 to 170 μm and the thickness of the zona pellucida is 15 μm . Our findings are in agreement with these data except

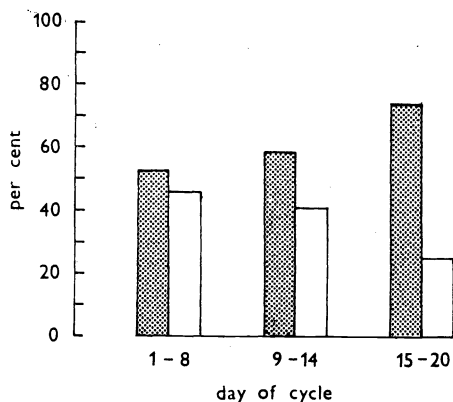


Fig 1. Morphologically unchanged (dotted columns) and degenerated (empty columns) oocytes with cumulus oophorus from small follicles

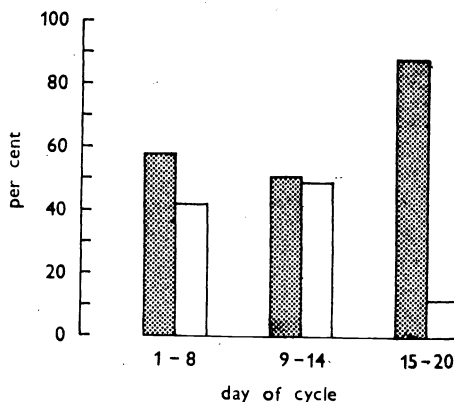


Fig 2. Morphologically unchanged (dotted columns) and degenerated (empty columns) oocytes with cumulus oophorus from medium size follicles

for the thickness of the zona pellucida which was somewhat greater in our material. In larger follicles larger oocytes with a thicker zona pellucida were found. The zona pellucida (and its sialic acid content) reportedly protects the oocyte from the autoimmune processes of the mother also after oocyte transfer (Sokolovskaya 1976). Our findings of thicker zona pellucida in oocytes from larger follicles seem to support her conclusion.

The presence of cumulus oophorus was the most important sign when evaluating quality of the oocytes as it is inevitable for maturation of the oocyte after ovulation, supports its fertilization ability and participates in the acrosomal reaction of spermatozoid (Motlík 1973).

The quality of oocytes recovered from small follicles is analyzed in Table 2 showing that from day 9 of the cycle more oocytes with cumulus oophorus were recovered than in earlier days of the cycle. Moreover, the number of morphologically unchanged oocytes with intact cumulus oophorus was highest when recovered in the middle phase of the cycle.

The quality of oocytes recovered from medium size follicles is given in Table 3 and Fig. 2. Interestingly, the number of morphologically unchanged oocytes with cumulus oophorus decreased from 58 % in the first phase of the cycle to 50 % in the middle phase, and increased sharply to 89.4 % in the last phase of the cycle.

Data summarized in Table 4 and Fig. 3 show the numbers of oocytes recovered from large follicles. In this group, the number of normal oocytes in the first phase of the cycle was extremely low and increased towards the end of the sexual cycle.

Recovery rates of normal oocytes with intact cumulus oophorus are summarized in Fig. 4.

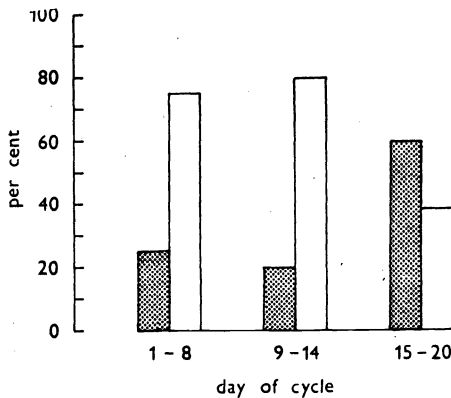


Fig. 3 Morphologically unchanged (dotted columns) and degenerated (empty columns) oocytes with cumulus oophorus from large oocytes

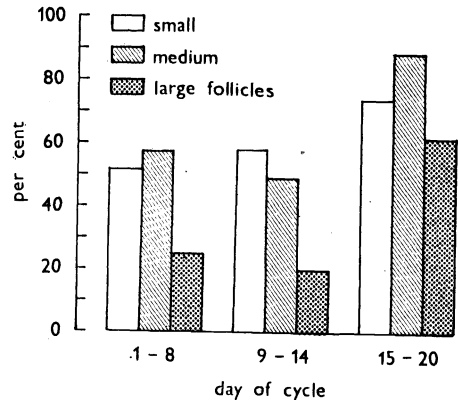


Fig. 4 Recovery rates of morphologically unchanged oocytes with cumulus oophorus in the course of the reproductive cycle

Evaluation of oocytes as far as their zona pellucida is concerned revealed that from 432 recovered oocytes damaged zona pellucida and loose deutoplasm was found in 8 oocytes (1.5 %). Among 433 oocytes from medium size follicles 22 were damaged (5.08 %). Among 251 oocytes from large follicles 11 had a ruptured zona pellucida (4.38 %). From a total number of 1 116 oocytes 41 (3.67 %) had a ruptured

zona pellucida. This finding is interesting especially in context with occurrence of sterility for unknown reasons (*sterilitas e causa ignota*) under which 5 to 10 per cent of dams are suffering (Kudláč et al. 1977).

Interesting facts emerge from the data summarized in Table 5: in each of 37 cows (20.9 %) from both ovaries 70 and more per cent of undamaged oocytes were recovered while in 42 animals (23.7 %) all oocytes were morphologically changed. The recovery rate of morphologically unchanged oocytes in the individual phases of the sexual cycle differed considerably: in the first phase 70 and more per cent of undamaged oocytes were found in 10 cows (18.2 %), in the second phase a decrease to 11.4 % but in the last phase an increase to 34.5 % (i.e. 19 animals) was observed. Also data on reverse situation, i.e. findings of morphologically changed oocytes on all occasions are of interest: in the first phase of the cycle such oocytes were found in 23.1 % of animals, in the second phase in 31.4 % and in the last phase only in 14.5 % of the cows.

Table 4
Quality of oocytes from large follicles

Phase of cycle (day)	No. of oocytes examined	With cumulus oophorus No. (%)	Without cumulus oophorus No. (%)	Oocytes with cumulus oophorus morphologically	
				unchanged No. (%)	degenerated No. (%)
1.—8.	39	16 (41,0)	23 (59,0)	4 (25,0)	12 (75,0)
9.—14.	76	49 (64,5)	27 (35,5)	10 (20,4)	39 (79,6)
15.—20.	83	53 (64,8)	30 (35,2)	33 (62,3)	20 (37,7)
Total	198	118 (59,6)	80 (40,0)	47 (39,8)	71 (60,2)

Table 5
Occurrence of undamaged oocytes with compact cumulus oophorus in cows in the course of their sexual cycle

Day of cycle	No. of animals	No. (%) of oocytes without morphological changes and with compact cumulus oophorus										
		0 %	1—10 %	11—20 %	21—30 %	31—40 %	41—50 %	51—60 %	61—70 %	71—80 %	81—90 %	91—100 %
		No. (%)	No. (%)	No. (%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1.—8.	52	12 (23,1)	0 (0)	11 (21,1)	2 (3,8)	6 (11,5)	8 (15,4)	3 (5,8)	0 (0)	7 (12,5)	0 (0)	3 (5,8)
9.—14.	70	22 (31,4)	0 (0)	5 (7,1)	6 (8,6)	14 (20,0)	12 (17,1)	1 (4,4)	2 (2,9)	4 (5,7)	0 (0)	4 (5,7)
15.—20.	55	8 (14,5)	0 (0)	3 (5,4)	4 (7,3)	4 (7,3)	8 (14,6)	1 (1,8)	8 (14,6)	11 (20,0)	0 (0)	8 (14,6)
Total	177	42 (23,7)	0 (0)	19 (10,7)	12 (6,8)	24 (13,6)	28 (15,8)	5 (2,8)	10 (5,6)	22 (12,4)	0 (0)	15 (8,5)

Considering the two growth phases of the follicles in ovaries and the data on degeneration and atresia of follicles (Kudláč et al. 1977; Horský and Presl 1978), it seems that in the first growth phase the occurrence of follicles with defective oocytes is greater than in the second growth phase. These findings are in agreement with data of Kudláč et al. (1977) and Hahn (1984) who obtained better results in oestrus synchronization and superovulation, higher recovery rates of early embryos for transfer and better impregnation results of recipients along with higher conception rates in oestrus synchronized with prostaglandins in cows that had been in the middle or late phase of the sexual cycle at the time of medication.

Výskyt morfologicky změněných oocytů ve folikulech na ovariích krav v průběhu pohlavního cyklu

V rámci studia fyziologie reprodukčního cyklu u skotu byly sledovány některé základní morfologické charakteristiky oocytů v průběhu ovariálního cyklu. Oocyty byly získány z vaječníků po porážce krav plemen české strakaté, černostrakaté nížinné a jejich kříženců ve stáří 3—9 roků. Po získání oocytů z malých (3—5 mm), středních (7—9 mm) a velkých (10—15 mm) folikulů bylo provedeno jejich posouzení. Byla sledována velikost oocytů, tloušťka zona pellucida, přítomnost kumulárních buněk a procento degenerovaných oocytů v průběhu ovariálního cyklu. Průměrná velikost oocytů činila 162,8 μm , tloušťka zona pellucida 20,38 μm .

Nález oocytů se zachovalým cumulus oophorus byl kolem 60 % u všech folikulů. Nejvíce morfologicky nezměněných oocytů se zachovalým cumulus oophorus jsme nacházeli u středních folikulů v poslední třetině cyklu (89,41 %), naopak nález morfologicky změněných oocytů byl nejvyšší u všech velikostních skupin folikulů uprostřed cyklu.

Tato zjištění by odpovídala i lepším výsledkům dosahovaným při použití biotechnických metod u krav, které byly v době ošetření uprostřed nebo spíše ve druhé polovině pohlavního cyklu.

Наличие морфологически измененных ооцитов в фолликулах яичников коров в процессе полового цикла

В рамках исследований физиологии цикла репродуктивности крупного рогатого скота изучались некоторые основные морфологические характеристики ооцитов в ходе овариального цикла. Ооциты были получены из яичников после убоя коров племени чешской пеструшки, чернопестрого низменного племени и их помесей в возрасте 3—9 лет. Получив ооциты из небольших (3—5 мм), средних (7—9 мм) и крупных (10—15 мм) фолликулов, проводили их оценку. Изучались размеры ооцитов, толщина зона pellucida, присутствие кумулярных клеток и процент дегенерированных ооцитов в ходе овариального цикла. Средние размеры ооцитов достигали 162,8 мкм, толщина зона pellucida — 20,38 мкм.

Наличие ооцитов с сохранившимся cumulus oophoricus достигало около 60 % у всех фолликулов. Больше всего морфологически не измененных ооцитов с сохранившимся cumulus oophoricus находили у средних фолликулов в последней трети цикла (89,41 %), самое большое количество морфологически измененных ооцитов находили у всех групп фолликулов в середине цикла.

Данные результаты соответствовали бы также лучшим результатом, достигаемым биохимическими методами у коров, находящихся в период ухода в середине или скорее во второй половине полового цикла.

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