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REGULAR ARTICLE

OVARIAN FOLLICLE ATRESIA IN DAIRY COWS IN RELATION TO THE BODY CONDITION

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ABSTRACT

The aim of the study was to define types of atresia in cow ovarian follicles in relation to their body condition. The ovaries were recovered from slaughtered cyclic Czech Fleckvieh dairy cows of three body condition scores: BCS1 (emaciation), BCS2 (tendency to emaciation) and BCS3 (average body condition status), classified according to a 5-point scale of BCS. The ovarian tissue sections were processed for histological analysis and stained with basic fuchsine and toluidine blue. For acidic mukopolysacharides a combination of PAS-technique with Alcian blue was used. In cows with BCS 1 the percentage of ovulated follicles was decreased up to 19% compared to the BCS 2 (76.6%) or BCS 3 (68.7%) animals. Among the non-ovulated follicles the ratio of cystic atresia was highly increased in cow with BCS 1 and 2 compared to the BCS 3. Also, the ratio of atresia associated with luteinization was substantially higher in cows with lower BCS grade (BCS 1), when compared with cows with average body condition status (BCS 3). No significant differences in the occurrence of obliterative atresia among cows with different BCS grades were observed. Higher occurrence of acidic mukopolysaccharides in small and large antral follicles from cows with lower BCS

(1 and 2) was revealed. Our observations indicate that in emaciated dairy cows the most frequent forms of follicle degeneration are cystic atresia and atresia with luteinization.

Key words: cattle, emaciation, body condition score, ovarian atresia

INTRODUCTION

Follicular atresia affects all steps of follicle development in cow ovaries and manifested by the arrest in development and follicle degeneration. Degenerative changes affect cells of membrane granulosa, theca, and their blood capillaries, as well as the basal membrane and ovum (**Irving-Rogers et al., 2009**). Antral follicles can be succumbed to preterm luteinization or to transform into luteal cysts. Estrous cycle, however, has regular running, or eventually has small abnormalities like anovulation. An occurrence of such atresia in the follicles with the oocytes can be increased under unfavourable life conditions, in particular at starvation.

Most critical period for dairy cows from the point of energy requirements is a period of early lactation, when negative energy balance may occur **(Butler, 2000**). The difference between energy needs and their supply with a feed of cows is compensated by a release of body reserves. This process positively affects productivity but, at the same time, is a predisposition factor for the ketosis and disorders in reproductive functions, which may lead to follicular atresia.

Body condition of dairy cows may affect their reproductive parameters: time of oestrus onset, conception rate and embryonal mortality (**Silke et al., 2002**). Body condition can be evaluated by five BCS (body condition score) grades. The scale from 1 to 5 is commonly used for dairy cattle and the scores have up-warding tendency according to the amount of fat reserves measured mostly over ribs, near tale base, in pelvic and hip area and using this approach body reserves can be determined (**Butler et al., 1981; Butler, 2000**). According to **Edmonson et al. (1989)** the BCS grades are defined as follows: the grade 1 – emaciation; the grade 2 – tendency to emaciation; the grade 3 – average body status; the grade 4 – tendency to adiposity; the grade 5 - adiposity.

Many authors studying the problematics of ovarian follicle atresia attempted to characterize follicles basing on changes in some cell structures. Basic categorization has been done by **Marion et al. (1968)**, who in the micromorphological study described bovine ovarian follicle system in relation to different forms of atresia. However, deep insight into morphological changes in ovarian follicle structure within individual grades of BSC is lacked.

The aim of the study was to define most common forms of degenerative alterations in ovarian antral follicles from dairy cows with different body condition.

MATERIAL AND METHODS

Biological material

As a source of biological material, the ovaries were recovered at slaughtering of cyclic dairy cows (n= 36) of Czech Fleckvieh breed at different times of the *post-partum* period. The cows had no pathological changes on sexual organs and were kept under normal feed regime. The animals were estimated as belonging into certain grade of body condition score (BCS) according to 5-point scale of BCS (Edmonson et al., 1989). For our experiment only dairy cows of BCS1 (emaciation; n= 9), BCS2 (tendency to emaciation; n= 13) and BCS3 (optimal body condition status; n= 14) were available. The data on these cows were taken from cow´s individual cards in farms and are following: average age - 6.2 years, 4.1 years and 5.7 and milk yield – 8814.7, 7937.3 and 7744.2 for BCS1, BCS2 and BCS3 respectively.

Histological analyses

For histological analysis ovarian samples were fixed in 10% neutral buffered formalin (Sigma-Aldrich), dehydrated in rising set of ethanol solutions (70% and 96% for 2 hours and 100% for 1 hour) and embedded into Technovit 7100 resin (Heraeus GmbH, CoKG, Werheim/Ts., Germany) according to producer’s manual. For light microscopy 1-5 µm sections were cut using AC-820 rotation microtome (American Corporation, USA) and stained with buffered basic fuchsine with toluidine blue in the ratio 3:2 prior to use (Bourne and St. John, 1978). For acidic mukopolysacharides (aMPS) a combination of PAS-technique with Alcian blue staining (Merck, Darmstadt, Germany) was used. Stained sections were mounted into Entelan and analyzed under a Jenaval light microscope (Carl Zeiss, DDR).

Atresia of antral follicles in histological specimens was evaluated on the basis of histopathological image of altered non-ovulated follicles, classified to the following four categories according to McKenzie and Kenney (1973) with partial modifications:

- Three forms of atresia without luteinization: (1) atresia of a first instance (initial atresia); (2)

obliterative atresia; (3) cystic atresia;

- Atresia associated with luteinization: (4) initial atresia with luteinization of granulosa and theca cells.

Statistics

The data for histopathological changes in non-ovulated antral follicles were statistically evaluated by Chi-square test using procFreq software (SAS/STAT statistical package). At the comparison of differences between different BCS grades a zero hypothesis was cancelled.

RESULTS

Using morphological assessment the following histopathological alterations characterizing atresia of antral follicles in comparison to follicles without changes have been observed (Fig. 1A). Initial form of atresia is characterized by degenerative changes in the nuclei of granulosa cells, more often in form of pyknosis on the surface and each layers of granulosa cells (Fig. 1B) and on the basal membrane (Fig. 1 C,D). The basal membrane of atretic follicles becomes separated from the basal layer of granulosa cells; it is swollen and often forms visible folds and undulations (Fig. 1 C, D). The apoptotic bodies are present in both basal and surface layers of *membrana granulosa* of antral follicles (Fig. 1 C, D). Following initial changes, obliterative or cystic form of atresia may be occurred, which lead to destruction of granulosa and theca cells.

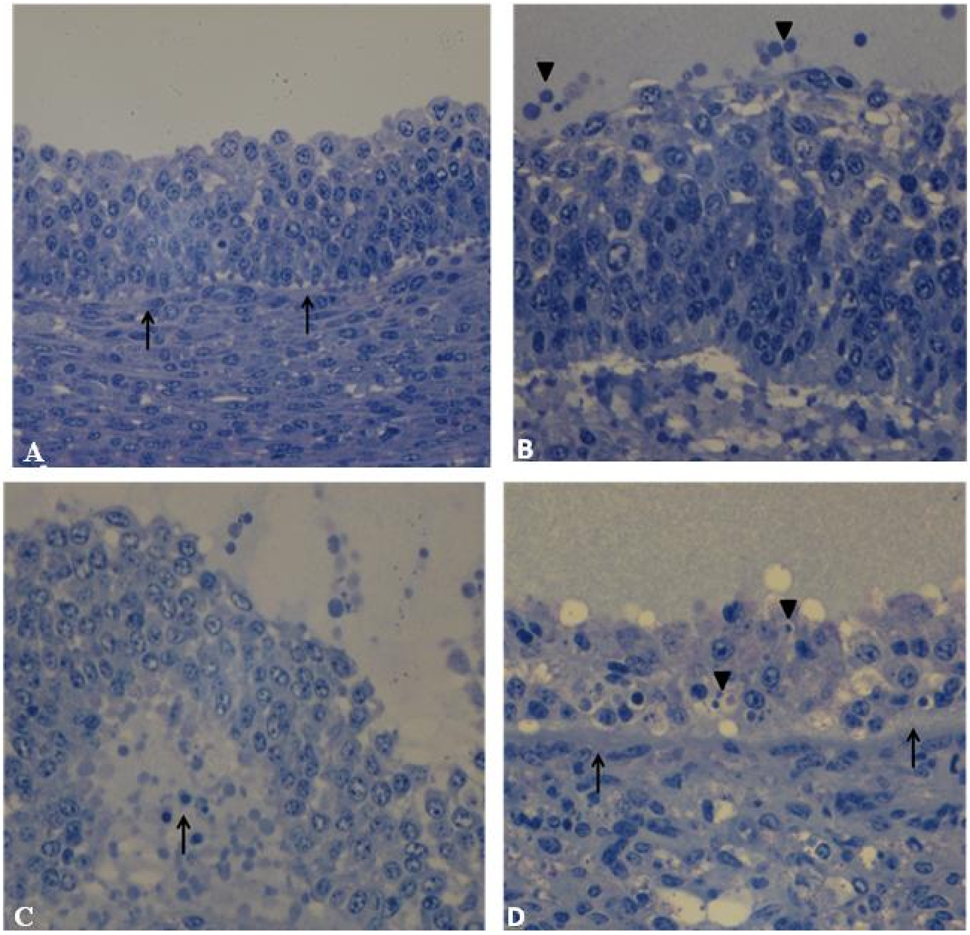

Figure 1 Forms of initial atresia in cow ovarian follicles. A – tertiary (Graafian) follicle of the control cow. Follicular multilayered epithelium of *membrana granulosa* which is formed by follicular cells and by external and internal theca cells without histopathological changes. The lower cell layer is settled on a thin basal membrane – *membrana propria* (arrows). **B** - atrophy of follicular cells in surface layers of the antral follicle *membrana granulosa* at initial stage of atresia (arrowheads). **C –** waived *membrana granulosa* with edematosis in the basal membrane and occurrence of apoptotic bodies (arrows). **D** – swollen basal membrane (arrows), necrosis of follicular cells in the basal and surface layers of *membrana granulosa* and the apoptotic body occurrence (arrowheads). Magnification: x 500.

Obliterative form of atresia is characteristic by a loss of intrafollicular pressure. Granulosa cells underwent degeneration and theca cells are surrounded by fibrotic connective tissue. The final step is a hyalinization of g*ranulosa* cells (the wall) and ingrowths of connective tissue cells into the formed cavity (Fig. 2 A).

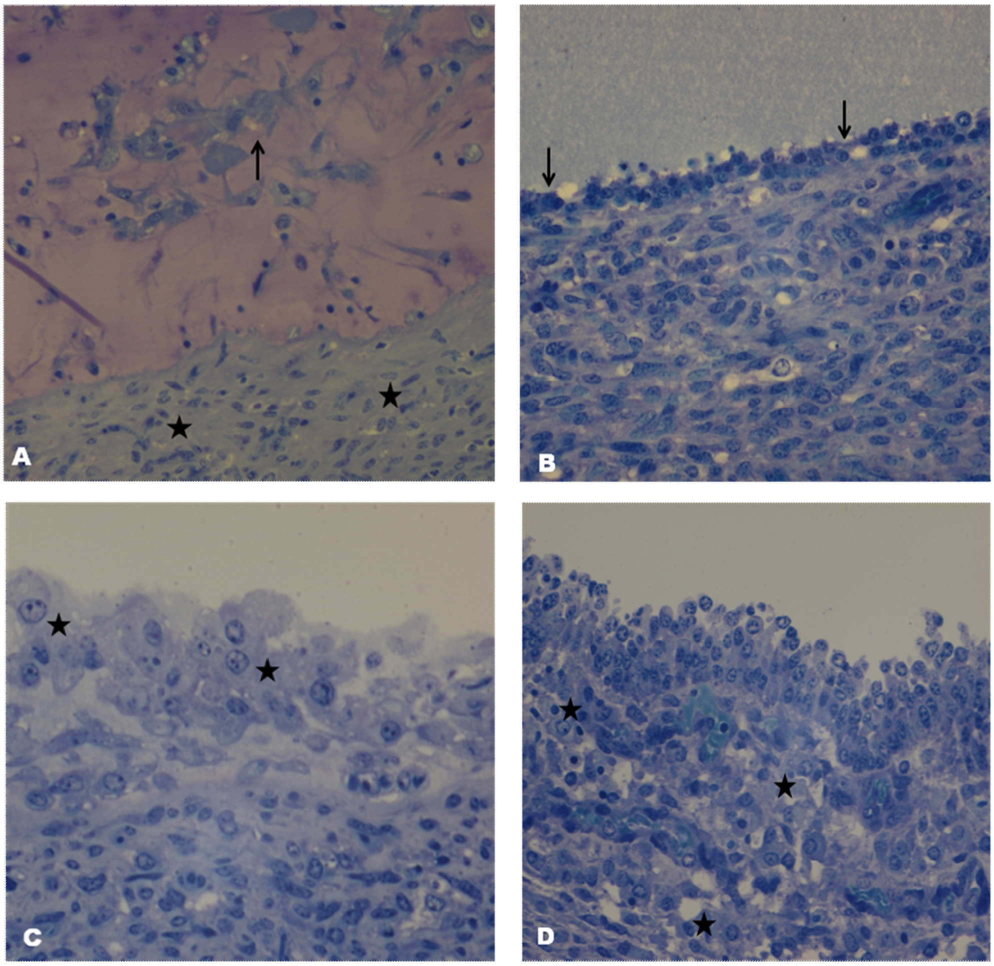

Figure 2 Atretic changes in cow ovarian follicles. **A** – obliterative atresia - numerous fibrotic tissue cells (asterisks) surrounding follicular cell remnants that are subjected to massive necrosis (arrow). Mukopolysaccharides in the extracellular matter filling the cavity of obliterating follicle (violet colour) after staining with basic fuchsine and toluidine blue. **B** – cystic atresia of the antral follicle with the epithelioid cell layer (arrows). **C** – atresia of the antral follicle associated with luteinization of granulosa cells (asterisks). **D** - atresia of the antral follicle associated with luteinization of both *theca interna* and *theca externa* cells (asterisks). Magnification: x 500.

Characteristic sign of cystic atresia is a loss of granulosa cells and partial loss of theca epithelial cells. The cavity is filled with a fluid under pressure. Cystic atresia affects the follicles larger than 2 mm, and this form of atresia should be distinguished from cystic degeneration, which damages follicles larger than 20 mm in diameter. Formation of follicular cyst is complete when the granulosa basal layer is transformed into epithelial layer (Fig. 2 B).

Atresia associated with luteinization is characterized by hypertrophy of granulosa and theca epithelial cells, which leads to a local rupture of the basal membrane and a disturbance of regular layout of granulosa cells. The loss of intrafollicular pressure leads to a cross mixing of theca and granulosa cells (Fig. 2 C, D).

The occurrence of atrophic changes in the non-ovulated ovarian follicles was dependent on a body condition score of cows. Thus, the percentage of ovulated follicles was decreased in cows with lowest body condition score, however the percentage of non-ovulated follicles was increased in the BCS 1 (81.0%) compared to the BCS 2 (76.6%) or BCS 3 (68.7%) animals. In the non-ovulated follicles the ratio of cystic atresia was highly increased in cow with BCS 1 (14.1%) and 2 (9.5%) compared to the BCS 3 (7.2%). Also, the ratio of atresia associated with luteinization was substantially increased in cows with lower BCS grade (33.7% and 26.1% for BCS 1 and 2 resp.), when compared with cows with average body condition status (BCS 3, 20.9%). No significant differences in the occurrence of obliterative atresia among cows with BCS 1 (32.6%), BCS 2 (36.9%) and BCS3 (37.7%) grades were observed.

At subjective evaluation of histochemical reaction using PAS-technique with Alcian blue higher occurrence of acidic mukopolysaccharides in small and large antral follicles in cows with lower BCS (1 and 2) compared to cows of BCS 3 was observed (Table 1). In several cases acidic mukopolysaccharides were revealed in the cavity of follicles subjected to obliterative atresia.

Table 1 Intensity of occurrence of acidic mukopolysaccharides (aMPS) in small and large antral ovarian follicles of cows with different BCS

Antral follicles	Body condition score of cows		
	1	2	3
small	+++	+++	++
large	+++	++	+

small follicle with initial antrum formation; large follicle with expanded antrum and differentiated *cumulus oophorus*; intensity of aMPS occurrence: „+“ - small; „++“- moderate; „+++“ - abundant

DISCUSSION

The issue of ovarian follicle atresia was studied earlier by several authors in attempting to characterize changes in some cell structures of ovarian follicles. Basic categorization has been done by **Marion et al. (1968),** who in the micromorphological study described bovine ovarian follicle system in relation to different grades of atresia. McKenzie and Kenney (1973) studied histological structure of ovarian follicles in heifers after gonadotropin injections and they characterized seven grades of atresia. Later, **Irving-Rodgers et al. (2001)** described two types of atresia: antral and basal atresia.

Using histological analysis we have detected signs of atresia in the surface and basal cells of *membrana granulosa*, which are similar to those described earlier (**Irving-Rodgers et al., 2001)**. We assume that these changes are preceded by morph-functional changes in the basal membrane, which in most cases has signs of waving and edematous dilation. It is supposed that physical-chemical properties of the basal membrane are subjected to changes, which lead to the alteration of their permeability.

Atretic changes affect granulosa cells of both antral and non-antral follicles. Necrotic changes are manifested mostly by pyknotic nuclei and fragmented cytoplasm (**Telfer, 1997).** Apoptotic changes in granulosa cells occur only in the antral follicles (**Yuan and Giudice, 1977)**. The other sign of atresia is increase in intercellular spaces (Peluso et al., 1980) and loss of gap junctions between the cells **(Merk et al., 1973).**

In our study all non-ovulated antral follicles were subjected to different forms of atresia, what corresponds to the findings of **McKenzie and Kenney (1973)**. However, atretic changes were detected not only in large antral follicles but also in small follicles basing on the presence of acidic mukopolysaccharides (aMPS). It is known, that accumulation of aMPS in ovarian follicular fluid accompanies follicular growth, and their amount may associate with follicular atresia **(Ax and Ryan, 1979)**. The naturally occurring MPS in biological samples are proteoglycans. In ovarian follicles, classified as atretic by morphological and steroidal criteria, the concentrations of proteoglycans in the follicular fluid are higher than in healthy follicles **(Salustri et al., 1999).** Therefore, histochemical evidence of aMPS is proper tool for early determination of atretic changes both in the antral and non-antral follicles (**Ax and Ryan, 1979; Salustri et al., 1999)**.

Increased occurrence of acidic mukopolysaccharides detected in small atretic follicles of emaciated dairy cows in our study just confirms already known results. The proportion of ovulated follicles in cows with BCS 1 and 2 in our study was substantially decreased, whilst the proportion of cystic atresia or luteinization-related atresia was subsequently increased.

Increased presence of antral follicle atresia with luteinization in dairy cows with a tendency to emaciation (BCS 1 and 2) may be caused by pre-term action of luteinization-regulated serum factors when permeability of the basal membrane of atretic follicles is changed. The other cause may be an incorporation of capillaries of the basal membrane of atretic follicles into spaces among granulosa cells **(Murphy, 2000).**

The effect of cow's body condition, evaluated by the BCS scale 1-5, on fertility of dairy cows is known **(Silke et al., 2002).** The loss of one point in this scale during first five weeks *post-partum* can lead to lengthening the onset of oestrus, lower conception rate and higher rate of embryonal mortality compared to dairy cows with a normal condition. Thus, the ratio of oocytes developed to blastocysts in dairy cows with BCS 1.5-2.5 is lower when compared to BCS 3.3-4.0 cows. Previous reports described histopathological alterations in ovarian folicles either from gonadotropin-treated **(McKenzie and Kenney, 1973)** or untreated (basal conditions; **Marion et al., 1968; Irving-Rodgers et al., 2001; 2009)** heifers or cows. A new aspect of our study is that we present histopathological characterization of antral ovarian follicles in relation to body conditions of dairy cows with a tendency to emaciation

In dairy cows of BCS1 and 2 compared to BCS3 cows, a higher occurrence of non-ovulated antral follicles was observed. Most of them were subjected to cystic atresia or atresia with luteinization. One of possible explanations may be a decreased gonadotropic activity and insufficient LH pulse frequency in cows with a tendency to emaciation at post-partum period, what causes atresia rather than ovulation **(Savio et al., 1990)**. Therefore, we may assume that levels of endogenous gonadotropins in cows both with a tendency to emaciation (BCS 2) or in emaciated (BCS 1) cows are insufficient for ovulation of follicles, which then subjected to different forms of atresia.

In conclusion, our observations indicate that in dairy cows with a tendency to emaciation (BCS 1 and 2) the occurrence of cystic atresia and atresia with luteinization is elevated, whilst initial atresia is less occurred compared to cows with average body condition.

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