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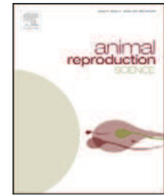

Heat shock protein patterns in the bovine ovary and relation with cystic ovarian disease

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ABSTRACT

The present study was performed to determine how the development of cystic ovarian disease (COD) affecting the ovarian expression of heat shock proteins (HSP) in cows were expressing extrous cycles. HSP27, HSP60, HSP70 and HSP90 were evaluated in different ovarian components by Western blot and semiquantitative immunohistochemical analysis. Greater expression of the HSP27 gene was detected in the granulosa and theca cells of primary, secondary, tertiary and cystic follicles, with decreasing amount in atretic follicles. HSP60, HSP70 and HSP90 showed a similar pattern of immunostaining, with moderate gene expression in primary and secondary follicles, increased expression in tertiary and atretic follicles with the greatest gene expression in cystic follicles. HSP were also localized in the corpus luteum, corpus albicans, interstitial tissue and tunica albuginea. The relative amount of protein in the follicular wall of small and large healthy follicles and cystic follicles as analysed by Western immunoblot was consistent with the immunohistochemical data. We speculate that altered expression of HSP genes decreases apoptosis in the follicular wall and leads to the delayed regression of cystic follicles. This study supports earlier observations suggesting that aberrant HSP gene expression, observed in cells of the cystic follicles, is probably associated with the intra-ovarian component of COD pathogenesis.

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1. Introduction

Heat shock proteins (HSP) are the products of several distinct gene families that are named according to the approximate relative molecular weight of their encoded proteins: HSP100, HSP90, HSP70, HSP60, HSP40 and the small heat shock proteins (HSP25/27) (Beere, 2004). HSP are involved in many cell functions and act as molecular chaperones interacting with diverse protein substrates to assist in their folding (Lindquist and Craig, 1988; Ellis, 1993; Welch, 1993).

In the context of reproduction, HSP participate in two key processes of ovarian physiology, being involved in the proliferation/apoptotic mechanisms and the action of the steroidal hormones mediated by their receptors. These biological processes are very important in whole ovarian physiology and particularly for follicular development and both have been implicated in the pathogenesis of cystic ovarian disease (COD) (Isobe and Yoshimura, 2007; D'Haeseleer et al., 2005; Salvetti et al., 2007, 2008; Ortega et al., 2009).

Many studies indicate that chaperones and stress proteins are involved in the regulation of cell growth and

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transformation (Helmbrecht et al., 2000). Generally, the prevalence of chaperones/HSP is increased in proliferating cells compared to those in the stationary state or differentiated cells (Wu and Morimoto, 1986; Hensold and Houseman, 1988; Murakami et al., 1991; Sainis et al., 1994; Helmbrecht and Rensing, 1999). In relation to apoptotic cell death, which mediates the elimination of damaged or unwanted cells, however, the underlying ability of HSP to maintain cell survival correlates with the inhibition of the mechanism of caspase activation (Garrido et al., 1999; Beere et al., 2000; Mosser et al., 2000; Beere, 2004). Because proliferation and apoptosis are part of the normal follicular development and atresia process, Isobe and Yoshimura (2007) assumed that disorders in the balance between cell proliferation and apoptosis may be associated with cystogenesis.

The ovarian steroid hormones perform several important functions related to reproduction through endocrine and paracrine mechanisms of action. The genomic effects of these hormones are mediated through interaction with specific intracellular receptors that are members of the nuclear receptor families (Ortega et al., 2009). In the ovary, sex steroids are important intra-ovarian regulators of follicle development, and disruption of their actions has been reported in cows with COD (Salvetti et al., 2007). Most of these receptors bind to a specific DNA sequence only after binding to ligands, and in the absence of the ligand, steroid receptors are held in a large multi-subunit complex containing HSP (Brosens et al., 2004).

Although the possible participation of HSP in the molecular mechanism of pathogenesis of several diseases (Yeyati and van Heyningen, 2008; Hirsh and Junger, 2008; Milioti et al., 2008; Saluja and Dudeja, 2008; Romanucci et al., 2008), including experimental models of cystic ovaries (Salvetti et al., 2008) has been established, no previous integral study on the expression of HSP members in bovine ovary was found. Thus, the present study was performed to determine how the presence of COD affects the expression of HSP in cows expressing estrous cyclic patterns. HSP27, HSP60, HSP70 and HSP90 were evaluated by Western blot (WB) and semiquantitative immunohistochemical (IHC) analysis, allowing the localization and quantification of the different proteins analyzed in each ovarian component, to determine any association with healthy follicles or cysts. The potential functional significance of the differential amount of HSP during follicular development and cystogenesis is discussed.

2. Materials and methods

2.1. Collection and preparation of tissues

Ovaries with normal ovaries ($n = 20$) and with spontaneous cystic follicles ($n = 10$) were collected at a local abattoir, within 20 min of death, from mixed breeds of *Bos taurus* cows. All cows were visually assessed as being non-pregnant, without macroscopic abnormality in the reproductive system, and were transported immediately to the laboratory. During dissection, the follicular diameter was measured with callipers and follicular fluid from each follicle was aspirated and stored separately at −20 °C.

Follicles were classified according to the criteria listed in the *Nomina Histologica* (International Committee on Veterinary Histological Nomenclature 1994) into the following groups: secondary (preantral), tertiary (antral), atretic, and cystic follicles (Silvia et al., 2002).

For WB, healthy follicles (i.e. well vascularized and with a transparent follicular wall and fluid) were dissected from normal ovaries and classified as small (<5 mm) and large (>10 mm) follicles (Parrott and Skinner, 1998). Follicular cysts were defined as follicles larger than 20 mm in diameter, in the absence of a functional corpus luteum (CL) in either the right or left ovary (Silvia et al., 2002; Vanholder et al., 2006). Tissues of follicular wall (intact theca and granulosa tissues) from these follicle types were immediately frozen at −80 °C until used in WB.

For histological studies, additional ovary sections were fixed in 4% buffered formaldehyde for 8 h at 4 °C and then washed in phosphate buffered saline (PBS, Cicarelli, Argentina). For light microscopy, fixed tissues were dehydrated in an ascending series of ethanol, cleared in xylene and embedded in paraffin. Four-micrometre thick sections were mounted on slides previously treated with 3-aminopropyltriethoxysilane (Sigma–Aldrich, St. Louis, MO, USA) and were either stained with haematoxylin–eosin for a preliminary observation of all ovarian structures, or for use in IHC procedures. The cystic follicles were evaluated macroscopically, histologically and hormonally and only those without signs of luteinization were used in this study (Ortega et al., 2007, 2008).

2.2. Immunohistochemistry for HSP27, HSP60, HSP70 and HSP90

The amount of protein of each HSP was detected with monoclonal or polyclonal antibody using appropriate dilutions (Table 1). The streptavidin–biotin immunoperoxidase method was performed as described previously (Salvetti et al., 2008; Ortega et al., 2009). Briefly, sections were deparaffinized, hydrated, and given microwave pretreatment for antigen retrieval. The endogenous peroxidase activity was inhibited with 1% H_2O_2 and nonspecific binding was blocked with 10% normal goat serum. All sections were incubated with the diluted primary antibody for 16–18 h at 4 °C and then for 30 min at room temperature with secondary antibody. An anti-mouse biotinylated antibody diluted 1:50 (Chemicon, Temecula, CA, USA) was used for HSP70 and HSP90 IHC and an anti-rabbit biotinylated antibody diluted 1:200 (Zymed, San Francisco, CA) was used for HSP27 and HSP60 IHC. Visualization of the antigen was achieved by the streptavidin-peroxidase method (BioGenex, San Ramon, CA) and 3,3-diaminobenzidine (DAB, Liquid DAB-Plus Substrate Kit, Zymed, San Francisco, CA) was used as the chromogen. Finally, the slides were washed in distilled water, counterstained with Mayer's haematoxylin, dehydrated, and mounted. To verify specificity, adjacent negative control sections were subjected to the same IHC method, replacing the primary antibody with mouse or rabbit non-immune serum. To exclude the possibility of non-suppressed endogenous peroxidase activity some sections were incubated with DAB reagent alone. Serial sections of similarly processed tissue samples of

Table 1
Antibodies including clone, source and dilution used for immunohistochemistry (IHC) and Western blot (WB) analysis of heat shock proteins (HSP27, HSP60, HSP70 and HSP90).

Antibodies	Clone	Source	Dilution IHC	Dilution WB
Primary antibodies				
HSP27	Polyclonal	Abcam, Cambridge, MA, USA	1:600	1:1500
HSP60	Polyclonal	Abcam, Cambridge, MA, USA	1:400	1:500
HSP70	BRM22	BioGenex, San Ramon, CA, USA	1:200	1:500
HSP90	AC88	Abcam, Cambridge, MA, USA	1:400	1:500
Actin	JLA20	Developmental Studies Hybridoma Bank, Iowa, IA, USA		1:50
Secondary antibodies				
Biotinylated anti-rabbit IgG	Polyclonal	Zymed, San Francisco, CA, USA	1:100	–
Biotinylated anti-mouse IgG	Polyclonal	Zymed, San Francisco, CA, USA	1:100	–
Anti-rabbit IgG peroxidase	Polyclonal	Amersham, Buckinghamshire, UK	–	1:500
Anti-mouse IgG peroxidase	Polyclonal	Amersham, Buckinghamshire, UK	–	1:500

ovaries were used as positive controls in each assay to normalize the image analysis.

2.3. Western blotting

Details of antibodies used are summarized in Table 1. Ovarian tissues from small and large healthy tertiary follicles and cystic follicles were homogenized in a radioimmunoprecipitation assay lysis buffer consisting of 1% (v/v) IGEPAL CA630 (octylphenyl-polyethylene glycol), 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 1 mM EDTA, 50 mM sodium fluoride (all from Sigma–Aldrich Corp., St. Louis, MO, USA), 0.1 M PBS and a protease inhibitor cocktail (Complete Mini Protease Inhibitor Cocktail Tablets, Roche, Mannheim, Germany). Ovarian homogenates were centrifuged at $12{,}000 \times g$ for 20 min and the protein concentration in the supernatants was estimated using fluorescence methods (Qubit™, Invitrogen). Sixty micrograms of protein, along with prestained molecular weight markers (Bio-Rad, Hercules, CA, USA), were separated by SDS-PAGE (12% resolving gel). Proteins were transferred to nitrocellulose membranes (Amersham, Buckinghamshire, UK), blocked for 1 h 30 min in 5% (w/v) non-fat milk in Tris-buffered saline (TBS) containing 0.05% (v/v) Tween 20 (Sigma–Aldrich Corp., St. Louis, MO, USA), and then incubated overnight at 4 °C with specific primary antibodies (Table 1). Following washing, membranes were treated for 1 h with corresponding secondary peroxidase-conjugated antibody. An anti-mouse antibody diluted 1:500 (Amersham, Buckinghamshire, UK) was used for HSP70 and HSP90 immunoblotting; and an anti-rabbit antibody diluted 1:500 (Amersham, Buckinghamshire, UK) was used for HSP27 and HSP60 immunoblotting. Immunopositive bands were visualized with a chemiluminescent detection kit (ECL, Amersham, Buckinghamshire, UK), using ECL film (Amersham, Buckinghamshire, UK), and were subsequently scanned into a computer.

2.4. Image analysis

Image analysis was performed using the Image Pro-Plus 3.0.1 system (Media Cybernetics, Silver Spring, MA, USA). Images were digitized by a CCD color video camera (Motic 2000, Motic China Group, China) mounted on top of a conventional light microscope (Olympus BH-2, Olympus Co., Japan) using an objective magnification of 40×. Image resolution was set to 1200 × 1600 pixels. Each pixel of the image corresponded to 0.13 μm at the respective magnification and each field represented a tissue area of 0.031 mm^2. The system captured each image and automatically corrected for the background. This prevented differential readings due to different lighting conditions. No further image processing was done.

The methodological details of image analysis as a valid method for quantifying amounts of protein have been described previously (Wang et al., 1999, 2000; Ortega et al., 2007, 2009; Salvetti et al., 2007, 2008).

To obtain quantitative data regarding IHC staining of HSP27, HSP60, HSP70 and HSP90 in the follicular wall, at least three sections for each specimen and antibody were evaluated. Primary, secondary, tertiary and atretic follicles were evaluated in healthy animals and only cystic structures were analyzed in cystic ovaries. The average density for each antibody reaction was calculated from at least 20 images of each area (granulosa and theca cells) in each slide as a percentage of the total area evaluated through color segmentation analysis, which extracts objects by locating all objects of a specific color (brown stain).

For the WB method, individual bands were quantified directly from exposed films by densitometry and the relative expression was determined using actin as the loading control.

2.5. Statistical analysis

A statistical software package (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL, USA) was used to perform the statistical tests. The statistical significance of differences between groups of data was assessed by one-way ANOVA, followed by Duncan's multiple range tests. The significant level was set at $P < 0.05$.

3. Results

Ovaries from normal cows contained follicles at various stages of development, including primary, secondary and tertiary follicles, corpora albicans and CL, as well as follicles with different degrees of atresia. In cows with COD, small follicles at early stages of development were observed, in addition to others showing evidence of atre-

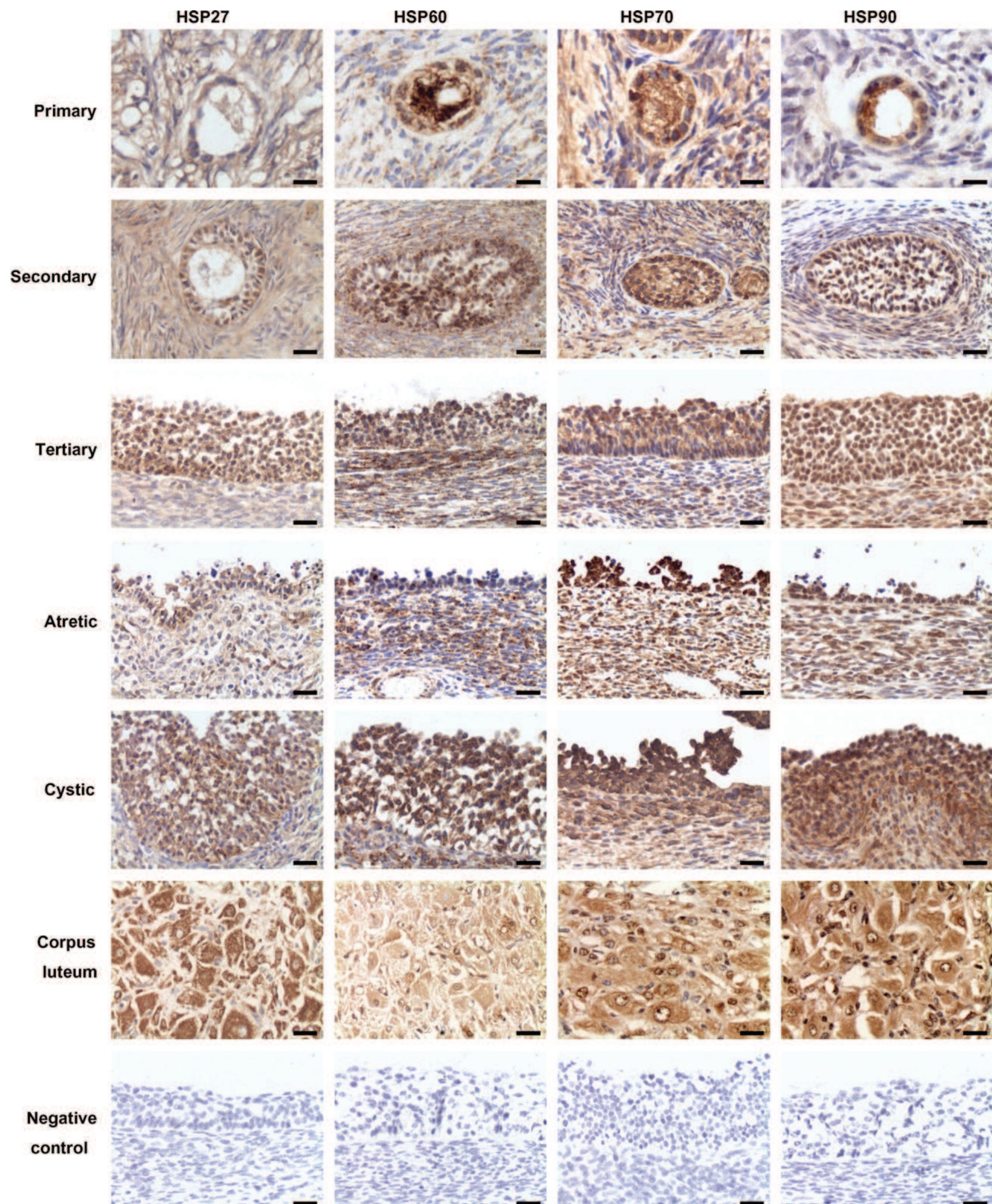

Fig. 1. Representative images of heat shock proteins (HSP27, HSP60, HSP70 and HSP90) immunostained in primary, secondary, tertiary, atretic and cystic follicles and corpus luteum. Negative control sections were subject to the same immunohistochemical method replacing primary antibodies by mouse and rabbit non-immune serum. Bar = 25 μm (except in primary = 20 μm).

sia, and one or two large cystic structures with a thickened or absent granulosa cell layer. A CL was absent in all COD cases.

A summary of the IHC expression of the different HSP is given in Figs. 1 and 2. The reactions were negative in all negative controls and positive in the positive tissue controls.

HSP27 was detected in the cytoplasm of almost all cells that comprise the ovary (Figs. 1 and 2). HSP27 was detected using IHC in the granulosa cells of primary, secondary and tertiary follicles, with significantly ($P < 0.05$) more staining in cystic follicles and less staining ($P < 0.05$) in atretic and primary follicles. A similar pattern was observed in the theca cells of secondary follicles and cells of the theca

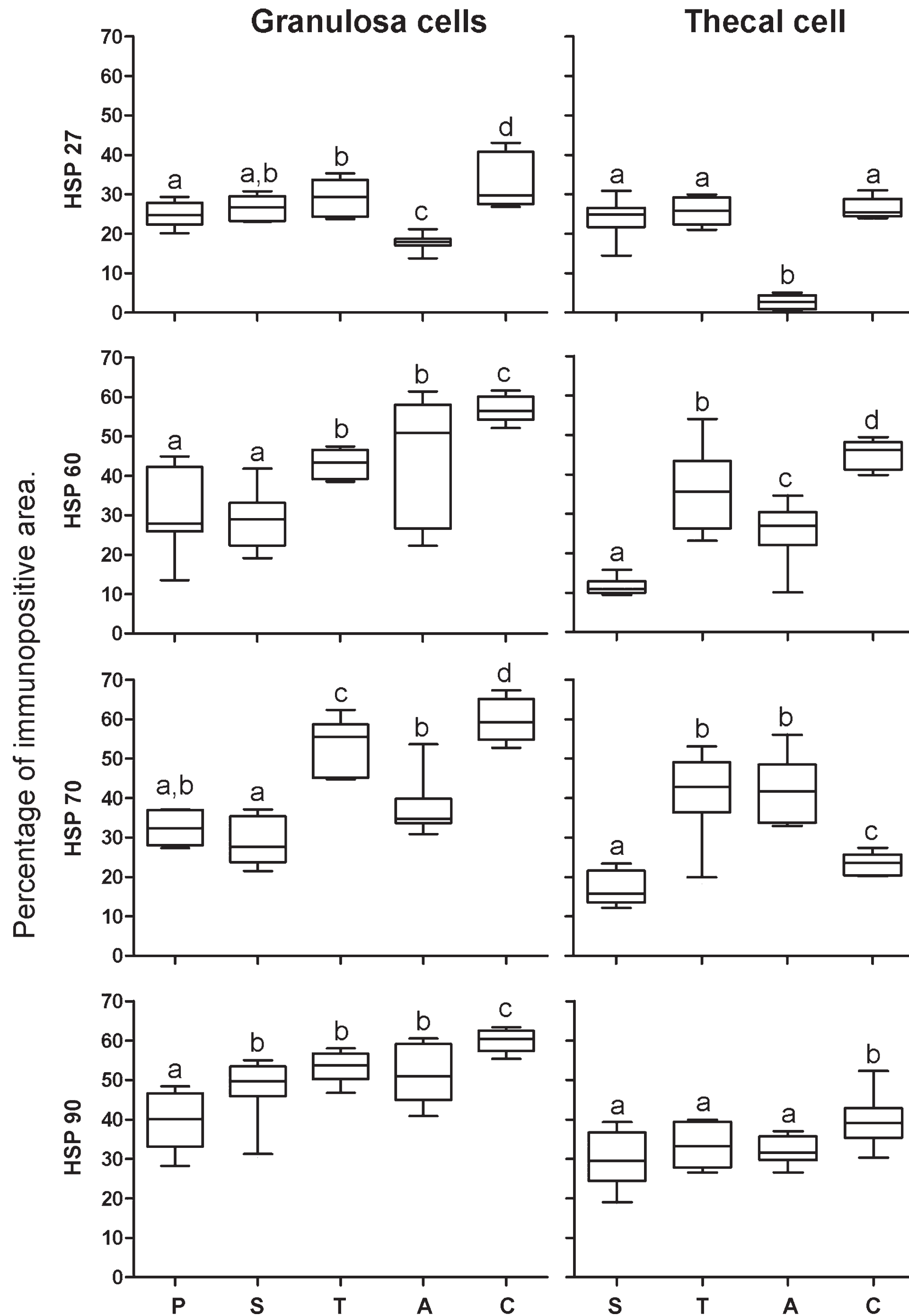

Fig. 2. Percentage of immunopositive areas detected by IHC for heat shock proteins (HSP27, HSP60, HSP70 and HSP90) in both granulosa and thecas of primary (P), secondary (S), tertiary (T), atretic (A) and cystic (C) follicles. Error bars indicate maximum and minimum values, horizontal lines indicate median values, and boxes indicate values between the 25th and 75th percentiles. Values with different letters are significantly different ($P < 0.05$).

interna of tertiary and cystic follicles with less ($P<0.05$) staining in the theca interna of atretic follicles. The corpus luteum, interstitial tissue and vascular walls showed intense immunostaining, while moderate reactivity was observed in the corpus albicans and some cells of the tunica albuginea. By WB, HSP27 protein was detected in a zone of 25 kDa; the antibody has been shown to react with both the phosphorylated and the non-phosphorylated forms of HSP27. The follicular wall of large antral healthy follicles is greater amount ($P<0.05$) than small antral and cystic follicular walls (Fig. 3).

HSP60 was detected in different components of the ovarian tissues (Figs. 1 and 2). Moderate amounts were observed in the granulosa of primary and secondary follicles, with more intense staining in tertiary and atretic follicles ($P<0.05$) and further increases in cystic follicles ($P<0.05$). A similar pattern was observed in the theca cells of secondary follicles, with increased staining in the theca interna of tertiary and atretic follicles and even more ($P<0.05$) staining in cells of the theca interna of cystic follicles. The corpus luteum, interstitial tissue and vascular walls showed moderate immunostaining, while weak reactivity was observed in the corpus albicans and some cells of the tunica albuginea. By WB, HSP60 protein was detected in a zone of 60 kDa. The follicular wall of small and large antral healthy follicles had greater amounts ($P<0.05$) than the cystic follicular wall (Fig. 3).

HSP70 was detected in almost all cells that comprise the ovary, in the nucleus as well as in the cytoplasm (Figs. 1 and 2). In healthy ovaries, significantly ($P<0.05$) greater amounts were detected in tertiary follicles compared with primary and secondary follicles; atretic follicles showed an intensity of staining similar to that in primary follicles. Significantly ($P<0.05$) greater amounts of immunostaining were also observed in the granulosa of cystic follicles. Theca cells showed a similar pattern to the granulosa, although the values in cystic follicles were less ($P<0.05$) than those of the tertiary and atretic follicles. The corpus luteum and vascular walls showed intense immunostaining, while moderate reactivity was observed in the corpus albicans, interstitial tissue, and tunica albuginea. By WB, a specific band was detected in a 70-kDa zone; the antibody recognizes both the constitutive (HSP73) and inducible (HSP72) forms of HSP70. The follicular wall of cystic follicles had greater amounts ($P<0.05$) than those from small and large follicles (Fig. 3).

HSP90 was detected in all cells that comprise the ovary, in the nucleus as well as in the cytoplasm (Figs. 1 and 2). Greater amounts were detected in granulosa cells compared to theca cells, with a significant increment that is consistent with follicular development. Atretic follicles showed staining similar to tertiary follicles, while cystic follicles had the greatest amount ($P<0.05$). No differences were found in the theca cells of secondary follicles and cells of the theca interna of tertiary and atretic follicles, while in cystic follicles, the cells of the theca interna had a significant increase in staining. The corpus luteum and vascular walls showed intense immunostaining, while moderate reactivity was observed in the interstitial tissue and corpus albicans. Weak staining was present in some cells of the tunica albuginea. By WB, a specific band was detected

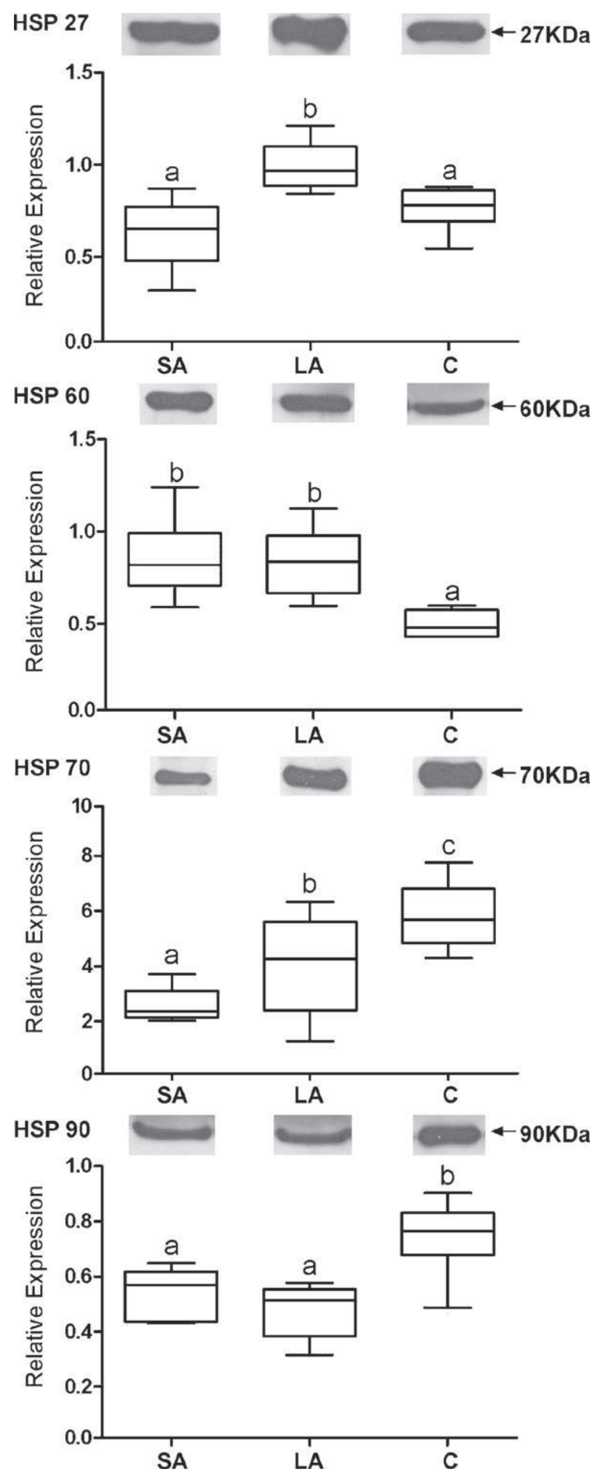

Fig. 3. Relative amounts of heat shock proteins analyzed by Western immunoblot in the wall of small antral (SA), large antral (LA) and cystic (C) follicles. The relative expression was normalized using actin as a loading control, to produce arbitrary densitometric units. In all tested homogenates the antibodies revealed single positive bands of appropriate sizes for each of the protein studied. Error bars indicate maximum and minimum values, horizontal lines indicate median values, and boxes indicate values between the 25th and 75th percentiles. Values with different letters are significantly different ($P<0.05$).

in a zone of 90 kDa; the antibody recognizes both HSP90 alpha and beta. Similar relative amounts of protein were found in small and large antral follicles with significantly ($P < 0.05$) greater amounts in cystic follicle walls (Fig. 3).

4. Discussion

The present study has shown that HSP27, HSP60, HSP70 and HSP90 are present in different follicular cells of the bovine ovary, with differences in their distribution through follicular development. The differential amounts of HSP was also observed in the wall of cystic follicles with specific differences with healthy follicles. The proteins analyzed by WB was consistent with the IHC data, identifying quantitative changes in the different components of the follicular wall. The selection of the follicle-size categories for WB (small and large antral) was based on the reported gonadotropin dependence and changes in steroidogenic enzyme and LH receptor mRNAs. Xu et al. (1995) showed that at these sizes follicle growth will be halted if follicle-stimulating hormone (FSH) is suppressed.

Although there have been several studies evaluating amounts of HSP in the ovaries of laboratory animals (Khanna et al., 1995; Ohsako et al., 1995; Paranko et al., 1996; Maizels et al., 1998; Salvetti et al., 2008), bovine oocytes (Wrenzycki et al., 2001), bovine follicular fluid (Driancourt et al., 1999; Driancourt, 2001; Maniwa et al., 2005) and human ovarian cells (Langdon et al., 1995; Kim et al., 1996), we found no previous studies on the constitutive amounts of these proteins in adult cattle ovaries.

In the present study, HSP27 is constant in the granulosa of secondary, tertiary and cystic follicles, while in the theca cells amount vary with follicular development. In addition, significantly lesser amounts were observed in the granulosa and theca cells of atretic follicles. In agreement with this pattern, lesser rates of apoptosis have occurred in cystic ovarian follicles in cows (Isobe and Yoshimura, 2007; Ortega et al., 2008). It has thus been suggested that a disorder of cell proliferation and apoptosis is associated with the occurrence of follicular cysts (Isobe and Yoshimura, 2007). Several different mechanisms may account for HSP27 antiapoptotic activity, suggesting that the inhibitory effect of HSP27 is rather specific for the cytochrome c, caspase-dependent, apoptotic pathway. Indeed, HSP27 specifically interacts with cytochrome c in the cytosol and this interaction has functional consequences since it prevents formation of the apoptosome (Garrido et al., 2001).

Intense immunostaining for HSP60 was seen in the granulosa of atretic follicles with less staining in primary and secondary follicles, while in the cells of the theca interna, greater amounts of staining were observed in tertiary and cystic follicles. The relative expression in WB did not follow the same pattern, possibly due to the changes detected by IHC, such that the changes were in certain compartments and not in the whole cyst wall. HSP60, also called chaperonin, was mostly contained within the mitochondrial matrix, although it has also been detected in extramitochondrial sites (Garrido et al., 2001).

HSP60 is present in human follicular fluid (Neuer et al., 1997) and is related to fertility (Neuer et al., 2000). Greater amounts of HSP60 were found in the oocytes of the single-layered primordial follicles and those of the growing preantral follicles of the rat. In addition, preovulatory oocytes remained distinctly immunoreactive. This suggests that these active cells contain a key element required for proper refolding of imported mitochondrial proteins (Paranko et al., 1996). Also, like other HSP, HSP60 can stabilize steroid receptors (Edwards et al., 1992); this stabilization deprives them of their capacity to enter into the nucleus and initiate their nuclear function (Kiang and Tsokos, 1998; Neuer et al., 2000) and their relation with PCOD pathogenesis is discussed later.

Although HSP70 immunoreactivity was observed in all follicular cells in the follicles studied, the intensity of staining was strongest in the granulosa of cystic follicles. WB analysis indicated greater amounts of this protein in tertiary and cystic follicular walls. In agreement with the present results, increased HSP70 has been described in the follicular fluid of cystic follicles of cattle (Maniwa et al., 2005). We have previously reported that HSP70 increases in induced cysts in rats (Salvetti et al., 2008), with a similar distribution in healthy ovaries to that found in cattle in the present study. HSP70 was present in the granulosa cells of human ovaries *in vitro* (Benifla et al., 2002), and was synthesized by oocytes and cumulus cells in cows (Edwards and Hansen, 1997), and by granulosa cells within follicles in rats (Yoon et al., 2002). The ability to inhibit apoptosis has become widely recognized as a function of HSP70, and this ability may contribute to its protective effect against cell death (Beere, 2004, 2005). As mentioned above, Isobe and Yoshimura (2007) reported that in the theca interna of ovarian follicles of cattle, a greater frequency of apoptosis was noted in the early cystic follicles, whereas this frequency was less in late cystic follicles. They concluded that the decrease in apoptosis cell rate may be responsible for the delay in follicular regression, and that control of apoptosis may be essential for reducing the incidence of cystic follicles. This observation is consistent with our results and other previous research (Maniwa et al., 2005; Salvetti et al., 2008), in which decreased apoptotic cell death was associated with increased HSP70 in cystic follicles.

HSP90 was also observed to have a similar staining distribution and relative amounts as HSP70. In the present study, HSP90 increased during follicular development, in agreement with previous findings (Driancourt, 2001), where HSP90 was also present in cattle ovaries, with follicular size- and atresia-related changes in its concentration. In addition, the granulosa cells were found to be more active producers of HSP90 than the theca cells. Therefore, HSP90 may be involved in follicular maturation. In rodents, HSP90 may be present in germinal (Curci et al., 1991) and somatic cells (Ben-Ze'ev and Amsterdam, 1989) of the ovary. To date, this is the first report that shows over-expression of the HSP90 gene in ovarian cystic structures.

Both HSP70 and HSP90 have been demonstrated to bind steroid receptors and modulate their function (Pratt and Toft, 2003). Because proliferation of the growing follicles in proestrous is believed to be one of the responses to sex steroids, HSP70 seems to act as a repressor of the steroidal effects. Atretic and cystic follicles had a low index of proliferation (data not published) with greater HSP70. In the

breast cancer cell line T47D, Bagchi et al. (1991) demonstrated that HSP70 associates with steroid receptors even after the dissociation of HSP90, and that those receptors complexed with HSP70 remain inactive. This is consistent with the study of Koshiyama et al. (1995) who found that in the endometrium, HSP70 is present in cells that do not react with sex steroid receptors. In addition, an increase in HSP70 and HSP90 was detected in cystic follicles according to the down-regulation of ERβ (Salvetti et al., 2007). Thus, there is a negative relationship between HSP70/HSP90 and the status of some sex steroid receptors in COD.

5. Conclusion

In agreement with Maniwa et al. (2005), it is important to emphasize that cows with follicular cysts are exposed to various kinds of stress such as oxidative stress, negative energy balance, poor liver function and low circulating insulin-like growth factor I (Silvia et al., 2002; Vanholder et al., 2006; Ortega et al., 2008). Although the relationship between these stresses and the increase in HSP in the ovary in the present study is unclear, it is speculated that altered expression of HSP genes decreases apoptosis in the follicular wall and leads to the delayed regression of cystic follicles. In addition, modifications in steroid receptor function by changes in the modulation of their function could affect important intra-ovarian regulatory mechanisms during follicle development.

The present study supports earlier observations in experimental models (Salvetti et al., 2007) suggesting that aberrant amounts of HSP, as observed in cystic follicles, is probably associated with the intra-ovarian component of COD pathogenesis.

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