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Levels and Subcellular Distributions of Detoxifying Enzymes in the Ovarian *Corpus Luteum* of the Pregnant and Non-pregnant Pig

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ABSTRACT. The levels and subcellular distribution of enzymes involved in defenses against reactive oxygen superoxide dismutase (SOD; E.C.1.15.1.1), glutathione peroxidase (GPX; E.C.1.11.1.9), catalase (CAT; E.C.1.11.1.6), and DT-diaphorase (DT; E.C.1.6.99.2) and of the conjugating enzymes glutathione transferase (GST; E.C.2.5.1.18) and *p*-sulphotransferase (*p*-ST; E.C.2.8.2.1) in the *corpus luteum* of ovaries from pregnant and non-pregnant pigs were investigated. In addition, non-protein thiols and glutathione reductase (GRD; E.C.1.6.4.2) were examined in the same manner. The total cytosolic activities of CAT, DT, GRD, and *p*-ST were significantly increased, whereas total GST activity was decreased in the pregnant *corpus luteum* compared to the corresponding activities in non-pregnant *corpus luteum*. In the case of the mitochondrial fraction from pregnant *corpus luteum*, GPX and GRD displayed significant increases in specific activity. Upon subfractionation of the mitochondrial fraction (i.e. mitoplast preparation), SOD activity was distributed equally between the mitoplasts and the supernatant. CAT and GPX activities were mainly recovered in the supernatant, while the major GRD activity pelleted with the mitoplasts. Microsomes from pregnant *corpus luteum* demonstrated increased specific GPX activity and decreased SOD activity compared to the non-pregnant *corpus luteum*. No differences in the non-protein thiol levels in the cytosolic, mitochondrial, or microsomal fractions from the *corpus luteum* were observed between non-pregnant and pregnant sows. BIOCHEM PHARMACOL **58**;8:1287–1292, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. pig; corpus luteum; pregnancy; detoxification; subcellular distribution

Reactive metabolites formed by cytochrome P450s in connection with their metabolism of xenobiotics, as well as of endogenous compounds, and ROS† have been implicated in the mechanisms underlying various diseases, as well as tissue damage. The reactive metabolites are detoxified by phase II enzymes, i.e. epoxide hydrolase and glutathione transferases, and often further conjugated by sulphotransferases and UDP-glucuronosyltransferases. At the same time, ROS are scavenged by certain molecules, e.g. antioxidant vitamins such as C, A, or E, or deactivated by antioxidant enzymes, including superoxide dismutase, catalase, and various peroxidases. ROS, antioxidant vitamins, antioxidant enzymes, and phase II enzymes are all known to be present in the mammalian ovary [1–3], where the levels of many of these molecules appear to be under hormonal regulation. Apart from being involved in the metabolism of toxic compounds, many isoforms of these enzymes also participate in normal ovarian processes, such as folliculogenesis, ovulation, luteinization, and maintenance of the pregnant *corpus luteum* (which is under the influence of endocrine, paracrine, and autocrine factors). Therefore, certain reactive intermediates formed from xenobiotics can also be potentially toxic towards the organism by exerting their effects indirectly, e.g. through disruption of hormonal homeostasis.

We have previously reported that porcine ovarian microsomal as well as mitochondrial fractions are able to metabolize the polycyclic aromatic hydrocarbon 7,12-dimethylbenz(*a*)anthracene to reactive metabolites [4] involving the cytochrome P450 system. The highest levels of 7,12-dimethylbenz(*a*)anthracene metabolism were observed in these subcellular fractions obtained from *corpora lutea*. As a consequence of these results, we wanted to further characterize the capacity of *corpora lutea* from porcine ovaries to deal with reactive intermediates. The present investigation was designed to investigate the levels and subcellular distribution of certain antioxidant enzymes and enzymes involved in defenses against reactive intermediates. The *corpus luteum* plays an important role in regulating not only the menstrual/estrous cycle, but also in maintaining the first phase of pregnancy. Therefore, it was

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† *Abbreviations:* SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase; DT, DT-diaphorase, NADPH dehydrogenase; GST, glutathione transferase; *p*-ST, *p*-sulphotransferase; GRD, glutathione reductase; NPT, non-protein thiol; ROS, reactive oxygen species; and CDNB, 1-chloro-2,4-dinitrobenzene.

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also of interest to explore possible differences in expression of these enzymes in non-pregnant and pregnant sows.

MATERIALS AND METHODS

Chemicals

Reduced and oxidized glutathione, CDNB, GRD, and [^{14}C]-1-naphthol were from Sigma Chemical Co. Bicinchoninic acid was from Pierce and 4-OH-phenylacetic acid from Merck. All other chemicals used were of reagent grade and purchased from common commercial sources.

Tissue Collection, Preparation of Subcellular Fractions, and Protein Determination

Porcine ovaries were collected from the local slaughterhouse (Scan, Uppsala) and immediately placed in ice-cold saline. The *corpora lutea* from non-pregnant or pregnant ovaries were dissected out and homogenized in 150 mM KCl, 1 mM EDTA, and 20 mM Tris–Cl, pH 7.6. Subcellular fractionation and mitoplast preparation were performed as described previously [4]. Briefly, mitoplasts (intact inner mitochondrial membranes enclosing the matrix) were prepared by digitonin treatment and the outer mitochondrial membrane/intermembrane space and any subcellular contaminants of the original mitochondrial fraction (i.e. peroxisomes, lysosomes, and microsomes) subsequently separated from the mitoplasts by centrifugation and designated the supernatant. Protein determination was performed employing the bicinchoninic acid procedure [5].

Enzyme Assays and Measurement of Non-protein Thiols

GLUTATHIONE TRANSFERASE. GST was measured spectrophotometrically at 340 nm, as the rate of CDNB conjugation with GSH [6].

GLUTATHIONE REDUCTASE. The enzymatic rate of oxidation of NADPH at 30° in the presence of GSSG was used to quantitate the activity of GRD [7].

GLUTATHIONE PEROXIDASE. GPX activity was assayed with cumene hydroperoxide as substrate in the presence of GSH, GRD, and NADPH. This reaction was monitored indirectly as the oxidation of NADPH at 340 nm. The background activity (i.e. in the absence of cumene hydroperoxide), accounting for approximately 10% of the total activity, was routinely subtracted [8].

SULPHOTRANSFERASE. Sulphation of [^{14}C]-1-naphthol was assayed to establish the subcellular distribution of *p*-STs [4]. The sulphated metabolites were separated from unconjugated [^{14}C]-1-naphthol by extraction and quantitated by scintillation counting.

DT-DIAPHORASE. DT-diaphorase activity was monitored spectrophotometrically by following the dicumarol-sensitive reduction of cytochrome *c*, with menadione as electron acceptor [9].

SUPEROXIDE DISMUTASE. SOD activity was measured by monitoring the formation of hydrogen peroxide using a coupled reaction involving formation of the fluorescent product 6,6′-diOH-(1,1′-biphenyl)-3,3′-diacetic acid from 4-OH-phenylacetic acid, hydrogen peroxide, and horseradish peroxidase [10].

CATALASE. The catalatic activity of catalase was monitored spectrophotometrically at 240 nm with hydrogen peroxide as substrate [11].

NON-PROTEIN THIOLS. Protein was removed from the different subcellular fractions by precipitation with trichloroacetic acid, and measurement of NPTs was subsequently performed with the colorimetric reaction employing Ellman's reagent [12].

Statistical analyses were performed using Student's *t*-test.

TABLE 1. Protein content in subcellular fractions from *corpora lutea* of non-pregnant and pregnant pig

	Non-pregnant	Pregnant
	(mg protein/g wet tissue)	
Microsomes	1.9 ± 0.3 (3)	3.6 ± 0.7 (2)
Mitochondria	0.5 ± 0.1 (3)	0.6 ± 0.2 (2)
Cytosol	14.4 ± 0.9 (3)	21.4 ± 1.4 (2)

The numbers in parentheses denote the number of individual preparations. Each preparation involved 6–8 pigs. The values are, when N = 3, means ± SD; when N = 2, values are means ± mean deviations.

RESULTS

Microsomal and cytosolic fractions prepared from pregnant *corpora lutea* showed significantly higher levels of protein (mg/g wet weight) than did the corresponding fractions from non-pregnant *corpora lutea* (Table 1). (For this reason, both specific and total enzyme activities are given for these fractions.) This phenomenon could not simply be explained as a higher degree of efficiency during homogenization and preparation of subcellular fractions from the pregnant tissue, since the levels of mitochondrial protein did not differ. These differences would rather seem to reflect a true increase in the total level of protein in the cytosol and endoplasmic reticulum of the *corpus luteum* in connection with pregnancy.

SOD was recovered predominantly in the cytosolic fraction, where this activity in pregnant *corpus luteum* appeared to be approximately twice as high as in the corresponding non-pregnant tissue (Table 2). Upon mitochondrial subfractionation, the SOD activity in this fraction was distributed equally between mitoplasts and the supernatant (Table 3). Cytosolic GPX activity was of the same order of magnitude as SOD activity in both non-pregnant and pregnant *corpus luteum*, with no significant

TABLE 2. Total and specific superoxide dismutase, glutathione peroxidase, catalase, glutathione transferase, sulphotransferase, and DT-diaphorase activities in microsomes and cytosol from *corpora lutea* from the ovaries of pregnant and non-pregnant pigs

	Microsomes		Cytosol	
Enzyme	Spec. activity	Tot. activity	Spec. activity	Tot. activity
Superoxide dismutase				
NP	73.6 ± 19.7†	4.9 ± 2.0¶	353 ± 145†	175 ± 60.1¶
P	19.8 ± 5.4††,†	1.6 ± 0.3¶	602 ± 12.3†	298 ± 4.3¶
Glutathione peroxidase				
NP	75.2 ± 7.1‡	4.9 ± 1.0**	547 ± 160‡	272 ± 58.3**
P	214 ± 28.5‡‡,‡	17.5 ± 2.4‡‡*	353 ± 22.7‡	174 ± 11.3**
Catalase				
NP	213 ± 34.9*	14.2 ± 1.8‖	29.0 ± 8.4*	19.3 ± 3.9‖
P	135 ± 44.1*	11.1 ± 3.6‖	136 ± 11.1‡‡*	67.0 ± 5.4‡‡‖
Glutathione transferase				
NP	0.15 ± 0.03*	10.2 ± 3.5**	3.11 ± 0.25*	1559 ± 45.0**
P	0.18 ± 0.07*	14.4 ± 5.3**	1.64 ± 0.31††*	808 ± 151‡‡**
Sulphotransferase				
NP	0.2 ± 0.23†	15.3 ± 21.8§	4.2 ± 0.77†	2110 ± 387‡
P	0.6 ± 0.02†	50.4 ± 1.7§	11.3 ± 1.65‡‡†	5570 ± 814‡‡§
DT-diaphorase				
NP	52.2 ± 27.6‡	3.6 ± 2.1**	100 ± 23.8‡	51.4 ± 16.8**
P	59.0 ± 3.7‡	4.8 ± 0.3**	302 ± 16.7‡‡,‡	149 ± 8.3‡‡**

Three independent experiments were performed in all cases. Enzyme activities were assayed as described under Materials and Methods. NP = non-pregnant *corpora lutea*, P = pregnant *corpora lutea*.

*μmol/min × mg; †pmol/min × mg; and ‡nmol/min × mg.

§pmol/min; ‖mmol/min; ¶nmol/min; and **μmol/min.

††$P < 0.05$ compared to the corresponding activities in non-pregnant *corpora lutea*.

‡‡$P < 0.01$ compared to the corresponding activities in non-pregnant *corpora lutea*.

difference observed between these tissues (Table 2). On the other hand, microsomal and mitochondrial GPX activities were significantly increased in pregnant compared to non-pregnant *corpus luteum* (Tables 2 and 4). Upon subfractionation of the mitochondrial fraction obtained from pregnant *corpus luteum*, most of the activity was recovered in the supernatant (Table 3). Total CAT activity in the pregnant *corpus luteum* was also located predominantly in the cytosol, where it was significantly (3.5-fold) higher than the corresponding activity in non-pregnant *corpus luteum* (Table 2). The microsomal fractions from non-pregnant and pregnant *corpus luteum* had similar activities. In the non-pregnant *corpus luteum*, the total microsomal catalase activity was of the same order of magnitude as the cytosolic, whereas the microsomal activity in the pregnant *corpus luteum* was 6-fold lower than the cytosolic. Conversely, the highest specific CAT activity was seen in microsomal fraction from

TABLE 3. Distribution of total superoxide dismutase, catalase, DT-diaphorase, glutathione peroxidase, glutathione transferase, sulphotransferase, and glutathione reductase activities upon subfractionation of the mitochondrial fractions from *corpora lutea* from the ovaries of non-pregnant and pregnant pigs

Enzyme	Mitoplast (%)	Supernatant (%)	Recovery (%)
Superoxide dismutase			
NP	43 ± 12	31 ± 15	74
P	39 ± 19	35 ± 4	74
Catalase			
NP	0.6 ± 0.0	88 ± 23	89
P	16 ± 2	48 ± 6	64
DT-diaphorase			
NP	30 ± 10	17 ± 5	47
P	48 ± 17	16 ± 3	64
Glutathione peroxidase			
NP	24 ± 11	34 ± 12	58
P	10 ± 0.4	67 ± 4	77
Glutathione reductase			
NP	46 ± 3	11 ± 1	57
P	56 ± 2	12 ± 2	68
Non-protein thiols			
NP	21 ± 4	64 ± 5	85
P	27 ± 0	72 ± 14	99

% = % of the value for the whole mitochondrial fraction. Three independent experiments were performed in all cases. NP = non-pregnant, P = pregnant.

TABLE 4. Specific activities of superoxide dismutase, glutathione peroxidase, catalase, glutathione transferase, sulphotransferase, and DT-diaphorase in the mitochondrial fraction of *corpora lutea* from the ovaries of non-pregnant and pregnant pigs

Enzyme	Non-pregnant	Pregnant
Superoxide dismutase	468 ± 92.4†	470 ± 41.6†
Glutathione peroxidase	101 ± 24.6‡	666 ± 64.3‖‡
Catalase	96.6 ± 19.1*	87.4 ± 10.0*
Glutathione transferase	0.12 ± 0.06*	0.05 ± 0.01*
Sulphotransferase	0.36 ± 0.28†	0.2†§
DT-diaphorase	67.1 ± 30.9‡	168 ± 83.5‡

*μmol/min × mg; †pmol/min × mg; and ‡nmol/min × mg.

§N = 1; for all other values, N = 3.

‖$P < 0.001$ compared to the corresponding activity in non-pregnant *corpora lutea*.

TABLE 5. Total and specific GSH reductase activity and non-protein thiol concentration in subcellular fractions of *corpora lutea* from the ovaries of non-pregnant and pregnant pigs

	GSH reductase activity					
	Specific (nmol/min × mg)		Total (μmol/min)		Non-protein thiols (μM)	
Fraction	Non-pregnant	Pregnant	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Microsomes	3.0 ± 2.1	4.0 ± 1.1	0.2 ± 0.13	0.3 ± 0.10	36 ± 4	ND†
Mitochondria	41.5 ± 2.4	73.3 ± 12.1*	0.7 ± 0.22	1.1 ± 0.15	140 ± 18	132 ± 28
Cytosol	20.5 ± 3.6	43.0 ± 1.5*	10.2 ± 1.35	21.2 ± 0.80*	354 ± 62	436 ± 68

*$P < 0.01$ compared to the corresponding activity in non-pregnant *corpora lutea*.
†ND (not detectable) < 20 μM. N = 3 in all cases.

non-pregnant *corpus luteum* (Table 2). No difference in the CAT activities of non-pregnant or pregnant mitochondrial fractions was observed (Table 4). Cytosolic GST activity, which had previously been reported by us and others to be remarkably higher in the non-pregnant *corpus luteum* than in ovarian follicles [13], was significantly lower in the pregnant *corpus luteum*. In fact, the cytosolic GST activity observed with the non-pregnant *corpus luteum* towards CDNB was 3- to 6-fold higher than the corresponding activities seen in rat ovary or liver [2]. With respect to microsomal GST activity, there were no significant differences between non-pregnant and pregnant *corpus luteum*. Furthermore, the total levels of the GST activities in this and the mitochondrial fraction were 100–1000 times lower than in the cytosol and the specific activities were 10- to 30-fold lower, indicating that mitochondria and endoplasmic reticulum are of relatively little quantitative importance in the total ability of the porcine *corpus luteum* to conjugate reactive electrophiles with glutathione. The other conjugating enzyme investigated, *p*-ST, demonstrated a 2.6-fold higher activity in cytosol from pregnant compared to non-pregnant *corpus luteum* (Table 2). *p*-ST activities in microsomal and mitochondrial fractions from non-pregnant and pregnant *corpus luteum* displayed no differences (Tables 2 and 4). Furthermore, these activities were, as in the case of GST, 100- to 200-fold lower than the corresponding cytosolic activities.

GPX and GST are dependent on GSH for their catalytic activities. For this reason, the level of GRD and an approximate indicator of GSH level (i.e. NPTs) were determined. The concentration of NPT was highest in the cytosol of both non-pregnant and pregnant *corpus luteum*, being roughly 2- to 3-fold as high as in the corresponding mitochondrial fractions. Mitoplast preparation revealed that two-thirds of the mitochondrial NPT fractionated with the supernatant (Table 3). There was no significant difference in the NPT levels in the cytosolic and mitochondrial fractions from non-pregnant and pregnant *corpus luteum*. In the microsomal fraction from pregnant *corpus luteum*, the level of NPT was undetectable, in contrast to the corresponding microsomal fraction from pregnant sows. Total GRD activity was also highest in the cytosol of both non-pregnant and pregnant *corpus luteum*, with this activity being twice as high in pregnant *corpus luteum* than in the non-pregnant tissue (Table 5). On the other hand, the specific activity in the mitochondrial fraction was approximately 2-fold higher in pregnant than in non-pregnant *corpus luteum*. When mitoplast preparation was performed, most of the GRD activity remained with the mitoplasts, i.e. inner mitochondrial membrane and matrix.

DISCUSSION

It is known that when follicle cells differentiate into *corpus luteal* cells during the estrous cycle, i.e. during formation of the non-pregnant *corpus luteum*, there is an accompanying hypertrophy of the agranular endoplasmic reticulum. During the first phase of pregnancy, this hypertrophy seems to be further enhanced [14, 15], which may provide at least a partial explanation for the increase in total protein observed here in the microsomal fraction from pregnant *corpus luteum*. To confirm this, electron microscopic studies designed to compare the ultrastructure of the cells of non-pregnant and pregnant *corpora lutea* will be required. This hypertrophy of the endoplasmic reticulum is suggested to reflect an increased demand for steroidogenesis. In the bovine *corpus luteum*, a correlation has been reported to exist between the levels of certain antioxidant enzymes and the progesterone level, and this has been suggested to indicate activation of antioxidant mechanisms to deal with ROS formed during steroidogenesis [15]. Furthermore, since ROS are known to be involved in luteolysis [16] (i.e. disruption of the persistent *corpus luteum*), which is undesirable during the early phase of pregnancy, the pregnant *corpus luteum* definitely requires such protection. Thus, the increase in cytosolic CAT and microsomal and mitochondrial GPX activities observed here in the pregnant *corpus luteum* might be related to increased steroidogenesis and, especially, to the increased production of progesterone which occurs during pregnancy. Furthermore, the porcine ovary is known to contain microperoxisomes [17, 18], which sediment in the microsomal fraction (30,000 × *g*) and might explain, at least in part, the relatively high specific GPX activity detected in this fraction. Upon subfractionation of the mitochondrial fraction, the major portion of the total mitochondrial GPX activity co-frac-

tionated with the supernatant. This activity could originate from peroxisomes, cytosolic contamination, and/or the intermembrane space.

It is known that in the rat *corpus luteum*, total SOD activity increases as pregnancy proceeds, with the Cu, Zn-SOD accounting for the major part of this activity (being almost 10-fold higher than the Mn-SOD) [19]. Judging from the present findings, this does not occur in the pregnant porcine *corpus luteum* (Table 2). The assay for SOD activity employed here does not distinguish between the two different isoforms and, therefore, the data presented represent total SOD activity. This is also seen by the fact that SOD activity is distributed equally between the mitoplasts (Mn-SOD) and supernatant (Cu, Zn-SOD) upon subfractionation of the mitochondrial fraction. The involvement of ST in conjugation of steroids is well documented [20], the steroid sulphates having lowered affinities for their receptors than the corresponding unconjugated steroids. The highest level of estrone sulphate is found during the luteal phase in the pig. Furthermore, measurement of urinary levels of estrone sulphate is a common method for diagnosing pregnancy [21]. These considerations might explain the increased ST activity observed here in the pregnant *corpus luteum*. Although we have assayed phenol ST activity and estrone sulphates are known to be produced by estrogen ST, in certain species these enzymes exhibit overlapping substrate specificities.

Taking together, the present findings suggest that pregnant *corpora lutea* possess, in general, increased defenses against reactive intermediates and/or a higher demand for these enzymes in order to participate in normal metabolic processes compared to non-pregnant *corpora lutea*. The only exception to this statement is a decrease in the level of cytosolic GST activity in connection with pregnancy. It is known that many of the genes for the enzymes investigated here contain the antioxidant responsive element in their upstream region. Nuclear transcription factors, including the Ah receptor, have been reported to bind to antioxidant responsive element [22, 23]. Furthermore, hormonal regulation of some of these genes, (e.g. mediated via the estrogen receptor [24]), has been reported. In most cases, these transcription factors have a positive (up-regulating) role in the expression of these genes, although a negative (repressive) effect is sometimes seen. Taking this into account, one might speculate that there could be coordinate regulation of the enzymes investigated even though the GST activity is decreased. The regulatory factors involved under the conditions employed here will be investigated in future studies.

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References

1. Graves-Hoagland RL and Hoagland TA, Relationship of plasma β-carotene and vitamin A to luteal function in postpartum cattle. *J Dairy Sci* **72:** 1854–1858, 1989.
2. Becedas L and Bengtsson-Ahlberg M, Hormonal influences of detoxication in the rat ovary on enzymes in comparison with the liver. *Biochem Pharmacol* **49:** 503–550, 1995.
3. Biswas SC, Singh D and Pandey RS, Subcellular distribution of oxygen free radical scavenging enzymes in the goat ovary. *Indian J Exp Biol* **33:** 785–787, 1995.
4. Eliasson M, Brock S and Bengtsson-Ahlberg M, Evidence for mitochondrial metabolism of 7,12-dimethylbenz(*a*)anthracene in porcine ovaries: Comparison with microsomal metabolism. *Toxicology* **122:** 11–21, 1997.
5. Smith PK, Kroh RI, Hermansson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC, Measurement of protein using bicinchoninic acid. *Anal Biochem* **150:** 76–85, 1985.
6. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferase. The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249:** 7130–7139, 1974.
7. Carlberg I and Mannervik B, Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J Biol Chem* **250:** 5475–5480, 1975.
8. Wendel A, Glutathione peroxidase. *Methods Enzymol* **77:** 325–333, 1981.
9. Schultzberg M, Segura-Aguilar J and Lind C, Distribution of DT diaphorase in the rat brain: Biochemical and immunohistochemical studies. *Neuroscience* **27:** 763–776, 1988.
10. Segura-Aguilar J, A new direct method for determining superoxide dismutase activity by measuring hydrogen peroxide formation. *Chem Biol Interact* **86:** 69–78, 1993.
11. Bergmeyer HU, Measurement of catalase activities. *Biochem Z* **327:** 255–258, 1955.
12. Djuric Z, Malviya VK, Deppe G, Malone JM Jr, McGunagle DL, Heilbrun LK, Reading BA and Lawrence WD, Detoxifying enzymes in human ovarian tissues: Comparison of normal and tumor tissues and effects of chemotherapy. *J Cancer Res Clin Oncol* **116:** 379–383, 1990.
13. Eliasson M, Stark T and DePierre JW, Expression of glutathione transferase isoenzymes in the porcine ovary in relationship to follicular maturation and luteinization. *Chem Biol Interact* **117:** 35–48, 1999.
14. Dharmarajan AM, Bruce NW and Waddell BJ, Quantitative changes in steroidogenic organelles in the *corpus luteum* of the pregnant rat in relation to progestin secretion on day 16 and in the morning and afternoon of day 22. *Am J Anat* **190:** 273–278, 1991.
15. Rapoport R, Sklan D, Wolfenson D, Shaham-Albalancy A and Hanukoglu I, Antioxidant capacity is correlated with steroidogenic status of the *corpus luteum* during the bovine estrous cycle. *Biochim Biophys Acta* **1380:** 133–140, 1998.
16. Brannian JD, Zhao Y and Burbash JA, Localization of lipid peroxidation-derived epitopes in the porcine *corpus luteum*. *Biol Reprod* **57:** 1461–1466, 1997.
17. Peterson SL and Stevenson PM, Changes in catalase activity and concentration during ovarian development and differentiation. *Biochim Biophys Acta* **1135:** 207–221, 1992.
18. Gulyas BJ and Yuan LC, Association of microperoxisomes with the endoplasmic reticulum in the granulosa lutein cells of the Rhesus monkey (*Macaca mulatta*). *Cell Tissue Res* **179:** 357–366, 1977.
19. Shimamura K, Sugino N, Yoshida Y, Nakamura Y, Ogino K and Kato H, Changes in lipid peroxide and antioxidant enzyme activities in *corpora lutea* during pseudopregnancy in rats. *J Reprod Fertil* **105:** 253–257, 1995.

20. Luu-The V, Bernier F and Dufort I, Steroid sulfotransferases. *J Endocrinol* **150**(Suppl)**:** S87–97, 1996.
21. Madej A, Romanowicz K, Einarsson S, Forsberg M and Barcikowski B, Urinary excretion of oestrone sulphate and cortisol in early pregnant gilts treated with glucocorticoids. *Acta Vet Scand* **39:** 61–70, 1998.
22. Jaiswal AK, Antioxidant response element. *Biochem Pharmacol* **48:** 439–444, 1994.
23. Buetler TM, Gallagher EP, Wang C, Stahl DL, Hayes JD and Eaton DL, Induction of phase I and phase II drug-metabolizing enzymes mRNA, protein, and activity by BHA, ethoxyquin, and oltipraz. *Toxicol Appl Pharmacol* **135:** 45–57, 1995.
24. Montano MM and Katzenellenbogen BS, The quinone reductase gene: A unique estrogen receptor-regulated gene that is activated by antiestrogens. *Proc Natl Acad Sci USA* **94:** 2581–2586, 1997.