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Original article

Concentrations of catecholamines, ascorbic acid, progesterone and oxytocin in the corpora lutea of cyclic and pregnant cattle

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Abstract — To determine if there are inter-relationships between progesterone, oxytocin (OT), dopamine (DA), noradrenaline (NA) and ascorbic acid, these compounds were measured in the corpus luteum (CL) from cattle at different stages of the oestrous cycle ($n = 42$) and from 1–5 months of pregnancy ($n = 27$). They were measured by radioimmunoassay (RIA), high performance liquid chromatography (HPLC) and colorimetric methods. Corpora lutea were collected from heifers and cows within 30 min of slaughter on days 1–5, 6–10, 11–16 and 17–21 of the oestrous cycle. The stage of pregnancy was determined on the basis of foetal size and development. Each CL was divided into four parts and stored in liquid nitrogen. For hormone estimation, the tissue was homogenised/powdered and suspended in phosphate buffer (for OT and progesterone), 0.1 M trichloracetic acid (TCA; for catecholamines) or in ice-cold metaphosphoric acid (for ascorbic acid). There were no significant differences in the measured parameters between cows and heifers, and so the data were combined. The concentration of DA was correlated with NA ($r = 0.66$; $P < 0.001$) during the oestrous cycle and was highest in newly formed CL ($P < 0.01$) as compared with early CL, regressed CL and CL of pregnant females. NA was negatively correlated ($P < 0.01$) with progesterone ($r = -0.53$) and OT ($r = -0.41$). In contrast, progesterone and OT were positively correlated with each other ($r = 0.81$; $P < 0.01$) during all stages of the oestrous cycle, but not during pregnancy. The lowest concentrations of ascorbic acid were observed in regressed CL. Ascorbic acid concentrations were correlated ($P < 0.01$) with those of progesterone ($r = 0.68$), OT ($r = 0.42$) and DA ($r = -0.37$). Luteal concentrations of ascorbic acid, progesterone and OT followed a pattern consistent with the development and regression of the CL. Luteal concentrations of catecholamines were not consistent with this pattern.

corpus luteum / catecholamines / progesterone / oxytocin / ascorbic acid / cattle

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Résumé — Concentrations en catécholamines, acide ascorbique, progestérone et ocytocine dans le corps jaune (CJ) chez les vaches cycliques et gestantes. Afin de déterminer d'éventuelles relations entre la progestérone, l'ocytocine (OT), la dopamine (DA), la noradrénaline (NA), et l'acide ascorbique, ces substances ont été mesurées dans les corps jaunes de vaches à différents stades du cycle œstrien ($n = 42$) et entre 1 et 5 mois de gestation ($n = 27$). Ces substances ont été mesurées respectivement par RIA, HPLC et méthodes colorimétriques. Les corps jaunes ont été collectés dans les 30 mn après l'abattage des vaches ou des génisses, aux jours 1–5, 6–10, 11–16 et 17–21 du cycle œstrien. Le stade de gestation était déterminé par la taille et le stade de développement du fœtus. Chaque CJ était divisé en quatre parties et gardé dans de l'azote liquide. Pour le dosage des hormones, les tissus étaient broyés et mis en suspension dans du tampon phosphate (pour OT et la progestérone), 0.1 M d'acide trichloracétique (pour DA et NA) ou dans l'acide métaphosphorique glacé (pour l'acide ascorbique). Il n'y a pas eu de différence entre vaches et génisses, et les résultats ont été regroupés. La concentration de DA est corrélée avec celle de NA ($r = 0,66$; $p < 0,001$) pendant le cycle et cette concentration est plus forte chez les corps jaunes neo-formés ($p < 0,01$) que chez les corps jaunes fonctionnels cycliques, régressés ou chez les CJ de gestation. NA est corrélée négativement avec la progestérone ($p < 0,01$) ($r = -0,53$) et l'OT ($r = -0.41$). À l'inverse, la progestérone et l'ocytocine sont positivement corrélées ($r = 0,81$; $p < 0,01$) pendant toutes les phases du cycle œstrien, mais pas pendant la gestation. La concentration la plus basse d'acide ascorbique est observée sur les corps jaunes regressés. La concentration d'acide ascorbique est corrélée avec celle de progestérone ($p < 0,01$; $r = 0,68$), l'OT ($r = 0,42$) et la DA ($r = -0,37$). Les concentrations lutéales d'acide ascorbique, de progestérone et d'OT suivent une évolution en accord avec le développement et la régression du CJ. Les concentrations lutéales des catécholamines ne montrent pas la même évolution. © Inra/Elsevier, Paris

corpus luteum / catécholamines / progestérone / ocytocine / acide ascorbique / vache

1. INTRODUCTION

Progesterone plays a key role in the regulation of the oestrous cycle and pregnancy in most mammals including cattle. Its synthesis and secretion are controlled by gonadotrophins but also supported by numerous locally produced factors which act in an autocrine and paracrine fashion. One such factor, oxytocin (OT), stimulates progesterone secretion [29, 36] in early and fully matured corpora lutea (CL); but in order to demonstrate this, the whole CL or luteal slices, rather than dispersed luteal cells, are required [34]. Adrenergic innervation of the adult ovary affects CL function in many species, as shown in studies carried out in vivo [16, 17] and in vitro [3, 5]. Moreover, noradrenaline (NA) is synthesised in bovine CL from its precursor dopamine (DA) [19]. Concentrations of NA and adrenaline in peripheral plasma do not vary significantly with the stage of the oestrous cycle in pigs [2]. In humans, however, plasma levels of NA are significantly higher during the luteal than during the follicular phase [8]. The ovarian content of NA, which reflects ovarian innervation, increases two to ten times during sexual maturation in rats [4] and declines during the ageing process [9]. Follicular differentiation in newborn rats, when ovaries are insensitive to gonadotrophins, depends on direct neurogenic influences [28].

It was found that both basic and gonadotrophin-stimulated progesterone secretion by luteal cells were higher after progesterone treatment [14, 38]. Recently, we discovered that progesterone stimulates its own synthesis in early CL, by an increase in 3β-hydroxysteroid dehydrogenase (3β-HSD) activity, whereas an antiprogesterone (onaprostone, Schering AG) decreases the activity of this enzyme [20]. Therefore, concentrations of both progesterone and OT in each CL provide an index of both luteal function and the degree of autocrine stimulation of activity.

Ascorbic acid is believed to act as an antioxidant, neutralising the oxidative by-products of cellular respiration in luteal cells, as an enzymatic cofactor in collagen synthesis and as a promotor of steroid and protein hormone synthesis [27]. It is present in cells involved in steroidogenesis, including those of the CL. Isolated CL from cattle [33], pig [13] and baboon [15] are able to secrete progesterone in a pulsatile manner. Thus, even though the CL is regulated by the hypothalamo-hypophysial system, it has a marked degree of autonomy and factors such as catecholamines, ovarian oxytocin, progesterone and ascorbic acid can operate locally to support its function. To understand the inter-relationships between local ovarian factors which may affect CL function, we measured progesterone, OT, DA, NA and ascorbic acid in bovine CL from different stages of the oestrous cycle and during the first 5 months of pregnancy.

2. MATERIALS AND METHODS

2.1. Corpora lutea collection

Ovaries with CL from heifers (n = 22) and cows (n = 20) of unknown reproductive status were collected within 30 min of death at a commercial slaughterhouse. Four stages of CL growth (days 1–5, n = 7; 6–10, n = 12; 11–16, n = 15; and 17–21, n = 8 of oestrous cycle) were estimated according to Ireland et al. [10]. The stage of pregnancy (end of month 1 until month 5; n = 27) was determined on the basis of foetal size and development. Immediately after collection, CL were perfused through the ovarian artery with ice-cold saline to remove blood; they were then divided into four parts and stored in liquid nitrogen.

2.2. Homogenisation of luteal tissue

The deep frozen tissue was homogenised (powdered) by means of a Vibratory Mill (Retsch MM-2). Portions of the weighed tissue powder were suspended in either phosphate buffer (for OT and progesterone determination), 0.1 M trichloracetic acid (TCA; for catecholamines) or in 3 % ice-cold metaphosphoric acid with 2.5 $mmol^{-1}$ EDTA (for ascorbic acid). Progesterone and OT were extracted from the powdered tissue as described by Tsang et al. [39]; recoveries averaged 90 and 85 %, respectively, and data were corrected for the procedural losses.

2.3. Hormone determinations

The concentrations of progesterone and OT were measured by radioimmunoassay (RIA) as described earlier [17, 19]. The sensitivities of the assays were 3 $pg \cdot mL^{-1}$ for OT and 0.3 $ng \cdot mL^{-1}$ for progesterone. Intra-assay variations were 7.5 and 7.4 %, respectively.

Tissue concentrations of NA and DA were determined by high performance liquid chromatography (HPLC) with electrochemical detection (HP 1049A; Hewlett-Packard). Catecholamines were extracted from the powdered luteal tissue (200–300 mg) with a ten-fold excess (w/v) of 0.1 M trichloroacetic acid containing 0.01 % $Na_2S_2O_5$ and 0.000 3 % ascorbic acid. The remainder of the procedure was as described earlier [19]. Recovery from this assay was 71 % and the final data were corrected for procedural losses.

For the ascorbic acid determination, powdered tissue was suspended in an ice-cold metaphosphoric acid 3 % w/v solution, containing 2.5 mmol $EDTA \cdot L^{-1}$. It was assayed by the colorimetric method of Smith [37] with the modification described by Luck and Zhao [26]. The sensitivity of the assay was 15–20 $mol \cdot L^{-1}$ and the intra-assay coefficient of variation was 7.6 %.

2.4. Statistical analysis

Data were evaluated by one-way ANOVA and differences between means were assessed by Tukey's test for unequal numbers of samples. The presence of significant variation shown by ANOVA was confirmed by the Kruskal-Wallis test because the normality of the data distribution could not be assumed.

3. RESULTS

There were no significant differences in the measured hormones between cows and heifers and between particular months of pregnancy, so these data were combined for

further analysis. The concentrations of DA and NA were correlated with each other (r = 0.66; $P < 0.01$) during the oestrous cycle. However, quantities of these substances were highest in newly formed CL ($P < 0.01$) compared with CL from other stages of the cycle and those of pregnant females. The concentration of DA was two to four times higher than that of NA in all CL except for those of pregnancy (*figure 1*). NA was negatively correlated ($P < 0.01$) with progesterone (r = –0.53) and OT (r = –0.41; *table I*), whereas progesterone and OT were correlated with each other (r = 0.81; $P < 0.01$) during all stages of the oestrous cycle, but not during pregnancy (*figure 2*; *table I*). The concentration of ascorbic acid (*figure 3*) followed those of progesterone (r = 0.68; $P < 0.001$) and OT (r = 0.42; $P < 0.01$). The lowest concentration of ascorbic acid was observed in regressed CL ($P < 0.01$) compared with CL from the second stage of the oestrous cycle (II) and pregnancy (*figure 3*).

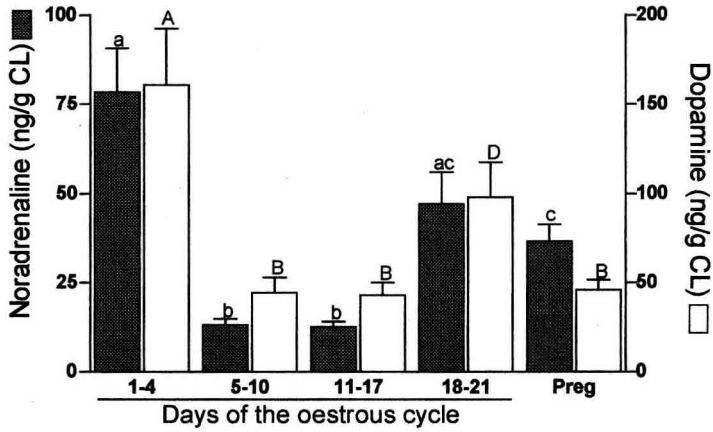

Figure 1. Noradrenaline (NA) and dopamine (DA) concentrations in the corpora lutea of cyclic (days 1–4, $n = 7$; 5–10, $n = 12$; 11–17, $n = 15$; 18–21, $n = 8$) and pregnant cattle ($n = 27$). Values with different superscripts are significantly different ($P < 0.05$). Note the different scales for NA and DA.

Table I. Coefficients of correlation for noradrenaline (NA), dopamine (DA), progesterone (P4), oxytocin (OT) and ascorbic acid (Aa) in corpora lutea from cyclic (four stages of the cycle combined; $n = 42$), and in pregnant cattle ($n = 27$).

Physiological status	Ratio of analysed factors							
	NA/DA	P4/NA	OT/NA	P4/OT	Aa/NA	Aa/DA	Aa/P4	Aa/OT
Oestrous cycle								
r =	0.66	–0.53	–0.41	0.81	–0.34	–0.37	0.68	0.42
P <	0.001	0.01	0.01	0.001	ns	0.01	0.001	0.01
Pregnancy	ns	ns	ns	ns	ns	ns	0.35 $P < 0.04$	ns

ns: not significantly different.

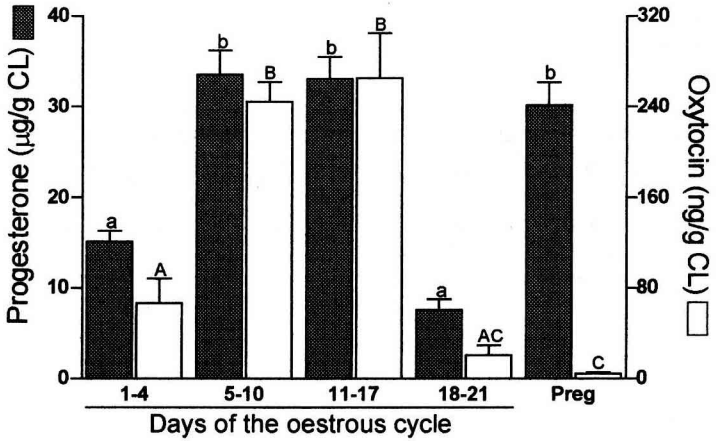

Figure 2. Progesterone and oxytocin concentrations in the corpora lutea of cyclic (days 1–4, $n = 7$; 5–10, $n = 12$; 11–17, $n = 15$; 18–21, $n = 8$) and pregnant ($n = 27$) cattle. Values with different superscripts are significantly different ($P < 0.05$). Note the different scales for progesterone and oxytocin.

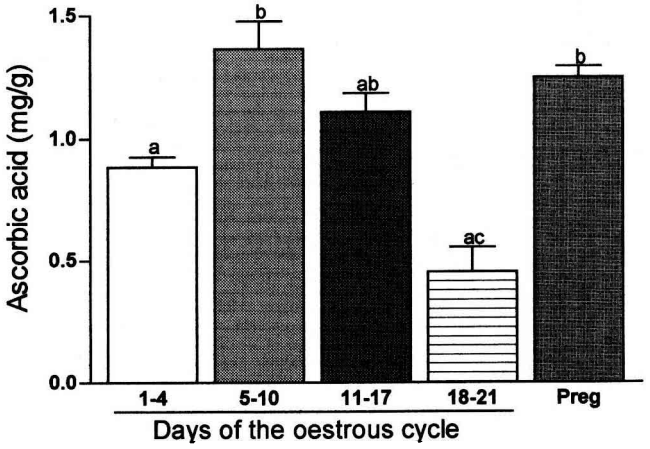

Figure 3. Concentrations of ascorbic acid in the corpora lutea from four stages of the oestrous cycle (days 1–4, $n = 7$; 5–10, $n = 12$; 11–17, $n = 15$; 18–21, $n = 8$) of heifers or cows. 'Preg' means 1–5 months of pregnancy ($n = 27$). Values with different superscripts are significantly different ($P < 0.05$).

4. DISCUSSION

Low concentrations of DA and NA were observed in fully developed CL during the oestrous cycle and gestation; between three and five times more of these catecholamines were found in newly formed and regressed CL. The present data confirm our earlier observations that although stimulation of CL β-receptors by catecholamines is important during all stages of its development, the most important role of the noradrenergic system does appear to be in the early CL. We also found that the amount of DA in the CL is two to four times higher than NA. The high correlation between DA and NA

(r = 0.66) and the presence of dopamine β-hydroxylase in bovine CL reported in another study [3], provide evidence that NA could be synthesised de novo in this tissue as shown earlier by Kotwica et al. [19]. The origin of DA in bovine CL is not clear. Battista et al. [3] and Denning-Kendall et al. [6] suggested that DA cannot be synthesised within the CL; they assumed that mast cells, stromal adrenergic nerves or the peripheral circulation are the source of DA, which is then preferentially taken up by luteal cells.

Although DA concentrations in CL are very high and can increase progesterone and OT secretion in vitro, DA receptors do not participate in this stimulation [19]. Thus, it is assumed that DA itself does not affect CL function directly. Since the CL contains dopamine β-hydroxylase and is the site of NA synthesis, we suggest that the only role of DA in the CL is that of a precursor for NA synthesis. However, in rat ovarian cells it was shown that DA and DA agonists increased progesterone in the media by an increase in 3β-HSD activity [1]. This would suggest some differences between species.

The present data showed that the level of ascorbic acid within the CL changed significantly with the stage of the oestrous cycle in a manner similar to changes in progesterone content both in heifers and in cyclic and pregnant cows. This may reflect the increasing need for antioxidants as steroidogenic activity increases within CL. Rapoport et al. [32] demonstrated that in bovine CL ascorbate was significantly correlated with cytochrome P-450scc and plasma progesterone levels. Thus, they proposed that the correlation between the levels of some antioxidant enzymes and compounds with progesterone levels indicates that antioxidative mechanisms are activated to cope with steroidogenesis in the bovine CL. The data by Luck and Jungclas [24] provide evidence for the involvement of ascorbic acid in the stimulation of progesterone synthesis and OT secretion from bovine granulosa cells in vitro [25]. Thus, maximal luteal and follicular function was associated with increased concentrations of total ascorbate within the tissue in pigs [30] and in cattle [26], although its precise role in steroidogenesis has not been established [22].

Catecholamines require ascorbic acid for their biosynthesis [22] and ascorbate seems to synergise with catecholamines in stimulating ovarian oxytocin secretion [7, 23, 24]. The lack of correlation between ascorbic acid and catecholamines in our study suggests that ascorbic acid is less important for catecholamine protection from oxidation within the CL. The concentration of ascorbic acid in CL is markedly decreased during luteal regression. It has been shown that prostaglandin $F_{2\alpha}$ (PGF) caused a rapid depletion of luteal ascorbate stores when given to rats [3, 21, 35] and pigs [31]. The ability of PGF to stimulate ascorbate secretion implies that this process is crucial for luteal regression.

The correlation of OT and progesterone in cyclic CL supports the concept that OT can be involved in the secretion of progesterone [34]. The OT prohormone is only synthesised in the ovaries during the first few days of the oestrous cycle [12] and once depleted during the cycle cannot be replaced [11]. Therefore, the correlation between OT and progesterone in pregnant animals (*table 1*) was not observed.

In conclusion, concentrations of catecholamines and ascorbic acid within the bovine CL vary during the oestrous cycle. Periods of maximal luteal function are associated with an increased antioxidant potential of ascorbic acid within the CL. Moreover, the time of luteolysis coincides with losses of ascorbic acid from the bovine CL. Ascorbic acid may promote luteal function during the oestrous cycle and help to maintain cyclic luteal function as suggested by its correlation with luteal progesterone and oxytocin content. However, its concentration is not correlated with those of catecholamines within the CL.

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REFERENCES

[1] Arakawa S., Yago N., Isobe S., Ohkawa R., Mori H., Okinaga S., Dopamine increases the ovarian progesterone synthesis of PMSG-treated rats by regulating 3β-HSD activity, Nippon Naibunpi Gakkai Zasshi 70 (4) (1994) 447–456.

[2] Bahr J.M., Ben-Jonathan N., Elevated catecholamines in porcine follicular fluid before ovulation, Endocrinology 117 (1985) 620–623.

[3] Battista P.J., Rexroad C.E., Poff J.P., Condon W.A., Support for a physiological role of endogenous catecholamines in the stimulation of bovine luteal progesterone production, Biol. Reprod. 41 (1989) 807–812.

[4] Ben-Jonathan N., Arbogast L.A., Rhoades T.A., Bahr J.M., Noradrenaline in the rat ovary: ontogeny and de novo synthesis, Endocrinology 115 (1984) 1426–1430.

[5] Condon W.A., Black D.L., Catecholamine-induced stimulation of progesterone by the bovine CL in vitro, Biol. Reprod. 15 (1976) 573–578.

[6] Denning-Kendall P.A., Wild M.D., Wathes D.C., Regional differences in catecholamine concentrations in bovine ovaries analysed by high-performance liquid chromatography, J. Endocrinol. 129 (1991) 221–226.

[7] Endo T., Aten R.F., Wang F., Behrman H.R., Coordinate induction and activation of metalloproteinase and ascorbate depletion in structural luteolysis, Endocrinology 133 (1993) 690–698.

[8] Fernandez-Pardal J., Gimeno M.F., Gimeno A.L., Catecholamines in sow Graffian follicles at prooestrus and at dioestrus, Biol. Reprod. 34 (1986) 439–445.

[9] Ferrante F., Bronzetti E., Cavalloti C., Ricci A., Amenta F., The noradrenergic innervation of the ovary in old rats, Mech. Ageing Dev. 54 (1990) 55–62.

[10] Ireland J.J., Murphee R.L., Coulson P.B., Accuracy of predicting stages of bovine oestrous cycle by gross appearance of the corpus luteum, J. Dairy Sci. 63 (1980) 155–160.

[11] Ivell R., Vasopressinergic and oxytocinergic cells: models in neuropeptide gene expression, in: Turner W.H. (Ed.), Neuropeptides and their Peptidases, Ellis Horwood Ltd, Chichester, UK and VCH, Weinheim, FRG, 1987, pp. 31–64.

[12] Ivell R., Brackett K.H., Fields M.J., Richter D., Ovulation triggers oxytocin gene expression in the bovine ovary, FEBS Lett. 190 (1985) 263–267.

[13] Jarry H., Einspanier A., Kangiebner L., Dietrich M., Holtz W., Wuttke W., Release and effects of oxytocin on estradiol and progesterone secretion in porcine corpora lutea as measured by an in vivo microdialysis system, Endocrinology 126 (1990) 2350–2358.

[14] Kawano T., Okamura H., Tajima C., Fukuma K., Katabuchi H., Effect of RU 486 on luteal function in early pregnant rat, J. Reprod. Fert. 83 (1988) 279–285.

[15] Khan-Dawood F.S., Gargiulo A.R., Dawood M.Y., Baboon corpus luteum: autonomous pulsatile progesterone secretion and evidence for intraluteal oscillator demonstrated by in vitro macroretrodialysis, J. Clin. Endocrinol. Metab. 79 (1994) 1790–1796.

[16] Kotwica J., Skarzynski D., Influence of oxytocin removal from the corpus luteum on secretory function and duration of the oestrous cycle in cattle, J. Reprod. Fertil. 97 (1993) 411–417.

[17] Kotwica J., Skarzynski D., Jaroszewski J., The coccygeal artery as a route for the administration of drugs into the reproductive tract of cattle, Vet. Rec. 127 (1990) 38–40.

[18] Kotwica J., Skarzynski D., Jaroszewski J., Kotwica G., Effect of noradrenaline on the release of progesterone and ovarian oxytocin in cattle, Anim. Reprod. Sci. 26 (1991) 179–191.

[19] Kotwica J., Skarzynski D., Bogacki M., Miszkiel G., Influence of dopamine as noradrenaline precursor on the secretory function of the bovine corpus luteum using in vitro model, Br. J. Pharmacol. 118 (1996) 1669–1974.

[20] Kotwica J., Miszkiel G., Skarzynski D., Bogacki M., Mechanism of action of progesterone on its own synthesis in bovine corpus luteum, Biol. Reprod. 58 (suppl. 1) (1998) 338.

[21] Leovit K., Badawy S., Laurence K., Alteration of corpus luteum function in the pregnant rat by antiluteinizing serum, Endocrinology 84 (1968) 405.

[22] Levine M., Morita K., Ascorbic acid in endocrine systems, Vitam. Horm. 42 (1985) 1–64.

[23] Luck M.R., Cholinergic stimulation, through muscarinic receptors, of oxytocin and progesterone secretion from bovine granulosa cells undergoing spontaneous luteinization in serum-free culture, Endocrinology 126 (1990) 1256–1263.

[24] Luck M.R., Jungclas B., The time-course of oxytocin secretion from cultured bovine granulosa cells stimulated by ascorbate and catecholamines, J. Endocrinol. 116 (1987) 247–258.

[25] Luck M.R., Jungclas B., Catecholamines and ascorbic acid as stimulators of bovine ovarian oxytocin secretion, J. Endocrinol. 114 (1987) 423–430.

[26] Luck M.R., Zhao Y., Identification and measurement of collagen in the bovine corpus luteum and its relationship with ascorbic acid and tissue development, J. Reprod. Fert. 99 (1993) 647–652.

[27] Luck M.R., Jeyaseelan E., Scholes R.A., Ascorbic acid and fertility, Biol. Reprod. 52 (1995) 262–266.

[28] Malamed S., Gibney J.A., Ojeda S.R., Ovarian innervation develops before initiation of folliculogenesis in the rat, Cell Tissue Res. 270 (1992) 87–93.

[29] Miyamoto A., Schams D., Oxytocin stimulates release from microdialysed bovine corpus luteum in vitro, Biol. Reprod. 44 (1991) 1163–1170.

[30] Petroff B.K., Dabrowski K., Ciereszko R.E., Ottobre J.S., Total ascorbate and dehydroascorbate concentrations in porcine ovarian stroma, follicles, and corpora lutea throughout the oestrous cycle and pregnancy, Theriogenology 47 (1997) 1265–1273.

[31] Petroff B.K., Ciereszko R.E., Dabrowski K., Ottobre A.C., Pope W.F., Ottobre J.S., Depletion of vitamin C from pig corpora lutea by prostaglandin F2 alpha-induced secretion of the vitamin, J. Reprod. Fert. 112 (2) (1998) 243–147.

[32] Rapoport R., Sklan D., Wolfenson D., Shaham-Albalancy A., Hanukoglu I., Antioxidant capacity is correlated with steroidogenic status of the corpus luteum during the bovine oestrous cycle, Biochim. Biophys. Acta 1380 (1) (1998) 133–140.

[33] Rossmanith W.G., Schick M., Benz R., Lauritzen Ch., Autonomous progesterone secretion from the bovine corpus luteum in vitro, Acta Endocrinol. (Copenh) 124 (1991) 179–187.

[34] Sakumoto R., Ando Y., Okuda K., Progesterone release of bovine corpus luteum in response to oxytocin in different culture systems, J. Reprod. Dev. 42 (1996) 199–204.

[35] Sato T., Iesaka T., Jyujo T., Taya K., Ishikawa J., Igarashi M., Prostaglandin-induced ovarian ascorbic acid depletion, Endocrinology 95 (1974) 417–420.

[36] Sauerwein H., Miyamoto A., Gunther J., Meyer H.H.D., Schams D., Binding and action of insulin-like growth factors and insulin in bovine luteal tissue during the oestrous cycle, J. Reprod. Fert. 96 (1992) 103–115.

[37] Smith J.A., An improved method of ascorbic acid measurement in the ovarian ascorbic acid depletion assay, J. Endocrionol. 55 (1972) 460–462.

[38] Tanaka N., Iwamasa K., Matsura K., Okamura H., Effects of progesterone and antiprogesterone RU486 on ovarian 3β-hydroxysteroid dehydrogenase activity during ovulation in the gonadotropin-primed immature rat, J. Reprod. Fert. 97 (1993) 167–172.

[39] Tsang P.C.W., Walton J.S., Hansel W., Oxytocin-specific RNA, oxytocin and progesterone concentrations in corpora lutea of heifers treated with oxytocin, J. Reprod. Fertil. 89 (1990) 77–84.