Regulation of lipid peroxidation by nitric oxide and PGF_{2α} during luteal regression in rats

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Corpus luteum regression is related to an increased generation of reactive oxygen species. Although several studies indicate that PGF_{2α} is involved in regression of the corpus luteum in mammalian species through an increase in reactive oxygen species, the exact mechanism remains unknown. In the present study, the relationship between nitric oxide and PGF_{2α} in regulation of lipid peroxidation was studied. Ovarian tissue from pseudopregnant rats at mid- (day 5) or late phase or at the time of regression (day 9 of pseudopregnancy) of corpus luteum development was used. Thiobarbituric acid reactants, used as a lipid peroxidation index, were higher on day 9 of pseudopregnancy than on day 5. In contrast, glutathione content (an antioxidant metabolite) was lower on day 9 than on day 5 of pseudopregnancy. These results indicate that there was an enhanced oxidative status in ovarian tissue during luteolysis. Administration of N^ω-nitro-L-arginine methyl ester (L-NAME: 600 μmol l⁻¹), a competitive nitric oxide synthase (NOS) inhibitor, led to a decrease in basal thiobarbituric acid reactant content in ovarian tissue from rats on day 9 of pseudopregnancy only, indicating that

during regression of the corpus luteum, NO could act as intermediary in ovarian lipid peroxidation. Administration of a luteolytic dose (3 μg kg⁻¹ body weight i.p.) of a synthetic PGF_{2α} increased thiobarbituric acid reactant content in ovaries from rats on day 9 of pseudopregnancy. As this effect was reversed partially by L-NAME, it is proposed that during regression of corpora lutea, PGF_{2α} and NO are involved in regulation of lipid peroxidation. As this effect was only reversed partially, it is possible that there is another mechanism involving PGF_{2α} (but not the NO–NOS pathway) in regulation of ovarian lipid peroxidation. Furthermore, the administration of PGF_{2α} enhanced ovarian NOS activity, whereas cyclooxygenase inhibition (by indomethacin treatment *in vivo*) reduced it. As western blotting of ovarian homogenates obtained from PGF_{2α}-injected rats increased inducible NOS (iNOS) content, it is concluded that PGF_{2α} enhances both activity and synthesis of NO in rat ovarian tissues during luteolysis. Taken together, these results indicate that in ovaries with regressing corpora lutea, both NO and PGF_{2α} are involved in part in regulation of lipid peroxidation.

Introduction

Luteal regression or luteolysis is a normal and necessary event in the mammalian reproductive cycle. This mechanism has been studied widely in relation to its involvement in early pregnancy dysfunction. Corpus luteum involution has been related to increased generation of reactive oxygen species, such as O₂⁻ and H₂O₂, by intact ovaries (Riley and Behrman, 1991; Sawada and Carlson, 1991a; Carlson *et al.*, 1993). One consequence of production of free radicals in ovarian tissue is lipid peroxidation (Kappus, 1985), which occurs within the plasma membrane of luteal cells and may be associated with loss of gonadotrophin receptors and decreased formation of cAMP, thereby decreasing the steroidogenic ability of the

corpus luteum during involution (Aulette and Flint, 1988; Wang HZ *et al.*, 1991). Protection against reactive oxygen species in cells is provided by enzymes (superoxide dismutase, catalase and glutathione peroxidase), metabolites (GSH) or antioxidant vitamins (Aten *et al.*, 1992) and may be regulated by hormones (Laloraya *et al.*, 1988; Sugino *et al.*, 1993, 1998, 1999).

Nitric oxide (NO) has emerged as an important regulator of many physiological processes, including vascular tone, platelet function, neurotransmission, host defence mechanisms (Moncada *et al.*, 1991), ovarian function (Van Voorhis *et al.*, 1994; Yamaguchi *et al.*, 1997) and embryonic development (Gouge *et al.*, 1998). NO is produced when nitric oxide synthase (NOS) catalyses the metabolism of L-arginine to citrulline (Marletta *et al.*, 1988). NO can react with molecular oxygen to give N₂O₃, which causes DNA deamination (Nguyen *et al.*, 1989), and with other oxygen

species, such as superoxide (O_2^-), yielding peroxynitrite ($ONOO^-$), which causes tissue injury (Ischiropoulos *et al.*, 1992; Nathan, 1992). NO exerts its biological effects by binding iron-containing guanylate cyclase resulting in an increase in intracellular cGMP content. In addition, a direct activation of haem-containing enzymes, such as cyclooxygenase 1 and 2, has been reported (Rettori *et al.*, 1992).

$PGF_{2\alpha}$ is involved in regression of corpora lutea (Oloffson and Selstam, 1988) at least in part through the increase in reactive oxygen species in rats (Sawada and Carlson, 1991b; Aten *et al.*, 1992). However, the mechanism by which $PGF_{2\alpha}$ and reactive oxygen species induce luteolysis is unknown (Shimamura *et al.*, 1995). There is growing evidence that $PGF_{2\alpha}$ is also important for involution of corpora lutea in humans (Bennegard *et al.*, 1984; Ichikawa *et al.*, 1990).

In previous studies of the mechanism of regression of corpora lutea, Motta *et al.* (1996) reported that oxytocin increased ovarian $PGF_{2\alpha}$ production during the late phase of pseudopregnancy in rats. This action was mediated by enhancing ovarian NOS activity (Motta *et al.*, 1997). It was also found that endogenous NO increases $PGF_{2\alpha}$ synthesis during luteolysis (Motta and Gimeno, 1997). By treatment of intrabursa ovarian sacs with two competitive NOS inhibitors and an NO generator, it was demonstrated that locally produced NO had an antisteroidogenic effect, decreasing serum progesterone concentrations and increasing ovarian $PGF_{2\alpha}$ production (Motta *et al.*, 1999). The aim of the present study was to clarify whether the NO system and ovarian $PGF_{2\alpha}$ production are related in modifying lipid peroxide concentrations during luteolysis, leading to morpho-functional involution of the corpus luteum. As the studies were performed in homogenates of whole ovarian tissue obtained from immature female pseudopregnant rats, it could be assumed that luteal cells were predominant in the assays.

Materials and Methods

Animals

The animal model was as described by Lahav *et al.* (1989). Briefly, immature (age 28–30 days) female rats of the Wistar strain were each given 15 iu pregnant mares' serum gonadotrophin i.p. (PMSG; Sigma Chemical Co., St Louis, MO). Day 0 of pseudopregnancy was 48 h after treatment. Administration of PMSG induced formation of a corpus luteum that remained functional for 9 ± 1 days as determined by Motta *et al.* (1996) by progesterone radioimmunoassay. Rats were housed under controlled temperature (22°C) and illumination (14 h light:10 h dark; lights on at 05:00 h) and were allowed free access to Purina rat chow and water. The animals were anaesthetized with ether and killed by cervical dislocation. Rats with ovarian tissue with corpora lutea at the mid-stage of development (day 5) or at the late stage or that were regressing (day 9 of pseudopregnancy) were used. All procedures involving animals were approved by the Animal Care and Use

Committee of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

$PGF_{2\alpha}$ -induced luteolysis

On day 9 of pseudopregnancy ten rats were injected i.p. with a luteolytic dose ($3 \mu\text{g kg}^{-1}$ body weight) of $PGF_{2\alpha}$ (synthetic derived: ILIREN, Hoechst, Roussel Vet) to define the conditions for $PGF_{2\alpha}$ -induced luteolysis. Animals were killed at different times after treatment (0–2.5 h after $PGF_{2\alpha}$ injection). Serum progesterone concentrations were determined by radioimmunoassay. Control animals were injected with saline solution.

Cyclooxygenase inhibition

On day 8 of pseudopregnancy (before luteolysis) ten rats were injected i.p. with a cyclooxygenase inhibitor (2.5 mg indomethacin kg^{-1} body weight; Sigma) between 22:00 h and 24:00 h. On day 9 of pseudopregnancy the rats received a second dose of indomethacin (2.5 mg kg^{-1} body weight) between 08:00 h and 10:00 h. At 2 h after the second injection the rats were anaesthetized with ether and killed by cervical dislocation. Ovaries were removed rapidly, pooled and held in ice for determination of NOS activity. Control animals were injected with saline solution.

Oxidative stress-related parameters

Glutathione content (GSH). The glutathione assay was performed as described by Tietze (1969). As the reduced form of glutathione (GSH) comprises the bulk of cellular protein sulphhydryl groups and accurate measurement of the tissue oxidized form of glutathione (GSSG) has proven difficult because of the much lower amounts of this form normally present and the absence of a convenient chemical feature such as that possessed by the reduced peptide, measurement of acid-soluble thiol is commonly used for estimation of GSH content in tissue extracts. Briefly, homogenates of pooled ovarian tissues (in 1.75 mol Tris buffer l^{-1} , pH 7.4) obtained at different stages of the luteal phase were incubated with NADPH and glutathione reductase to yield the enzymatic reduction of the oxidized form (GSSG) to GSH. Ellman's reagent (a sulphhydryl reagent 5,5'-dithiobis-(2-nitrobenzoic acid; Sigma), was added to the incubation medium. The chromophoric product resulting from the reaction between Ellman's reagent and GSH possesses a molar absorption at 412 nm that is linear to the first beyond 6 min; after this the reaction remains constant. Results are expressed as $\mu\text{mol GSH (g ovarian tissue)}^{-1}$.

Thiobarbituric acid reactants. The amount of malondialdehyde, which is formed from the breakdown of polyunsaturated fatty acids, may be taken as an index of peroxidation reaction. The method used in the present study, as described by Buege and Aust (1978), quantifies malondialdehyde as the product of lipid peroxidation that reacts with trichloroacetic acid–thiobarbituric acid–HCl

(Sigma: 15% w/v, 0.375% w/v and 0.25 mol l^{-1} , respectively), yielding a red compound that absorbs at 535 nm.

Homogenates of ovarian pooled tissue (in 2.5% (w/v) trichloroacetic acid) obtained at different stages of the luteal phase were treated with trichloroacetic acid–thiobarbituric acid–HCl and heated for 15 min in a boiling waterbath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 g for 10 min. The absorbance of the sample was determined at 535 nm. Content of thiobarbituric acid reactants (TBAR) is expressed as μmol malondialdehyde formed g^{-1} ovarian tissue.

Progesterone radioimmunoassay

Progesterone was measured in serum samples (Abraham *et al.*, 1971). Briefly, the blood was allowed to clot and the serum was removed and frozen until used. Serum was extracted with 2 ml diethyl ether and progesterone concentrations were determined by radioimmunoassay. The progesterone antiserum (provided by G. D. Niswender, Colorado State University, Fort Collins, CO) was highly specific for progesterone and showed low crossreactivity ($< 2.0\%$ for 20α -dihydro-progesterone and deoxycorticosterone and 1.0% for other steroids normally found in serum). The sensitivity was 5–10 pg per tube and 2–5 μl serum was assayed routinely.

Nitric oxide synthase activity

NOS activity in ovarian pooled homogenate was determined by monitoring the formation of $\text{L-[}^{14}\text{C]citulline}$ from $\text{L-[}^{14}\text{C]arginine}$ as described by Salter *et al.* (1991). Briefly, the frozen tissue was homogenized (Tissuemizer Tekmar; Thomas Scientific, New Jersey) at 0°C in 3 volumes of 50 mmol Hepes l^{-1} , 1 mmol DL-dithiothreitol l^{-1} , 1 mmol NADPH l^{-1} (pH 7.5) and L-valine (50 mmol l^{-1}) to minimize any interference from arginase. Samples were incubated at 37°C with 10 μmol ^{14}C arginine l^{-1} (0.3 μCi ; 1 Ci = 37 GBq). After 15 min of incubation, samples were centrifuged for 10 min at 1000 g and then applied to 1 ml DOWEX AG50W-X8 (Na^+ form) resin. The radioactivity was measured by liquid scintillation counting. Intra- and interassay variations were both $< 8.0\%$.

Western blotting for inducible NOS

Western blotting of ovarian homogenates was performed according to the procedure described by Olson *et al.* (1994) to quantify changes in protein content. Briefly, ovaries from control rats (PMSG, no $\text{PGF}_{2\alpha}$ injection, saline injection) and from treated rats (PMSG, $\text{PGF}_{2\alpha}$ injection) were placed in 300 μl ice-cold homogenization buffer (20 mmol Tris base l^{-1} , pH 7.4; 1 mmol EDTA l^{-1} ; 1 μg aprotinin ml^{-1} ; 10 μg soybean trypsin inhibitor ml^{-1} ; 10 μg caproic acid ml^{-1}), homogenized and sonicated on ice, and then centrifuged at 7800 g for 10 min at 4°C . The protein content of the resulting supernatant was determined using BSA as the standard. Equal amounts of protein (100 μg) from

homogenized ovaries were diluted in $4 \times$ SDS sample buffer (2.5 ml of 0.5 mol Tris–HCl l^{-1} , pH 6.8: 10% (w/v) SDS; 5% (v/v) β -mercaptoethanol; 0.3% (v/v) bromophenol blue dye; 20% (v/v) glycerol) and heated at 95°C for 4 min. Protein fractions were loaded on to 4.0–7.5% (w/v) gradient SDS-PAGE gels and separated by electrophoresis for 2 h at a constant current (20 mA). Rainbow-coloured protein mass markers (14.3–200.0 kDa; BioRad, CA) were run on each gel. Separated proteins were electrotransferred onto nitrocellulose membranes (Sigma) at 4°C overnight at constant voltage (40 V) in a buffer containing 25 mmol Tris l^{-1} , 192 mmol glycine l^{-1} and 20% (v/v) methanol. The membranes were blocked in 5% (w/v) milk powder in T–TBS (0.1% (v/v) Tween + TBS (10 mmol Tris l^{-1} and 100 mmol NaCl l^{-1})) for 1 h at 25°C . The presence of iNOS was confirmed by incubating the membranes with polyclonal antibody anti-iNOS (dilution 1:1000, Transduction, Lexington) overnight at 4°C . The blots were incubated for 1 h with alkaline-phosphatase conjugated anti-rabbit IgG (dilution 1:4000, Sigma), washed in T–TBS. The iNOS contents were detected after the blots were exposed to βCIP and NBT (Sigma) and quantified by densitometry scanning. This relationship was linear. Positive controls for iNOS included homogenates of LPS-activated RAW 264-7 macrophages.

Statistical analysis

Student's t test or ANOVA were used to assess the statistical significance of differences between means. The level of significance was $P < 0.05$.

Results

The oxidative status of the ovarian tissue during mid- (day 5) and late (day 9) pseudopregnancy is shown (Fig. 1). The GSH content was significantly higher ($P < 0.001$) on day 5 than on day 9 of pseudopregnancy (Fig. 1a). Conversely, TBAR content was higher ($P < 0.001$) on day 9 than on day 5 of pseudopregnancy (Fig. 1b).

The effect of a competitive NOS inhibitor (N^G -nitro-L-arginine methyl ester; L-NAME; 600 μmol l^{-1}) added to the incubation medium 1 h before determination of TBAR content is shown (Fig. 2). Ovarian TBAR content was not modified in the mid-stage of pseudopregnancy by L-name (Fig. 2a), whereas on day 9 of pseudopregnancy, TBAR content in ovaries incubated with L-NAME decreased abruptly ($P < 0.001$) (Fig. 2b). Specificity was demonstrated by the fact that the enantiomer (D-NAME; 600 μmol l^{-1}) did not have this effect (Fig. 2b).

Rats were injected i.p. with 3 μg $\text{PGF}_{2\alpha}$ kg^{-1} body weight on day 9 of pseudopregnancy to define the conditions for $\text{PGF}_{2\alpha}$ -induced luteolysis (Fig. 3). Serum progesterone concentrations decreased significantly ($P < 0.001$) at 30 min after $\text{PGF}_{2\alpha}$ treatment and continued to decrease. The greatest decrease in progesterone concentrations was observed at 2 h after injection, when concentrations fell to < 5 ng ml^{-1} ; concentrations remained constant after this

Fig. 1. (a) Glutathione (GSH) content and (b) thiobarbituric acid reactants (TBAR) content of ovarian tissue from rats at mid- (day 5 of pseudopregnancy) and late (day 9 of pseudopregnancy) stages of corpus luteum development. Data represent GSH or TBAR content (mean $\pm$ SEM) determined from ten animals at each stage. MDA: malondialdehyde. *Value is significantly different from day 5 value ($P < 0.001$) by Student's *t* test.

time and so ovarian tissues obtained from rats after 2 h of $\text{PGF}_{2\alpha}$ injection were used.

TBAR content in incubated ovarian tissues from rats in the following groups was used to study the role of $\text{PGF}_{2\alpha}$ on lipid peroxidation and its possible relationship with the NO–NOS system: (i) rats without treatment (control); (ii) rats injected with $\text{PGF}_{2\alpha}$ 2 h before ovarian tissues were obtained; (iii) rats injected with $\text{PGF}_{2\alpha}$ whose ovaries were treated *in vitro* for 1 h with $600 \mu\text{mol L-NAME l}^{-1}$; (iv) rats with $\text{PGF}_{2\alpha}$ whose ovarian tissues were treated *in vitro* for 1 h with $600 \mu\text{mol D-NAME l}^{-1}$. $\text{PGF}_{2\alpha}$ administration increased ovarian TBAR content (Fig. 4); addition of L-NAME to the incubation medium partially reversed this effect. The fact that D-NAME could not mimic this effect led us to confirm the specificity of this result.

Ovarian tissue obtained from $\text{PGF}_{2\alpha}$ -treated animals (on day 9 of pseudopregnancy) showed a significant ($P < 0.001$) increase in NOS activity (Fig. 5a). In contrast, cyclooxygenase inhibition significantly decreased ($P < 0.001$) ovarian NOS activity. $\text{PGF}_{2\alpha}$ also enhanced synthesis of iNOS (Fig. 6).

Discussion

The results obtained in the present study show that ovarian tissue with functional corpora lutea has high GSH content and a lower TBAR content, whereas during regression of corpora lutea, GSH content is low and TBAR content

Fig. 2. Effect of a nitric oxide synthase (NOS) inhibitor (L-NAME; $600 \mu\text{mol l}^{-1}$) and the enantiomer (D-NAME; $600 \mu\text{mol l}^{-1}$) on thiobarbituric acid reactants (TBAR) content from ovaries in (a) mid- (day 5 of pseudopregnancy) and (b) late (day 9 of pseudopregnancy) corpus luteum development. Ovarian tissue was incubated for 1 h in Krebs' ringer phosphate buffer with L-NAME or D-NAME at 37°C in a Dubnoff metabolic shaker. Data represent TBAR content (mean $\pm$ SEM) determined from ten animals at each point. *Value is significantly different from corresponding value on day 5 of pseudopregnancy ($P < 0.001$) by ANOVA test.

increases. These results are in agreement with previous studies of rat (Wu *et al.*, 1993; Shimamura *et al.*, 1995) and human (Vega *et al.*, 1994) corpora lutea.

The fact that a specific NOS inhibitor decreased basal TBAR content in the late phase of corpus luteum development led to the suggestion that endogenous NO could be involved in lipid peroxidation. This possibility was not confirmed in ovarian tissue with functional corpora lutea (day 5 of pseudopregnancy). Considering that under appropriate conditions NO reacts with superoxide (O_2^-) resulting in the generation of peroxynitrite, a potent oxidant capable of enhancing lipid peroxidation (Hogg and Kalyanaraman, 1999; Poderoso *et al.*, 1999), it is proposed that during involution of corpora lutea, the lower superoxide dismutase activity (Shimamura *et al.*, 1995) would induce higher O_2^- content, thus yielding highly oxidant peroxynitrites. In contrast, on day 5 of pseudopregnancy when superoxide dismutase activity was at its

Fig. 3. Serum progesterone concentrations in rats that had been treated with a luteolytic dose of $\text{PGF}_{2\alpha}$ (Δ); ●, controls. The rats were injected i.p. with $3 \mu\text{g} \text{PGF}_{2\alpha} \text{kg}^{-1}$ body weight on day 9 of pseudopregnancy. The animals were anaesthetized with ether and 1 ml samples of blood were collected at 30 min intervals between 0 h and 2.5 h after injection. Data represent serum progesterone concentrations determined by radioimmunoassay (mean $\pm$ SEM; $n = 10$). *Value is significantly different from value at $t = 0$ ($P < 0.001$) determined by ANOVA.

Fig. 4. Thiobarbituric acid reactants (TBAR) content during spontaneous (control) and $\text{PGF}_{2\alpha}$ -induced luteolysis in rats on day 9 of pseudopregnancy. $\text{PGF}_{2\alpha}$: rats received $3 \mu\text{g} \text{PGF}_{2\alpha} \text{kg}^{-1}$ body weight. $\text{PGF}_{2\alpha} + \text{L-NAME}$: rats received $3 \mu\text{g} \text{PGF}_{2\alpha} \text{kg}^{-1}$ body weight plus the NOS inhibitor L-NAME ($600 \mu\text{mol l}^{-1}$). $\text{PGF}_{2\alpha} + \text{D-NAME}$: rats received $3 \mu\text{g} \text{PGF}_{2\alpha} \text{kg}^{-1}$ body weight plus the NOS inhibitor D-NAME ($600 \mu\text{mol l}^{-1}$). After injection of $\text{PGF}_{2\alpha}$ ovarian tissue was incubated for 1 h in Kreb's ringer phosphate buffer with L-NAME or D-NAME at 37°C in a Dubnoff metabolic shaker. Data represent TBAR content (mean $\pm$ SEM) determined from ten animals at each point. *Value is significantly different from control value ($P < 0.001$) by ANOVA.

highest (Shimamura *et al.*, 1995) this mechanism was not present as O_2^- concentrations were too low. The $\text{PGF}_{2\alpha}$ inducing luteolysis increased lipid peroxidation during the late phase of corpus luteum development. As L-NAME (a

Fig. 5. (a) Nitric oxide synthase (NOS) activity in ovarian homogenates from rats treated with $\text{PGF}_{2\alpha}$ ($3 \mu\text{g} \text{kg}^{-1}$ body weight) on day 9 of corpus luteum development. (b) NOS activity in ovarian homogenates in which cyclooxygenase activity was inhibited. Rats were injected on day 8 and day 9 of pseudopregnancy with indomethacin ($2.5 \text{mg} \text{kg}^{-1}$ body weight). Data represent NOS activity (mean $\pm$ SEM) determined from ten animals at each point. *Value is significantly different from control value ($P < 0.001$) by Student's *t* test.

specific NOS inhibitor) decreased in part the effect of $\text{PGF}_{2\alpha}$ on TBAR content it can be assumed that ovarian NO could be related to this action of $\text{PGF}_{2\alpha}$. It is important to point out that L-NAME treatment did not completely inhibit the effect of $\text{PGF}_{2\alpha}$ on lipid peroxidation, so it is concluded that there is probably another pathway independent to the NOS-NO system in the mechanism by which $\text{PGF}_{2\alpha}$ induces lipid peroxidation.

The fact that indomethacin reduced NOS activity on day 9 of pseudopregnancy led to the suggestion that endogenous $\text{PGF}_{2\alpha}$ content, which increases in ovarian tissue towards the time of luteolysis (Motta *et al.*, 1996), could enhance NOS activity, leading to enhanced lipid peroxidation. This last observation is in agreement with our previous report (Motta *et al.*, 1999). $\text{PGF}_{2\alpha}$ not only increased NOS activity but also enhanced iNOS synthesis, as was demonstrated by the immunoblot assay. It is important to consider that macrophages are present as normal components of developing corpora lutea of several species and increase in number during luteolysis (Brännström *et al.*, 1994; Hehnke *et al.*, 1994). By means

Fig. 6. Effect of a luteolytic dose of PGF_{2α} on steady state of ovarian iNOS from rats on day 9 of pseudopregnancy. (a) Rats received an i.p. injection of a synthetic derived PGF_{2α} on day 9 and were killed 2 h later. The ovaries were removed and homogenates (100 µg protein) were subjected to 4.0–7.5% (w/v) gradient SDS-PAGE followed by immunoprobining with a polyclonal iNOS antibody. Lane 1: 8 µl positive control LPS/RAW 264.7 macrophages. Lane 2: saline injected control group ($n = 7$). Lane 3: rats injected with PGF_{2α} ($n = 8$). (b) Quantitative analysis of ovarian iNOS expression after PGF_{2α} treatment. Control: rats injected with saline. PGF_{2α}: rats injected with PGF_{2α}. Two experiments were carried out and the data were analysed by Student's t test. *Value is significantly different from control ($P < 0.05$).

of cytokine production macrophages modulate NO production by inducing both activity and iNOS production in a variety of tissues (Fosterman *et al.*, 1991; Nathan, 1992). Exposure of the corpora lutea to PGF_{2α} results in an influx of immune cells (Wang *et al.*, 1991) that could amplify the pathway described.

The involvement of PGF_{2α} in regression of corpora lutea (Oloffson and Selstam, 1988) at least in part through the increase of reactive oxygen species in rats (Sawada and Carlson, 1991b; Aten *et al.*, 1992) has been reported by other authors. The novel finding reported in the present study is the implication of the NO–NOS system as a potential mediator of lipid peroxidation induced in rat corpora lutea by PGF_{2α} during luteolysis (spontaneous or induced).

The authors would like to thank M. I. Casella and R. Morales for their technical support and C. Pokrant and S. Zorz for reviewing this manuscript. These studies were supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 4076, PEI 0045/97) and Programa Latinoamericano de Capacitación e Investigación en Reproducción Humana (PLACIRH, Re-Entry Grant PRE-032/99).

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Received 22 August 2000.

First decision 19 September 2000.

Accepted 27 November 2000.