Nonmitochondrial oxygen utilization by rabbit blastocysts and surface production of superoxide radicals

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A minimum value for nonmitochondrial oxygen utilization in rabbit blastocysts at day 6 *post coitum* was determined by measuring oxygen consumption in the presence of cyanide. A microcathode oxygen electrode was used to monitor oxygen concentration continuously during blastocyst incubation in a newly devised culture medium, and the uninhibited blastocyst was found to consume $2.79 \pm 0.09 \mu\text{l O}_2 \text{ h}^{-1} \text{ cm}^{-2}$. This rate was reduced by 51% in the presence of $1 \text{ mmol KCN l}^{-1}$. The addition of nitroblue tetrazolium to the cyanide-containing medium reduced net oxygen consumption by an additional 23% as the nitroblue tetrazolium was reduced to formazan. The ability of rabbit blastocysts to reduce nitroblue tetrazolium in the presence of cyanide was investigated using a spectrophotometric assay. Fractionation of blastocyst cells revealed that the enzymatic activity chiefly responsible for formazan production partitioned with the membrane/particulate fraction and could be solubilized by the detergent NP40. The enzyme was NAD(P)H-dependent, did not require divalent cations for activity, and appeared to contain no haeme moiety. The rate of formazan production in the spectrophotometric assay was markedly reduced by the presence of superoxide dismutase. The oxygen electrode and spectrophotometer data indicate that there is a superoxide-generating NAD(P)H oxidase on the blastocyst surface. Calculations based on the average surface area of rabbit blastocysts at day 6 show that these embryos can produce at least 8 nmoles of superoxide per embryo h^{-1} . Potential decidual effects of blastocyst-derived superoxide and its dismutated product, hydrogen peroxide, are discussed.

Introduction

In rabbits, the earliest signs of an endometrial reaction to the blastocyst have been detected at day 6 *post coitum*; this is 24 h before the trophoblast penetrates the blastocyst covering and begins to fuse with the endometrial epithelium (Schlafke and Enders, 1975). At day 6 *post coitum*, the rapidly expanding rabbit blastocysts are spaced along the uterine horns and are virtually immobilized because of their size. At this time the endometrium directly apposed to a blastocyst shows a fivefold increase in phospholipase activity (Hoffman *et al.*, 1984), as well as selective expression of the 25 kDa gap junction protein (Winterhager *et al.*, 1988). These early responses are followed by increased vascular permeability, detectable by day 6.5 *post coitum* (Hoos and Hoffman, 1980), and by marked local thickening of the endometrial stroma (the decidual reaction) by day 8 (Amoroso, 1964).

Among the chemical signals that may be involved in this blastocyst–endometrial interaction, prostaglandins appear to have a central role (Kennedy, 1983). In rabbits, the blastocyst (Dey *et al.*, 1980; Racowsky and Biggers, 1983) and the endometrium (Hoffman *et al.*, 1984) can synthesize prostaglan-

dins. Arachidonic acid, the substrate for prostaglandin synthesis, is a strong decidual agent in the uterus of the progestational rabbit (Hoffman *et al.*, 1977). Since arachidonic acid is released from membrane phospholipids by phospholipases, chiefly by phospholipase A₂ (Halliwell and Gutteridge, 1989), the local increase in phospholipase activity found on day 6 *post coitum* (Hoffman *et al.*, 1984) could initiate the decidual cell reaction in the endometrium. Thus, on day 6, the blastocyst signal should be capable of stimulating phospholipase activity.

A role for reactive oxygen species in blastocyst signalling in rabbits has been implicated by the cytochemical finding of hydrogen peroxide production on the surface of rabbit blastocysts facing the endometrium (Manes, 1992). Hydrogen peroxide is the central member of the reactive oxygen species group, being formed by the dismutation of superoxide, with which it can subsequently react to yield hydroxyl radicals (Halliwell and Gutteridge, 1989). The presence of hydrogen peroxide on the trophoblast surface therefore implies the presence of the other two species. Hydrogen peroxide appears between day 4 and day 5 *post coitum* and persists for the remainder of the preimplantation period (Manes, 1992). Not only do reactive oxygen species stimulate phospholipase activity (Carlson and Wu, 1991), but an inorganic source of

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reactive oxygen species, such as copper wire (Oster and Oster, 1974), elicits an intense decidual response in the endometrium of the progestational rabbit (Tobert, 1975).

The study reported here was undertaken to confirm and quantitate the cytochemical finding. The enzymatic activity producing hydrogen peroxide on the trophoblast is cyanide resistant (Manes, 1992); thus, a primary aim of this study was to determine whether cyanide-resistant oxygen consumption by rabbit blastocysts at day 6 could be detected in a microrespiration cell. Nitroblue tetrazolium was used to estimate minimum amounts of superoxide and hydrogen peroxide produced by individual rabbit blastocysts at day 6, as well as to characterize the NAD(P)H oxidase chiefly responsible for superoxide production in a spectrophotometric assay (Oberley and Spitz, 1985).

Materials and Methods

Embryos

Blastocysts were collected from normally ovulating New Zealand White does at day 6 ± 2 h *post coitum* and placed immediately into the defined sterile culture medium described by Manes (1992) and modified for these studies by omitting copper and manganese. The culture was maintained at $37\text{--}38^\circ\text{C}$ on a warming tray. The blastocysts were washed twice in 10 ml of this medium to remove adherent blood cells. The maximum and minimum diameters of each blastocyst were measured with an ocular micrometer; surface areas were calculated using the formula for a prolate spheroid.

Measurement of oxygen uptake

Pooled, washed blastocysts were divided randomly into groups of 3–5 for determination of oxygen uptake. The group of blastocysts was placed into a microrespiration cell (Strathkelvin Model RC200, Glasgow) containing 1.0 ml of the culture medium (Manes, 1992) maintained at 38.5°C . The medium was stirred gently throughout the period of the assay. Oxygen tension in the medium was determined continuously with a microcathode oxygen electrode (Strathkelvin Model 1302) and monitored by a Strathkelvin Instant Oxygen Meter (Model 781). When required, $10\text{ }\mu\text{l}$ from $\times 100$ stock solutions were added to the medium in the chamber using a Hamilton syringe with the needle inserted through a slot in the electrode seal. For all measurements, the blastocysts remained fully expanded throughout the period of the assay.

The rate of change of oxygen tension in the respiration chamber was determined from the slope of the first order regression from the digitized data. Oxygen utilization by the microcathode electrode in culture medium alone was measured immediately before introducing each group of embryos into the respiration chamber and was subtracted from the experimental values for that group. Oxygen consumption by blastocysts was calculated from their total surface area (cm^2) divided by the combined products of the culture medium volume (1 ml), the rate of change in oxygen tension (Torr h^{-1}) and the oxygen solubility coefficient of the culture medium ($1.2\text{ }\mu\text{mol O}_2\text{ l}^{-1}\text{ Torr}^{-1}$).

Determination of NAD(P)H oxidase activity

Groups of 10–15 washed blastocysts were pooled, removed from the zonae pellucidae with needles, opened widely to allow escape of all fluid, and then transferred by capillary pipette to a 10 ml wash in PBS. These collapsed blastocysts were then transferred to a 400 μl Eppendorf tube and pelleted by centrifugation at $15\,000\text{ g}$ for 3 s in a microfuge. The PBS supernatant was removed by aspiration.

For lysate assays, the cell pellet was resuspended in 100–300 μl of 50 mmol sodium phosphate l^{-1} buffer, pH 7.5, containing 0.3% NP40 (v/v) (Particle Data Laboratories, Elmhurst, IL) and 5 μg soybean trypsin inhibitor ml^{-1} (Sigma, St Louis, MO) and allowed to stand for 10 min in ice. The cell suspension was then agitated for 5 s on a vortex mixer followed by centrifugation at $15\,000\text{ g}$ for 5 min. Aliquots of this supernatant were assayed for activity in reducing nitroblue tetrazolium in a microcuvette providing a 1 cm light path through a volume of 50 μl (Beckman, Fullerton, CA). The reaction mixture consisted of 50 μl embryo lysate, 25 μl of 150 μmol hypoxanthine l^{-1} (Sigma) in water, and 1 μl of 4.25 mmol nitroblue tetrazolium l^{-1} in 10% (v/v) dimethylformamide, giving a final concentration of nitroblue tetrazolium of $5.6 \times 10^{-5}\text{ mol l}^{-1}$ (Oberley and Spitz, 1985). Assays were carried out at room temperature, recording absorbance at 560 nm at intervals of 1 min. NADH, NADPH and superoxide dismutase (from beef heart, Sigma S2139) were added in 1 μl volumes. The final concentrations of NADH and NADPH were 190 $\mu\text{mol l}^{-1}$. Superoxide dismutase (Sigma) was diluted in water to provide 1 U μl^{-1} . As a control for nonspecific protein effect, superoxide dismutase was held in a boiling water bath for 10 min. All assays were performed at least in duplicate. Blank values were obtained by substituting lysis buffer alone, and were subtracted from experimental values. Blastocyst cellular protein is reported to correlate closely with blastocyst surface area (Morgan and Kane, 1993); therefore, all values for absorbance at 560 nm were normalized to 100 mm^2 of blastocyst surface area.

Crude fractionation of the embryonic cell pellet was carried out by resuspension in 150 μl of 50 mmol sodium phosphate buffer l^{-1} , pH 7.5, containing 5 μg soybean trypsin inhibitor ml^{-1} but without NP40. The cells were allowed to swell for 10 min on ice; they were then homogenized with a sintered glass pestle and centrifuged at $15\,000\text{ g}$ for 5 min in a microfuge. The supernatant was aspirated and designated as the soluble fraction. The pellet was then resuspended in the complete buffer containing NP40 and processed as described above. NADPH was used as substrate in all assays of soluble or pellet fractions.

When required, reaction mixtures were adjusted to 1 mmol KCN l^{-1} , 3 mmol EDTA l^{-1} (J. T. Baker, Phillipsburg, NJ), 50 mmol imidazole l^{-1} (Sigma), 200 mmol pyridine l^{-1} (J. T. Baker), or 1 mmol allopurinol l^{-1} (Sigma) by adding the appropriate chemical in 1 μl of a $\times 77$ stock solution. Allopurinol was solubilized in 100 mmol NaOH l^{-1} .

Statistical analyses

Mean values for oxygen consumption by rabbit blastocysts are expressed $\pm$ SEM. Statistical comparisons were determined

Fig. 1. Oxygen tension in 1 ml of culture medium as a function of time of incubation of rabbit blastocysts. For the measurements illustrated here, three blastocysts at day 6 *post coitum* recovered from a single doe having a combined surface area of 1.134 cm² were used. Additions to the culture medium were made at the times indicated (arrows).

by Student's *t* test with the limit of significance set at $P < 0.05$.

Results

A typical recording of oxygen concentration in the culture medium during blastocyst incubation is shown (Fig. 1). The results of a large series of such measurements of oxygen utilization by the rabbit blastocyst at day 6, expressed as a function of blastocyst surface area, are summarized (Table 1). Under the conditions used, 1 mmol KCN l⁻¹ did not abolish embryonic oxygen consumption but reduced it to about half the control value. The addition of nitroblue tetrazolium to the respiration chamber reduced oxygen consumption to about half the KCN-resistant value (Table 1). The blastocysts become blue during the first 10 min of the incubation with nitroblue tetrazolium. Allopurinol also diminished KCN-resistant oxygen consumption, and in combination with nitroblue tetrazolium resulted in an inhibition that, whilst not additive, was greater than that caused by either compound alone (Table 1).

The enzymatic activity responsible for reduction of nitroblue tetrazolium was detectable in homogenates of rabbit blastocysts at day 6. The activity partitions with the particulate/membrane fraction and can be solubilized with NP40 (Fig. 2a). NADH and NADPH markedly stimulated the rate of reduction of nitroblue tetrazolium by blastocyst NP40 lysates (Fig. 2b). The rate of reduction in the presence of NAD(P)H was diminished by including native superoxide dismutase in the reaction mixture, whereas the boiled enzyme has no effect (Fig. 2b). The rate of reduction of nitroblue tetrazolium was not affected by 1 mmol KCN l⁻¹, 3 mmol EDTA l⁻¹, 50 mmol imidazole l⁻¹, 200 mmol pyridine l⁻¹ (Fig. 3), or 1 mmol allopurinol l⁻¹ (Fig. 4a). Some inhibition of nitroblue tetrazolium reduction by allopurinol was detectable in the absence of NAD(P)H (Fig. 4b).

Discussion

At day 6 *post coitum* the rabbit blastocyst is virtually a monolayer of about 80 000 trophoblast cells (Daniel, 1964). The two-layered inner cell mass occupies less than 10% of the

surface area, and widely spaced extra-embryonic endoderm cells have migrated no more than half-way along the inner surface of the trophoblast. The properties described as those of whole blastocysts are therefore principally those of the trophoblast cell population.

The magnitude of the cyanide-resistant fraction of oxygen consumption was not expected, since in earlier work 0.1 mmol cyanide l⁻¹ totally abolished oxygen consumption by rabbit blastocysts (Fridhandler *et al.*, 1957; Benos and Balaban, 1980). The reasons for the differences between the results in the work presented here and those of previous investigators are not clear but are likely to include differences in embryo handling and in the culture medium used for respiration measurements. The serum-free medium used to obtain the results reported here is an extensive modification of Ham's F-12 solution with an amino acid composition altered to mimic that of uterine fluids at this stage of pregnancy in rabbits (Jaszczak *et al.*, 1972; Miller and Schultz, 1987). Other modifications are the inclusion of taurine and soybean trypsin inhibitor (Manes, 1992). Blastocysts were collected, maintained and assayed for oxygen uptake in this medium. Blastocysts expand at approximately two-thirds the rate *in vivo* during culture for 24 h and display minimal evidence of cell death in the inner cell mass in this medium without serum or other supplements (C. Manes, unpublished). Cyanide at 1 mmol l⁻¹ is more than adequate to inhibit mitochondrial cytochrome oxidase (Mahler and Cordes, 1966). The nonmitochondrial oxygen consumption detected in the present work is thus due to cellular oxygenases and oxidases. However, since several of these potential contributors to oxygen utilization are also sensitive to cyanide (Tanaka and Knox, 1959; Yamamoto and Hayaishi, 1967; Badwey *et al.*, 1981), the cyanide-resistant value of 1.38 µl O₂ h⁻¹ cm⁻² must be viewed as a minimum value for nonmitochondrial oxygen utilization. The cyanide-sensitive fraction was not characterized further.

Reduction of nitroblue tetrazolium has been used for many years to evaluate the cyanide-resistant cell surface NAD(P)H oxidase activity in neutrophils (Baehner and Nathan, 1967). In that situation, nitroblue tetrazolium (NBT) is reduced to formazan by superoxide anions (O₂⁻) formed on the surface of activated neutrophils:

Thus, O₂ involved in the production of O₂⁻ would be regenerated on an equimolar basis in the presence of nitroblue tetrazolium. In our measurements of O₂ concentration in the respiration chamber during blastocyst incubation, net O₂ consumption would be reduced to the extent that nitroblue tetrazolium can react with O₂⁻. As the blastocysts become blue due to formazan formation, net KCN-resistant O₂ consumption is reduced by about half. This fraction is not only a measure of superoxide production but is also indicative of the availability of superoxide on the blastocyst (trophoblast) surface. Nitroblue tetrazolium is a divalent cation that traverses cell membranes with difficulty (Halliwell and Gutteridge, 1989); in the time span of the oxygen uptake measurements, most of the nitroblue tetrazolium added to the medium would have remained outside the cells. These findings indicate that about 25% of the oxygen consumed by the rabbit blastocyst at day 6 is used for superoxide production at the trophoblast surface.

Table 1. Effects of KCN, nitroblue tetrazolium and allopurinol on oxygen consumption by rabbit blastocysts at day 6 *post coitum*

Additions to the incubation medium	Number of determinations	Total number of blastocysts used	Oxygen consumption ($\mu\text{l O}_2 \text{ h}^{-1} \text{ cm}^{-2}$) mean $\pm$ SEM
Series 1	49	178	
None			2.79 ± 0.09
1 mmol KCN l^{-1}			1.38 ± 0.06^a
Series 2	5	19	
1 mmol KCN l^{-1}			1.91 ± 0.05
+ 200 μmol nitroblue tetrazolium l^{-1}			1.02 ± 0.09^a
Series 3	6	26	
1 mmol KCN l^{-1}			1.69 ± 0.09
+ 1 mmol allopurinol l^{-1}			0.75 ± 0.09^a
Series 4	7	30	
1 mmol KCN l^{-1}			1.71 ± 0.08
+ nitroblue tetrazolium + allopurinol as above			0.62 ± 0.07^a

^aSignificantly different from control, $P < 0.05$.

Fig. 2. (a) Reduction of nitroblue tetrazolium by soluble ($\bullet$, $n = 3$ assays) and pellet ($\blacksquare$, $n = 5$ assays) fractions prepared from rabbit blastocysts at day 6 *post coitum*. NADPH was the substrate provided in these reactions. For these assays, 26 blastocysts recovered from four rabbits were used. (b) Reduction of nitroblue tetrazolium by NP40 lysates of rabbit blastocysts at day 6 *post coitum*. Additions to the reaction mixture were NADH ($\blacktriangle$, $n = 2$ assays), NADPH ($\blacksquare$, $n = 13$ assays), NADPH plus superoxide dismutase ($\blacktriangledown$, $n = 5$ assays), or NADH plus boiled superoxide dismutase ($\blacklozenge$, $n = 3$ assays). The control reaction containing no additions is indicated by ($\bullet$, $n = 7$ assays). For these assays, 54 blastocysts recovered from eight rabbits were used. Values are means $\pm$ SD.

The direct measurements of oxygen consumption support the cytochemical results as evidence of a superoxide-generating enzyme that is not inhibited by 1 mmol KCN l^{-1} . In this respect, the blastocyst enzyme resembles the cell surface NAD(P)H oxidases of neutrophils and sea urchin eggs (Briggs *et al.*, 1975; Turner *et al.*, 1985). In other respects, however, it differs fundamentally. Its insensitivity to 3 mmol EDTA l^{-1} indicates that it does not require divalent cations, in contrast to the calcium-dependent NADPH oxidase in the sea urchin egg plasma membrane (Turner *et al.*, 1985). Furthermore, its insensitivity to 50 mmol imidazole l^{-1} or to 200 mmol pyridine l^{-1} contrasts with the neutrophil surface NAD(P)H oxidase, which

is strongly inhibited by these agents by virtue of its haeme centre (Iizuka *et al.*, 1985). The blastocyst enzyme thus appears to be a new member of the group of cell surface NAD(P)H oxidases.

It should be noted that the blastocyst enzyme would be well supplied with substrate. Rabbit blastocysts actively use the hexose monophosphate pathway (Fridhandler, 1961). The high rate of nucleic acid synthesis occurring in these embryos (Karp *et al.*, 1973) implicates this pathway in the provision of the requisite ribose. The NADPH generated must be reoxidized to maintain the hexose monophosphate pathway. A model for the trophoblast surface oxidase that is consistent with its two

Fig. 3. Reduction of nitroblue tetrazolium by NP40 lysates of rabbit blastocysts at day 6 *post coitum* in the presence of (a) 1 mmol KCN l⁻¹ (3); (b) 3 mmol EDTA l⁻¹ (3); (c) 50 mmol imidazole l⁻¹ (2) and (d) 200 mmol pyridine l⁻¹ (3). NADPH was the substrate provided in all of the reactions shown. Numbers in parentheses refer to the number of assays carried out. For these assays, 33 blastocysts recovered from five rabbits were used. Values are means $\pm$ SD.

functions would place the NAD(P)H binding site on the cytosolic face of a transmembrane protein and the oxygen-binding site on the exterior.

The enzymatic activities responsible for the remainder of the KCN-resistant oxygen utilization have not been completely identified. Allopurinol increased the inhibition caused by nitroblue tetrazolium alone. A low rate of allopurinol-inhibitable reduction of nitroblue tetrazolium was detectable in the absence of NAD(P)H in the cuvette assay. These findings, together with the recent identification of xanthine oxidase cDNA clones in a recombinant library prepared from rabbit blastocysts at day 6 (C. Manes, unpublished), indicate that xanthine oxidase is present and active in rabbit embryos at this stage. The low activity seen in the cuvette assay contrasts with the prominent effect on oxygen consumption in the respiration chamber, and suggests that allopurinol may have nonspecific effects on oxygen utilization beyond its specific inhibition of xanthine oxidase. It should be stressed that allopurinol shows no inhibition of NAD(P)H-stimulated reduction of nitroblue tetrazolium which clearly differentiates this enzyme from xanthine oxidase. It is very likely that other enzymes are also

contributing to KCN-resistant oxygen utilization by rabbit blastocysts.

The surface area of the average rabbit blastocyst at day 6 is 25 mm² (Daniel, 1964; and present data). Given a total oxygen consumption of approximately 2.8 μ l h⁻¹ cm⁻², the average blastocyst at day 6 would consume 30 nmol O₂ h⁻¹. If, as indicated by the data presented, approximately 25% of this O₂ is being converted to superoxide at the trophoblast surface, superoxide is being produced at a rate of about 8 nmol h⁻¹. Two moles of superoxide are required to yield one mole of hydrogen peroxide by dismutation (Halliwell and Gutteridge, 1989). The potential yield of hydrogen peroxide from the surface of the average blastocyst at day 6 is thus about 4 nmol h⁻¹.

Any hydrogen peroxide generated at the trophoblast surface would be separated from the endometrial epithelium only by the thinning blastocyst covering (Denker and Gerdes, 1979) through which it can diffuse with the same ease as water molecules (Halliwell and Gutteridge, 1989). Transient or steady-state concentrations of hydrogen peroxide in this trophoblast-endometrial space are unknown, but it should be

Fig. 4. Reduction of nitroblue tetrazolium by NP40 lysates of rabbit blastocysts at day 6 *post coitum* in the presence (■, $n = 2$ assays) or absence (●, $n = 2$ assays) of 1 mmol allopurinol l⁻¹. (a) NADPH was provided as substrate in these reactions. (b) NADPH was not provided. For the assays shown in (a) and (b), 40 blastocysts recovered from five rabbits were used. Values are means $\pm$ SD.

noted that hydrogen peroxide can exert significant biological effects at micromolar concentrations. At 100 $\mu\text{mol l}^{-1}$, hydrogen peroxide mimics many of the effects of insulin on adipocytes (Mukherjee, 1980). At 300 $\mu\text{mol l}^{-1}$ it exerts maximum stimulation of cyclooxygenase, the initial enzyme in prostaglandin synthesis (Morse *et al.*, 1977; Cherouny *et al.*, 1988), while at 400 $\mu\text{mol l}^{-1}$, it stimulates cytosolic guanylyl cyclase (Burke and Wolin, 1987), activates transcription factor NF- κ B (Schreck *et al.*, 1991) and induces the transcription of proto-oncogenes *c-myc* and *c-fos* (Rao and Berk, 1992). Catalase activity in the endometrial secretions or epithelium would not necessarily negate a paracrine role for hydrogen peroxide. In fact, Burke and Wolin (1987) found that catalase is required for the stimulation of guanylyl cyclase activity by hydrogen peroxide. It appears that in this system the hydrogen peroxide-catalase complex is the effective activator of guanylyl cyclase. The sensitivity and relevant responses of the endometrium of the pregestational rabbit to exogenous hydrogen peroxide remain to be investigated.

Of equal importance in the blastocyst-endometrial interaction is the possibility of generating hydroxyl radicals via the Fenton reaction, in which the reaction of hydrogen peroxide with superoxide is catalysed by trace metals (Halliwell and Gutteridge, 1989). Hydroxyl radicals are capable of initiating peroxidation of membrane fatty acids, which then sets in motion a chain reaction of further lipid peroxidation (Halliwell and Gutteridge, 1989). The lipid hydroperoxides that result can directly stimulate phospholipase A₂ at 100 $\mu\text{mol l}^{-1}$ (Chakraborti *et al.*, 1989). Lipid hydroperoxides have been found to stimulate cyclooxygenase activity *in vitro* at concentrations as low as 10⁻⁸ mol l⁻¹ (Marshall *et al.*, 1984).

In summary, we have demonstrated that cyanide-resistant oxygen consumption by rabbit blastocysts at day 6 can be readily detected and quantitated. About half of this cyanide-resistant fraction results in the production of superoxide at the blastocyst (trophoblast) surface. In conjunction with

the cytochemical evidence of hydrogen peroxide on the trophoblast surface (Manes, 1992), it is reasonable to infer the additional presence of hydroxyl radicals. The initiating enzyme is a cyanide-resistant NAD(P)H oxidase on the trophoblast surface which has properties that distinguish it from previously described cell surface NAD(P)H oxidases. This enzyme is active at the time when early endometrial responses to the blastocyst are detectable, and possible roles for the reactive oxygen species generated include stimulation of phospholipase and cyclooxygenase activities, which appear to be central to the decidual response.

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