Oxygen tension in the oviduct and uterus of rhesus monkeys, hamsters and rabbits

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Oxygen tension was measured using flexible polarographic microelectrodes within the oviductal and uterine lumen in rhesus monkeys (n = 9), golden hamsters (n = 21) and rabbits (n = 6), during the reproductive cycle (monkey), during oestrus and pseudopregnancy (hamsters, rabbits) and during pregnancy (hamsters). In general, oxygen tensions in each species were much less than half of atmospheric O\textsubscript{2}, ranging from high values of about 60 mm Hg (8.7\% O\textsubscript{2}) in the rabbit oviduct, rabbit and hamster uterus, to as low as 11 mm Hg (1.5\% O\textsubscript{2}) in the monkey uterus. Oxygen tensions did not vary significantly between left and right sides of the reproductive tracts (all species), nor between pregnant and pseudopregnant states nor between oviduct and uterus (hamsters). Differences owing to reproductive stage were found in the monkey oviduct, hamster oviduct and uterus, and rabbit uterus. Oxygen tensions were consistently very low (11–14 mm Hg) in the monkey uterus throughout the menstrual cycle. In hamsters and rabbits, intrauterine O\textsubscript{2} decreased significantly at about the normal time of blastocyst formation and implantation, to 37 mm Hg (5.3\% O\textsubscript{2}) and 24 mm Hg (3.5\% O\textsubscript{2}), respectively. This study indicates that embryos develop in vitro under low oxygen concentrations, especially during the peri-implantation period. The data have implications for investigations of embryo metabolism and for improving embryo development in vitro.

Introduction

Availability of accurate data on the intraluminal oxygen tension within the mammalian female genital tract has attracted increasing attention during recent years, for two reasons. First, it has become apparent from in vitro studies that, depending on species and stage, preimplantation embryos vary widely in their metabolic characteristics, including substrate uptake and utilization, and oxygen consumption. Because we may expect that metabolism and viability are strongly influenced by the oxygen tension to which embryos are exposed, as in somatic cells (Cooper et al., 1958; Packer and Fuehr, 1977; Knighton et al., 1983; Balin et al., 1984), the question is raised: what is the oxygen tension under which preimplantation embryos develop in vitro? Second, in vitro production of embryos from a wide variety of species is becoming increasingly important, for economic as well as for scientific reasons. Although much progress has been made during the last two decades in the ability to support embryonic development in vitro, the viability of cultured embryos is still clearly inferior to that of embryos grown in vivo, irrespective of the species involved (see Biggers, 1987; Fischer, 1987; Kane, 1987; Boatman, 1987; Carney and Foote, 1990; Winston et al., 1991). In vitro culture conditions deviate from the in vivo situation in many respects, but one of the critical factors appears to be the oxygen concentration in which embryos grow in vitro. Oxygen is toxic to many types of cell at atmospheric levels, probably owing to formation of free oxygen radicals. Several studies have reported improved embryo development in culture using oxygen concentrations much lower than that found in air, which is approximately 21\%. These studies examined embryos from mice (Auerbach and Brinster, 1968; Whitten, 1971; Quinn and Harlow, 1978; Pabon et al., 1989; Umaoka et al., 1992), cattle (Wright et al., 1976; Thompson et al., 1990), sheep (Terlizzi et al., 1972; Thompson et al., 1990), rabbits (Daniel, 1968; Li and Foote, 1993; Lindena and Fischer, in press) and hamsters (McKierman and Bavister, 1990). Oxygen concentrations used in these studies were usually selected empirically, for example 1–10\% O\textsubscript{2}.

Physiological data from measurements of oxygen tension in vivo are needed to provide conditions closely resembling those found naturally. Although there are several such reports (Bishop, 1956; Mastroianni and Jones, 1965; Mitchell and Yochim, 1968a; b; Maas et al., 1976; Garris and Mitchell, 1979; Hammer et al., 1981; Kaufman and Mitchell, 1990), within each study, the data are limited to the oviduct or the uterus and to only one species, making general conclusions difficult to reach. Furthermore, no data on oxygen tension are available for rodent oviducts or for the primate uterus, nor any measurements made during pregnancy or pseudopregnancy, which are of special interest for studies of embryo development in vitro. More extensive data collected in a single study could help resolve the
similarities and differences within and between species, and serve as a valuable reference for in vitro techniques. We therefore measured the intraluminal oxygen tension in vivo within the oviduct and uterus in a primate (rhesus monkey), a rodent (golden hamster) and the rabbit; embryos from each of these species have been used to study development in vitro. Measurements were made during different reproductive phases: during the follicular–luteal cycle (monkey), during oestrus and pseudopregnancy (hamster, rabbit), and during pregnancy (hamster). Preliminary results from this study were reported by Bavister and Fischer (1991).

**Materials and Methods**

**Measurement of oxygen tension**

Oxygen tension was measured with flexible polarographic microelectrodes (Innovative Sensors Inc. (ISI) Anaheim, CA) designed for continuous intravascular recording of in vivo \( P_{O_2} \) in human clinical monitoring. The sensor readings, according to the manufacturer, are not affected by changes in solution viscosity and only ± 0.5% by differences in pH. The readings are, however, influenced by temperature changes. Measurements on hamsters and rabbits were made with the animals maintained at 37°C within an "Isolette" infant incubator (model C-86, Air-Shields Inc., Hatboro, PA) to avoid hypothermic effects of anaesthesia. Oxygen tension measurements on rhesus monkeys were performed at room temperature (approximately 22°C). Ketamine anaesthesia, used for uterine oxygen measurements, does not depress core temperature, which was determined using a rectal thermometer to be 37.4 ± 0.2°C (mean ± SEM for nine female monkeys at three stages of the menstrual cycle). Halothane–oxygen anaesthesia, used for oviductal oxygen measurements, induces slight hypothermia; core body temperature recordings were therefore made during anaesthesia to approximate the temperature of the reproductive tract. Temperatures were taken at 30 and 60 min after the onset of anaesthesia, corresponding to the period of oviductal oxygen probe measurements. During this 60 min, the halothane–oxygen mixture depressed core temperature 2.1 ± 0.2°C (n = 9 animals). Accordingly, oxygen probe values were corrected (× 1.03) for the reduced temperature that occurred during the oxygen measurements.

Electrodes were connected to a potentiometric oxygen analyser with two channels for simultaneous recording (ISI). The bipolar sensor was equipped with a silver anode and cathode and covered with a hydrophilic membrane. The electrodes had an outer diameter of 0.6–0.7 mm, and could pass through an 18-gauge hypodermic needle. Analyser and electrodes were equilibrated for approximately 1 h before use by placing them in a reference bath consisting of a 250 ml glass beaker filled with 157 mM NaCl held in a water bath at 38°C. The NaCl solution was constantly bubbled with room air to saturate it with \( O_2 \).

After equilibration, the electrodes were calibrated by adjusting the oxygen analyser output reading to 149 mm Hg; this is the \( P_{O_2} \) of saline solution under standard atmospheric pressure at 38°C (corrected for water vapour pressure), which corresponds to 20.9% \( O_2 \). Only electrodes giving stable reference bath readings were used for \( O_2 \) measurements. Measurements were corrected for the barometric pressure prevailing at the altitude of the laboratory.

Measurements of oxygen tension within the oviduct and uterus were taken after 1–2 min of equilibration within the organs. Where appropriate, ovulation and the presence of corpora lutea were ascertained before oxygen tension measurements were made. While making measurements, occlusion of the tip of the electrode within the organs by oviductal or uterine tissue was carefully avoided. After taking a reading, the electrode was briefly rinsed in NaCl solution containing a small amount of detergent (Liqui-Nox: Alconox Inc., New York, NY) to remove biological fluids, and then replaced in the reference bath. Oxygen tension readings were accepted as valid only if the reference bath reading after retraction from the organ and following equilibration for approximately 2 min in the reference bath did not deviate by 10% from the calibrated value of 149 mm Hg.

**Animals**

*Rhesus monkeys* (Macaca mulatta). Nine naturally cyclic females were used, between 5.5 and 19 years of age (mean 12 ± 2 SEM), from the colony of the Wisconsin Regional Primate Research Center. Time of ovulation was estimated by sex skin breakdown, which occurs in > 80% of the females approximately 1–2 days after ovulation (see Boatman, 1987). For oviduct \( O_2 \) measurements, the animals were initially anaesthetised with 15 mg kg\(^{-1}\) body weight with ketamine HCl (Ketaset: Aveco Co. Inc., Fort Dodge, IA) and 0.4–0.6 mg xylazine kg\(^{-1}\) (Rompun; Miles Laboratories, Shawnee, KS), together with 100 μg atropine, all injected i.m. Anaesthesia was maintained by inhalation of 1–2% halothane in oxygen. Oxygen electrodes were introduced into the oviducts using a modification of our laparoscopic embryo transfer procedure. The abdomen of the animal was insufflated with compressed air (7–8 mm Hg pressure) and a laparoscope was inserted to view the oviducts and ovaries. A curved, blunt-ended 19-gauge needle was passed through the body wall and its tip was inserted through the fimbrial ostium to lie approximately 5 mm inside the ampulla. An oxygen electrode was threaded through this cannula until its tip projected 3–5 mm beyond the end of the cannula, as indicated by a calibration mark made on the electrode. Possible distortion of the oviductal oxygen tension measurements by the oxygen used for anaesthesia was examined by repeating oxygen tension measurements after withdrawing the halothane–oxygen anaesthesia mixture for a few minutes. The intra-oviductal \( O_2 \) readings obtained did not change noticeably. Bilateral oviductal \( O_2 \) measurements were made during a single operation in each of eight monkeys; in the ninth animal, one of the oviducts could not be cannulated and so this was accomplished during a subsequent operation.

For uterine oxygen tension measurements, animals were lightly sedated with ketamine and held in a prostrate position. Oxygen electrodes were inserted non-surgically into the uterine cavity through the cervix using a modification of the embryo flushing procedure routinely used at the Wisconsin Primate Center (Goodeaux et al., 1990). After removing excess cervical mucus by suction, a blunt-ended 18-gauge steel hypodermic needle was threaded carefully through the twisting
cervical canal until its tip lay just beyond the internal os, as
detected by rectal palpation. The electrode was then passed
through this cannula until its tip lay approximately 5 mm beyond
the end of the cannula. Readings were taken as described for
oviducts.

In each of six animals, both oviductal and uterine O₂
measurements were made at the same cycle stage, that is
mid-follicular (cycle day 6 or 7), early post-ovulatory (days 14–16)
or mid-luteal (days 18–21), although for practical reasons the
uterine measurements were made during a later cycle. In the
other three monkeys used for oviductal O₂ measurements, it
was not possible to pass a cannula through the cervix; three
new animals were substituted to provide the uterine O₂ values
(one mid-follicular, two early post-ovulatory).

**Rabbits.** Sexually mature New Zealand White rabbits (≥ 4
months of age) were either untreated (oestrus; n = 2) or treated
with 75 i.u. hCG (Sigma Chemical Co., St Louis, MO) to induce
ovulation and pseudopregnancy (n = 4). Sedation was attained
with 10 mg ketamine HCl kg⁻¹ and 3 mg xylazine kg⁻¹, both
i.m. Fifteen minutes later, full anaesthesia was induced by step-
wise intravenous injections of ketamine–xylazine (2 mg and
0.6 mg, respectively, per step). The abdomen was opened
through a mid-line incision. An electrode was inserted through
the fimbria until its tip lay 1–2 cm deep into the ampulla;
oviductal O₂ readings were then taken. Uterine readings
were taken in the same animal during the same operation. The
electrode was placed 2–3 cm into the uterus lumen after
puncturing the uterine wall with an 18-gauge hypodermic
needle. The tip of the electrode was freely movable in the
lumen. Care was taken to avoid bleeding and to prevent drying
of organs during manipulations. The organs were repositioned
into the abdominal cavity while the measurements were being
made. Animals were killed without recovery from the anaes-
thetic when measurements were completed with an overdose of
pentobarbital.

**Golden hamsters.** Regularly cyclic golden hamsters (bred
in-house) were used for this study. Oxygen tension measurements
in oestrous females were taken on the day on which mating
would normally have taken place during the evening, i.e. on day
4 of the cycle (Orsini, 1961). Other females were mated with
fertile or with vasectomized males to induce pregnancy and
pseudopregnancy, respectively. Matings were confirmed by
the presence of vaginal plugs the next morning. Measurements
were performed on each day of pregnancy or pseudopregnancy
(in different animals) from the day after mating (day 1) each
day to day 5 and finally on day 8. The hamsters were anaesthetized
with sodium pentobarbital (Nembutal, 75 mg kg⁻¹). Bilateral
dorsal incisions were made about 2 cm lateral to the vertebral
column and the upper third of each uterine horn together with
the oviduct and ovary were pulled out (see Farrell and Bavister,
1984; Seshagiri and Bavister, 1990). For insertion of the
electrode into the oviducts, the procedure described by Farrell
and Bavister (1984) for embryo transfer was followed, in which
the fimbriae are externalized through a puncture made in the
ovarian bursal membrane. Insertion of the electrode was
performed using a dissecting microscope inside the Isolette
incubator. After insertion of the electrode until its tip lay several
millimetres into the ampulla of the oviduct, it was secured in
place by tying a ligature (no. 5 braided silk thread) around
the fimbria. Bleeding and drying of the organs was avoided by
frequently wetting them with 0.9% NaCl solution.

For uterine measurements, a puncture was made with a hypo-
dermic needle just below the uterotubal junction, and the elec-
trode was passed through this hole into the lumen until its tip
lay about 1 cm distal to the puncture site. For both oviductal
and uterine measurements, which were made in the same ani-
mal, the organs with electrodes in place were carefully replaced
inside the body cavity while the readings were being made.
Twenty-one females were studied, but in one case, only uterine
readings could be taken. Animals were killed without recovery
from the anaesthetic when measurements were completed with
an overdose of pentobarbital.

**Data analysis**

For each species, data for left and right oviducts were com-
pared (randomized block ANOVA), and the average of the two
measurements was used for subsequent analyses. For each
species, differences between oviductal and uterine oxygen
measurements were tested using analysis of variance with sub-
ject blocks. For each species, effects of stage of cycle or
pregnancy/pseudopregnancy (pregnant and pseudopregnant
values were pooled for hamsters because no significant differ-
ences were found) were analysed for each organ (oviduct or
uterus). Multiple comparisons were made by a protected Fisher’s
least significant difference test; P < 0.05 was considered to
represent statistically significant differences.

**Results**

In rhesus monkeys, oviductal oxygen tensions on the ovulatory
and non-ovulatory sides were not significantly different
(55 ± 6 mm Hg (mean ± SEM) and 53 ± 5 mm Hg, respec-
tively, n = 7 for each side); data for left and right sides were
therefore pooled for analysis. Mean oxygen tension readings in
the oviducts (all cycle stages) were higher than those for the
uterus (Fig. 1a). Oxygen tension in the oviducts was lower dur-
ing the mid-follicular phase of the cycle (mean 35 ± 5 mm Hg)
than at either early post-ovulatory or mid-luteal phases (57 ± 5
and 61 ± 5 mm Hg, respectively) (Fig. 1b). However, oxygen
tension in the uterus was consistently very low (mean 13 ± 2 mm Hg) throughout the cycle and no cycle differences
were observed (Fig. 1c).

In hamsters, there was no difference in oxygen tension between
left and right oviducts (41 ± 3 mm Hg, n = 19 and
43 ± 3 mm Hg, n = 17, respectively), between left and right
uterine horns (42 ± 3 mm Hg, n = 21 and 41 ± 3 mm Hg,
42 ± 3 mm Hg, n = 19, respectively), or between pregnant and pseudopregnan-
t females (day 2–day 8 only) (46 ± 2 mm Hg, n = 13
40 ± 3 mm Hg, n = 13, respectively, for oviductal readings
and 41 ± 3, n = 13 and 44 ± 3, n = 16, respectively, for uteri-
ne readings). Accordingly, data were combined within main
categories (oviducts, uterus) for analysis of differences between
cyclic (day 0) and pregnant/pseudopregnant (P/P) phases.
There was high variability among animals for day 0 measure-
ments, both for oviductal (mean 45 ± 14 mm Hg, n = 3) and
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uterine (37 ± 7 mm Hg, n = 3) oxygen tension. Because of this, the data for day 0 were not statistically analysed with the P/P data. Data analysis was performed on P/P from day 1 to day 8. For both oviductal and uterine O₂, there was a rise from day 1 to day 3 (highest levels reached: 53 ± 3 mm Hg and 60 ± 6 mm Hg, for oviducts and uteri, respectively), followed by a pronounced drop on day 4 (Fig. 2a,b). There was no difference for either organ among days 4, 5 and 8.

In rabbits, mean oxygen tensions were not different for left and right oviducts (48 ± 4 mm Hg, n = 6 and 55 ± 7 mm Hg, n = 6, respectively) nor for left and right uteri (43 ± 7 mm Hg, n = 6 and 42 ± 6 mm Hg, n = 6 respectively); data from the left and right sides were therefore combined within organ categories. Overall mean oxygen tensions were higher in the oviducts than in the uterus (53 ± 3 and 42 ± 5 mm Hg, respectively) (Fig. 3a). Although no differences in mean oviductal oxygen were found across days (day 0, day 1 and day 6 of pseudopregnancy) (Fig. 3b), uterine oxygen tensions were lower on day 6 than on day 1 (24 ± 2 and 60 ± 4 mm Hg, respectively) (Fig. 3c).

Discussion

In this study, using sensitive polarographic electrodes, we found that in all three species, mean oxygen tension in the female reproductive tract was only 40% or less of atmospheric O₂. This confirms previous observations by other investigators. Oxygen tension has been previously measured within the oviduct of rabbits (Bishop, 1956; Mastroianni and Jones, 1965) and rhesus monkeys (Maas et al., 1976), and the uterus of rats (Mitchell and Yochim, 1968a, b; Hammer et al., 1981; Kaufman and Mitchell, 1990), guinea-pigs (Garris and Mitchell, 1979) and hamsters (Kaufman and Mitchell, 1990). However, there are some important procedural differences between the present and earlier studies, and our data and conclusions are at variance with some of the earlier findings. To provide useful comparative data, we measured O₂ tension in oviducts and uteri of the same species, which was not done previously, except for a few preliminary observations by Bishop (1956). In addition, we measured oxygen tension in hamsters and rabbits during pregnancy/pseudopregnancy, which is obviously relevant to embryo development; with one possible exception (Bishop, 1956), there are no previous reports in any species on the oxygen tension in the female reproductive tract during these conditions. However, our data are different from the values found by Bishop (1956). Furthermore, our measurements of oxygen tension within the rhesus monkey oviduct are at variance with data from a previous study by Maas et al. (1976). Finally, we also present the first oxygen tension data from the oviducts of a rodent species; this information should be helpful in view of the widespread use of rodent preimplantation embryos for investigation of development of preimplantation embryos in vitro.
although tension was zero. It be postulated that the oviductal pressure changes from 0.05 to 60, and 80r oxygen may be more. Some researchers have postulated that the oxygen tension in the oviductal ampulla via the fimbria for a distance of several millimetres, until the first ampillary flexure was encountered. In the study reported here, we examined the effects of daily changes in oxygen tension on day 3, that is between the eight-cell–morula stages and blastocyst formation (Bavister et al., 1983). This increase in oxygen tension from day 1 to day 3 may be important for embryo metabolism during the early cleavage stages, in both oviduct and uterus. On day 4, there was a marked drop in oxygen tension, again in both organs, corresponding to the time when blastocysts are fully developed and implantation begins. The parallel changes in oxygen tension seen in the oviduct and uterus indicate that a systemic (endocrine) mechanism is responsible, rather than some local action of the developing embryos, as all embryos are in the uterus by day 3 (Bavister et al., 1983). This observation is consistent with the data of Mitchell and Yochim (1968a) showing that uterine oxygen tension in rats is highly influenced by the endocrine status of the animal (oestrogen- or progesterone-dominated). The oxygen tension was examined for progesterone- and placental-dominated rats. In the hamster uterus at oestrus, we found a mean oxygen tension of 37 ± 7 mm Hg (5.4% O_2) which is similar to the range (25.7 ± 4.2) reported by Mitchell and Yochim (1968b) for the rat uterus during oestrus.

The mean oxygen tension in the rabbit oviduct was higher than in the uterus, but did not differ statistically over the three days examined. Our data are different from the values reported by Bishop (1956) for the rabbit oviduct. This author found an average of 30 mm Hg for O_2 under progesterone-dominated conditions; 'progesterone dominance' was not closely defined by this worker, and we do not know if this corresponds to the use of pseudopregnant rabbits, as in our study. Bishop (1956) stated that oviductal oxygen tension was lower in progesterone-dominated rabbits than during oestrogen dominance (average of 30 and 45 mm Hg, respectively). We observed a similar trend in oviductal oxygen tensions between oestrus and luteal conditions, but the data were not significantly different; mean values ranged between 42 and 62 mm Hg (6–9% O_2) over the 3 days studied. In contrast to the oviductal situation, uterine oxygen tension was significantly lower on day 6 of pseudopregnancy than on day 1. This period of reduced oxygen tension (24 mm Hg, 3.5% O_2) is near to the time when
progesterone concentrations reach peak values (see Fischer et al., 1985) and implantation begins.

Several authors have measured intrauterine oxygen tensions following ovariectomy and hormone replacement (for example, Mitchell and Yochim, 1968a; Garris and Mitchell, 1979; Kaufman and Mitchell, 1990). In all three species studied (rats, guinea-pigs, and hamsters), there was a sharp increase in uterine \( P_{O_2} \) after ovariectomy; however, the reaction after oestrogen replacement was very different in these animals, comprising increased (guinea-pig: Garris and Mitchell, 1979), or drastically decreased (rat: Mitchell and Yochim, 1968a; hamster: Kaufman and Mitchell, 1990) intrauterine oxygen tensions. Although such studies can be very informative about the hormonal regulation of intrauterine oxygen tension, conditions obtained under specific hormone dose regimens in ovariectomized animals may not correspond to the physiological conditions of the luteal phase following mating, that is early pregnancy. Measurement of intraluminal \( O_2 \) in naturally cycling and P/P animals, as in the present study, should provide the closest approximation to normal situations.

In all three species examined in this study, intrauterine oxygen tension was lowest at about the time corresponding to late blastocyst development and implantation (hamster: 37 mm Hg; 5.3% \( O_2 \); rabbit: 24 mm Hg; 3.5% \( O_2 \) ) or was consistently low throughout the cycle (monkey: 11–14 mm Hg; 1.5–2.0% \( O_2 \) ). This conclusion is at variance with the observation that uterine oxygen tension in guinea-pigs sharply increased in the mid-luteal phase, that is at about the time of implantation (Garris and Mitchell, 1979). This disparity could be due to species differences, or possibly to a difference in oxygen tension between pregnant and pseudopregnant animals, as used in the present study, and nonpregnant animals used in the work of Garris and Mitchell (1979).

The functional significance of reduced intrauterine oxygen tension at about the time of implantation is unknown, but it may serve to protect the peri-implantation blastocyst from oxygen toxicity, which could be one reason for the inferior development of embryos in culture. Several studies have shown that reduced \( O_2 \) (1–10%) improves development of cultured embryos, in a number of species. In a study with hamster embryos cultured under 20% \( O_2 \) or reduced (5 or 10%) \( O_2 \), no detrimental effect of high oxygen on development was observed until the blastocyst stage was reached (McKieran and Bavister, 1990). A striking effect of \( O_2 \) toxicity was shown by briefly (1–2 h) exposing mouse one-cell embryos to ambient (20%) \( O_2 \) before culturing under 5% \( O_2 \); toxicity was not manifested until the morula or blastocyst stages (Pabon et al., 1989), further underscoring the sensitivity of the preimplantation embryo to \( O_2 \) concentrations. However, several studies have reported that there is no difference in embryo development under reduced \( O_2 \) (for example, mouse: Legge and Sellens, 1991; cat: Johnston et al., 1991), but this could be due to other rate-limiting factors in the culture environment, masking a detrimental effect of oxygen. Although Daniel (1968) reported that day 5 and day 7 rabbit blastocysts can tolerate a broad range of \( O_2 \) concentrations in vitro (up to 95%), the optimum \( O_2 \) concentration is much lower as evidenced by Li and Foote (1993) and Lindenau and Fischer (in press). Rabbit one-cell embryos develop into blastocysts much more frequently (Li and Foote, 1993) and blastocysts cultured from the morula stage had a significantly higher cell proliferation (Lindenau and Fischer, in press) when incubated under 1% or 5% \( O_2 \) than in 20% \( O_2 \). Taken together, all of these findings indicate that the \( O_2 \) concentration has to be considered very critically in studies using in vitro embryo culture. Attempts to establish optimal \( O_2 \) concentrations for in vitro embryo development need to consider possible interrelations with species, stage of embryo development and other culture-associated conditions like composition and volume of the culture media, time of culture, presence or absence of cocultured somatic cells, and oxygen radical formation in vitro. The latter two conditions have attracted considerable attention during recent years. Coculture greatly complicates or obscures investigation of the \( O_2 \) requirements and tolerances of developing embryos (Bavister, 1993). In vivo, embryos may be protected against free radicals by mechanisms such as oxygen scavengers in oviductal fluid (Legge and Sellens, 1991). However, when mouse embryos were cultured in vitro, the intracellular concentration of \( H_2O_2 \), was increased (Nasr-Esfahani et al., 1990). The protective effect of radical scavengers such as superoxide dismutase on cultured mouse embryos has been demonstrated by several workers (Iannaccone, 1986; Nasr-Esfahani et al., 1990; Noda et al., 1991; Umaoka et al., 1992).

In vivo measurements of oxygen tension in the oviduct and uterus could help the understanding of the metabolism of embryos at different stages of development. Our data indicate that peri-implantation blastocysts in these three species would be exposed to quite low oxygen tension in vivo, which may play a role in regulating metabolism. Studies on the metabolism of embryos carried out in vitro may be misleading unless physiological oxygen tensions are used. For example, studies performed on blastocysts under oxygen tensions that are near atmospheric values (21%) may 'drive' the embryos into metabolic behaviour that is abnormal (see Gott et al., 1990). Unfortunately, virtually no information is available on effects of different oxygen tensions on respiration and metabolism of mammalian preimplantation embryos (a rare exception is the study by Khurana and Wales, 1989). This information is critically needed to ascertain whether present concepts about embryo metabolism are influenced by unphysiological oxygen tensions used during the experiments.

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Oxygen tension within the oviduct and uterus 679

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