Article

Observations on intrauterine oxygen tension measured by fibre-optic microsensors



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Abstract

Understanding the biology of reproductive organs is essential for the development of assisted reproductive techniques. There is at present no direct evidence for either the concentration and dynamics of intrauterine oxygen tension at the endometrial surface, nor its importance for the receptiveness of the endometrium. In this study a new method measured mid-cycle (ranging from day 12–18) endometrial surface oxygen tension in 21 patients referred to intrauterine insemination (IUI). Time series was measured online for a period of 5–10 min. The (mean) individual oxygen tension among patients varied from 4 to 27% air saturation. Overall mean oxygen tension among all patients was 11.8% air saturation. Within the same patient, considerable time-related variations were observed. Some patients exhibited rhythmic oscillations with a frequency in the order of 1 min, whereas others did not show any regular patterns. A good description of endometrial surface oxygen concentration and dynamics was thus obtained, but given the relatively small number of participants, an association with pregnancy following insemination could not be established. Further studies using this new method could elucidate the association between individual intrauterine activity, embryo implantation and endometrial surface oxygen tension.

Keywords: *intrauterine, IUI, oxygen, uterus*

Introduction

The non-pregnant uterus is an active organ with rhythmic contractions (Ayoubi *et al.*, 2003), wave-like activity (Ijland *et al.*, 1996; van Gestel *et al.*, 2003) and varying vascular impedance (Ozturk *et al.*, 2004). Non-invasive sonographic imaging can be used to evaluate the endometrial thickness, wave-like activities and contraction frequencies, and Doppler assessment has been suggested as a measure of the vascular impedance of the reproductive organs. Doppler assessment of endometrial vascularization has been reported to associate with increased implantation and pregnancy rates (Yang *et al.*, 1999; Schild *et al.*, 2000; Ozturk *et al.*, 2004), although conflicting findings have also been published (Zaidi *et al.*, 1995; Aytoz *et al.*, 1997; Contart *et al.*, 2000; Schild *et al.*, 2001).

Some ultrasound studies indicate that spiral artery blood flow activity may be the most relevant measure of uterine receptivity, rather than endometrial thickness and general uterine blood flow (Zaidi *et al.*, 1995; Schild *et al.*, 2001). Also endometrial wave-like movements have been investigated during the menstrual cycle (Ijland *et al.*, 1996) and in relation to IVF cycles (van Gestel *et al.*, 2005). Association between wave-like activity and endometrial receptiveness remains unclear, but it has been speculated that such movements facilitate the supply of nutrients and oxygen to the preimplantation embryo, and perhaps assist a favourable positioning of the preimplantation embryo inside the uterus (Ijland *et al.*, 1996). Variability between studies, and even within studies, illustrates the current difficulties linked to ultrasound techniques. Therefore, additional methods to evaluate uterine tissue perfusion, vascularization and activity, should be considered.

Spiral artery blood flow is expected to associate with endometrial oxygen tension. The actual endometrial surface oxygen tension is a complex function of arterial blood supply, local consumption, and diffusion barriers from the spiral arteries towards the surface

of the endometrial tissue. Information on actual intraluminal oxygen tension is especially relevant in embryo transfer settings as the metabolism and viability of the preimplantation embryo is largely affected by the oxygen tension (Houghton *et al.*, 2004; Karagenc *et al.*, 2004). Previous studies from rodents and primates have shown a large interspecies variation among intrauterine oxygen tension (Fischer and Bavister, 1993), but data from the human uterus have not been presented. However, oxygen tension appears to be one important parameter where in-vitro culture conditions (e.g. 5% CO_2 in air) often deviate from the lower in-vivo oxygen tensions.

Oxygen tension measurements at the endometrial surface may provide a direct and objective method to evaluate the vascularization and perfusion of the inner uterine lining and its association with uterine activity. It may also provide data from the very surface of the endometrium where the complicated process of in-vivo preimplantation embryo development and implantation takes place. So far, easily applicable methods for such measurements have not been available, and data on human intrauterine oxygen tension have not been reported previously. In this study an easy and gentle method based on a new technology is introduced, which enables recording of time series of intrauterine oxygen tension, measured at the endometrial surface, and presents data from intrauterine insemination (IUI) patients at the day of insemination, where the endometrium is expected to be well developed.

Materials and methods

The study was a prospective observational study on 21 women referred to IUI at the Aarhus University Hospital Public Fertility Clinic. The study was approved by the Regional Ethics Committee.

Patients being treated with IUI in stimulated and non-stimulated cycles were asked to participate in the study. Protocols in stimulated cycles were, in general, clomifen citrate (Pergotime® 100mg/day, Serono Nordic, Denmark) cycle day 3–7 followed by recombinant FSH (Gonal-F® 75 IU/day, Serono Nordic) cycle day 8–9. HCG (Ovitrelle®, Serono Nordic), was administered in all cycles, stimulated or non-stimulated, at a follicular size of >18 mm. The patient was inseminated with 0.5 ml prewashed spermatozoa (Suprasperm, Medicult A/S, www.medicult.com) using a concentration of at least 4×10^6/ml). The intrauterine oxygen tension was measured with a specially devised type of fibre-optic microsensor, made of thin flexible insulated optical fibres with an outer diameter of 0.9 mm. These miniaturized implantable probes are designed for various applications, e.g. insertion into the blood circuit or tissue, and yield online real time oxygen measurements. The detection principle is based on quenching by oxygen of the fluorescent dye (a fluorophore) immobilized at the tip of the fibre and subsequently coated with an optical insulation to avoid interference from intrinsic fluorescence (PreSens, Regensburg, Germany, www.presens.de) (Klimant *et al.*, 1997). The decrease of the fluorescent quantum yield of the fluorophore [ruthenium(II)-tris-(4,7-diphenyl-1,10-phenanthroline], as well as the fluorescent decay time is proportional to the concentration of oxygen bound to the matrix in which the dye is immobilized. The fluorescent decay time was recorded with an oxygen meter Microx TX3 (www.presense.de), which was connected to a laptop computer. Measuring characteristics have been determined by the manufacturer. Measuring range of the sensor was 0–500% air saturation (air sat.). Due to the measuring principle of the sensor, resolution varies with partial pressure. Resolution at 1, 30, 100, 250% air sat. was 0.05, 0.1, 0.5 and 1.7% air sat. respectively. Resolution in the uterine cavity was thus better than 0.1% air sat. Accuracy was within ± 1% air sat. The 0–90% response time was <5–10 s. The sensor shows no cross-sensitivity for carbon dioxide or any ionic species (www.presense.de).

Intrauterine oxygen tension measurements were conducted immediately before insemination. Prior to insertion in the uterus, the fibre-optic oxygen sensor was placed inside a standard insemination catheter (Frydman memory catheter 4.5, Laboratoire CCD, Paris), aligning the oxygen sensitive tip with the catheter tip. The catheter was then inserted through the cervix and placed in the uterine cavity mimicking a routine insemination procedure. Following insertion the oxygen sensitive tip of the optical fibre was exposed by retracting the catheter (not the microsensor) 5–10 mm. It is unlikely that any significant oxygen was introduced in the uterine cavity during the measurements. Oxygen recordings were conducted during a period of 5–10 min. Thereafter the oxygen sensor was retracted from the uterus, whereas the catheter remained in the uterus for subsequent insemination. Accordingly, no extra operations or manipulations of the patient were required, only a brief waiting period of 5–10 min. Following measurement, a two-point calibration of the oxygen sensor was performed in dinitrogen-purged water and in water at air saturation. Subsequent clinical pregnancy was detected at a week 7 ultrasound scan.

Results

Relevant patient and cycle characteristics are shown in **Table 1**.

The mean oxygen tension (n = 21) in the uterine cavity was 11.8% (95% CI: 8.9–14.6) air sat. or partial pressure 18.9 mmHg, 95% CI: 14.6–23.2. The (mean) individual oxygen tension varied considerably from patient to patient, within the range of 4 to 27% air saturation (6.4 to 32 mmHg) (**Figure 1**).

However, within the same patient, a considerable time-related variation was often noticeable, with large and occasionally rhythmic oscillations in the oxygen tension. **Figure 2** shows examples of such individual fluctuation patterns from eight patients (similar axis scales). Apparently, both the mean concentration and oscillation patterns varied from one patient to another.

Although the main purpose of the study was to measure and describe the concentration and dynamics of oxygen at the endometrial surface, an association with pregnancy among the participating patients was investigated. The present dataset did not show a significant difference in mean intrauterine oxygen tension between the pregnant (n = 4) and non-pregnant group (n = 17) following insemination. A subjective evaluation of individual oscillation patterns in oxygen tensions did not indicate any substantial difference between the pregnant and non-pregnant group. Univariate statistical analysis (Odds ratios calculated by logistic regression using the STATA software for windows, www.stata.com) showed that no patient characteristic (body mass index, hormone stimulation, age, smoking habits, or

Table 1. Patient and cycle characteristics.

	All (n = *21*)	*Pregnant group* (n = *4*)	*Not pregnant group* (n = *17*)
Age in years	31.9 (29.9–33.8)	31.5 (27.5–35.5)	31.9 (29.6–34.3)
Body mass index	23.3 (21.4–25.2)	24.7 (19.9–29.6)	23.0 (20.7–25.2)
Cycle day of oxygen measurement and insemination (range)	11–18	13–14	12–18
Cycle day of oxygen measurement and insemination	13.7 (12.8–14.5)	13.3 (11.9–14.8)	13.7 (12.7–14.7)
Washed sperm count 10^6/ml	12.2 (6.6–17.9)	18 (1.0–37.2)	11.4 (5.6–17.3)
Oxygen tension as % air saturation	11.8 (8.9–14.6)	9.0 (0.9–17.1)	12.4 (9.0–15.8)

Values are means (95% CI), unless otherwise stated.

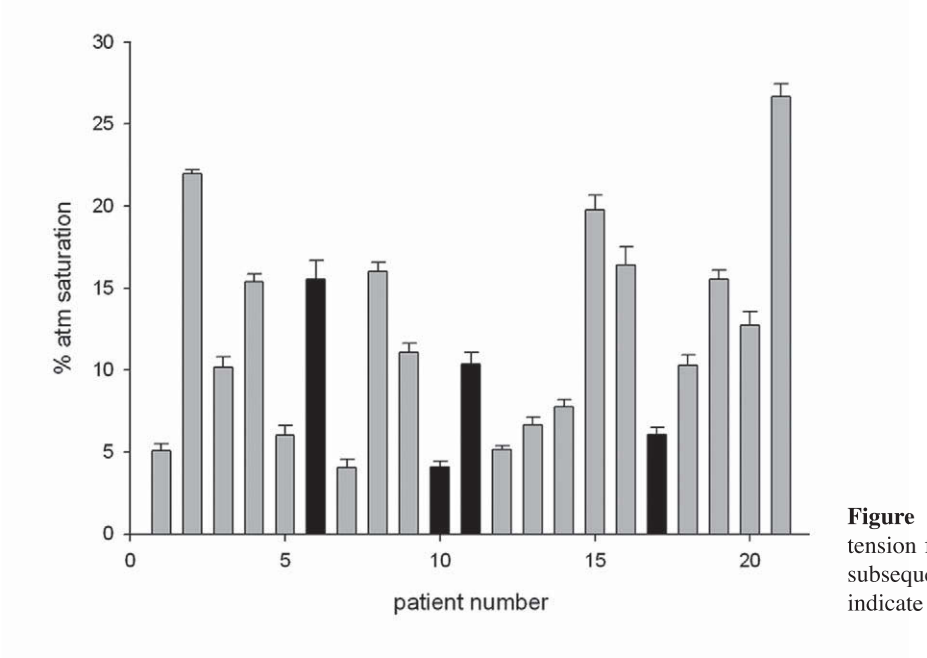


Figure 1. Mean endometrial surface oxygen tension for all 21 patients. Black bars indicate subsequent clinical pregnancy. Error bars indicate 95% CI for the individual mean.

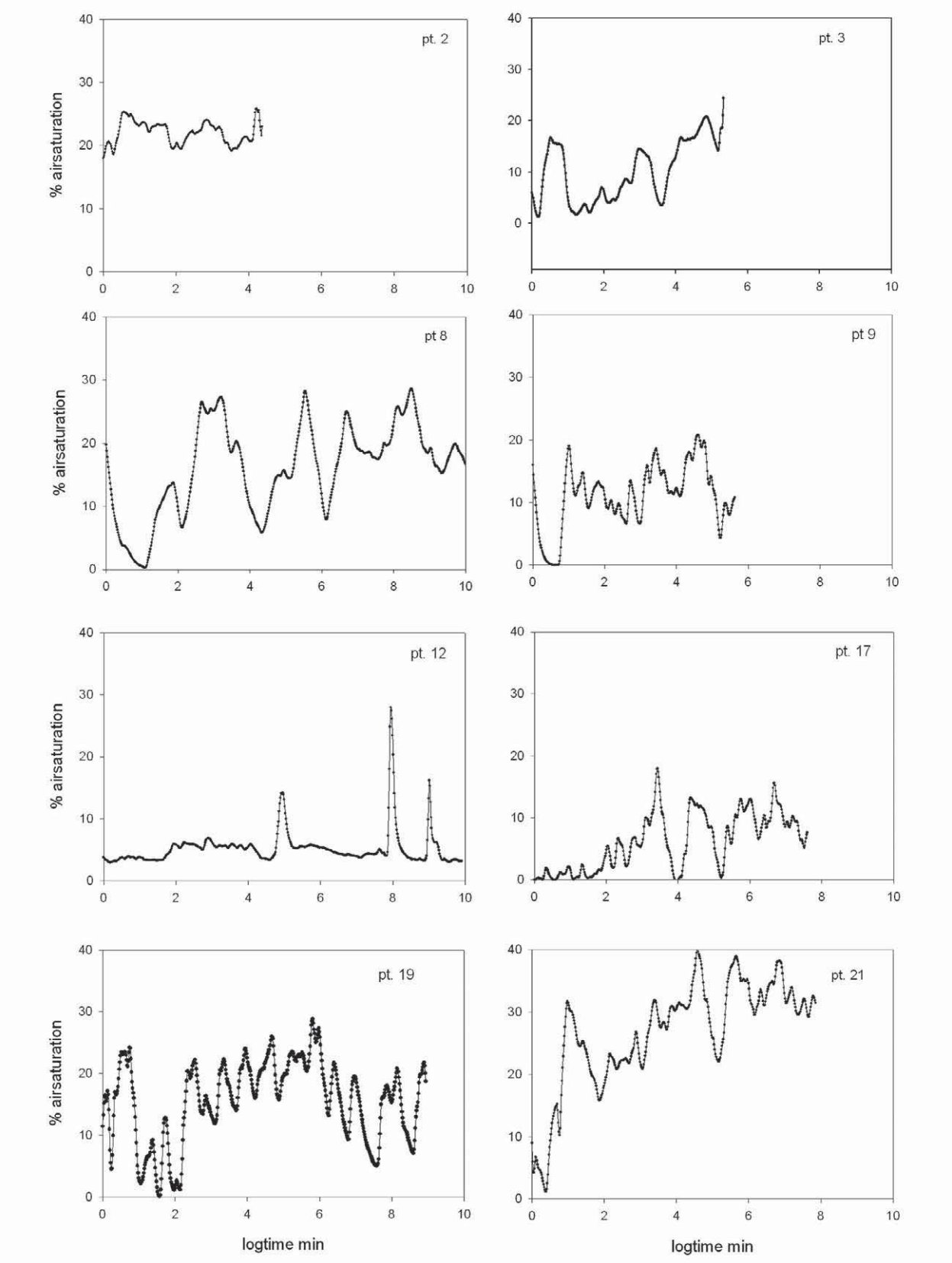


Figure 2. Examples of intrauterine oxygen dynamics from eight patients (pt).

infertility reason) was associated with mean intrauterine oxygen tension ($P > 0.05$, analysis data not shown). Furthermore, there was no statistically significant difference in mean oxygen tension between stimulated and non-stimulated cycles.

Discussion

The present study describes human intrauterine oxygen concentration in the uterine cavity and for the first time demonstrates dynamic and in some cases rhythmic variation in mid-cycle intrauterine oxygen tension. Where a pattern was found, the frequency was in the order of minutes or higher (several each minute). Contraction frequency studies in six women suggested mid-cycle uterine contraction frequencies within this order (Ayoubi *et al*., 2003). These uterine contraction patterns may cause the rhythmic variations in oxygen tension demonstrated in the present study. Also studies of endometrial wave-like activity suggest rhythmic activity, which may affect intrauterine oxygen tension (Ayoubi *et al*., 2003; van Gestel *et al*., 2003; van Gestel *et al*., 2005). The overall average of intrauterine oxygen tension among the 21 patients (18.9 mmHg) agreed well with findings by Yedwab *et al*. (1976) who recorded an average pO_2 in the human uterus around 15 mmHg (10% atm. sat.). In a primate (Rhesus monkey) Fischer and Bavister (1993) reported intrauterine oxygen tension of 15–20 mmHg (10–12.5% air sat.).

The method introduced is applicable for intrauterine oxygen measurements, or other semi-invasive measurements. In contrast to classic electrochemical oxygen electrodes, this oxygen sensor probe is a flexible optical fibre that fits inside a standard insemination catheter. With an outer diameter of 0.9 mm, the physical disturbance incurred during measurement is believed to be small, if any, but it is difficult to quantify. Fibres up to 10 m may be used, enabling computer and oxygen meter to be kept outside normal working area around the patient during measurements. Another advantage over classic oxygen electrodes is that these probes do not consume oxygen during measurement. Accordingly, no stirring sensitivity or drifting signal is caused by local depletion of oxygen imposed by the sensor itself. The probes can be sterilised in an autoclave and can stand routine hospital equipment sterilization procedures. No patients felt uncomfortable due to the measurements.

The present method is semi-invasive, and it is difficult to decide whether the insertion of a standard insemination catheter into the uterine cavity affects the blood flow patterns in the endometrium, and thus possibly the oxygen tension. The oxygen concentration is recorded quite locally, within a few mm^2, so larger scale heterogeneity in oxygen concentration within different parts of the uterus would necessitate systematic and repeated measurements in defined locations of the uterus. However, the huge variation over time in oxygen concentration at a given location makes it difficult to record a spatial variation, and transect measurements of oxygen from the uterine fundus towards the cervix did not show systematic variation and could not clarify on this issue (data not shown).

Compared with ultrasound techniques such as power Doppler, which yield information on uterine blood flow characteristics, intrauterine oxygen measurements are completely objective and readings can be obtained continuously for as long as necessary or experimentally possible. The fact that the present method probably provides a direct measure at the endometrial surface is a stimulus to further research on the possible relationship between oxygen tension and embryo implantation.

Reports on concentrations of respiratory gases in the uteroplacental tissue later in the early pregnancy period (week 7–10) indicate pO_2 values in the coelomic fluid around 20 mmHg (12.5% air sat.), and approximately 15–18 mmHg (9–11% air sat.) in amniotic fluid at week 11–16 (Jauniaux *et al*., 2001, 2003). It thus appears that low oxygen partial pressure is an in-vivo condition for the early embryo, which persists from fertilization to at least 10 weeks of gestation. Although indispensable for energy metabolism in higher forms of life, aerobic respiration generates reactive oxygen species (ROS) known to stress cellular function in the preimplantation embryo (Johnson *et al*., 1994; Guerin *et al*., 2001; Burton *et al*., 2003). Even though contradictory reports exists, it seems that in-vitro culture of mammalian preimplantation embryos, from fertilization to blastocyst, is favoured by 'near' physiological oxygen tension, typically 5% (24% air sat. or 38 mmHg), as compared with 20% (95% air sat. or 153 mmHg) (Umaoka *et al*., 1992; Dumoulin *et al*., 1999; Catt and Henman, 2000; Orsi and Leese, 2001; Petersen *et al*., 2005). Furthermore, it has previously been shown that fetal cells (syncytiotrophoblasts) lack antioxidant enzymes in the first 2 months of gestation, and thrive poorly under ambient oxygen (21%) (Watson *et al*., 1998).

This study, in concert with others, suggests that low ambient oxygen tension is the actual condition of life to which the early embryo seems adapted. Accordingly, an immediate effect of elevated intrauterine oxygen tension on subsequent pregnancy rate following insemination was not observed.

Acknowledgements

This work was supported by the Danish Medical Research Council, The Beckett Foundation, The Foundation of 17/12–1981, The Toyota Foundation, the AP Møller Foundation for the advancement of Medical Science, Karen Elise Jensens Foundation and Clinical Institute Aarhus University.

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Received 30 March 2006; refereed 25 April 2006; accepted 30 May 2006.