***In-vivo* oxygen, temperature and pH dynamics in the female reproductive tract and their importance in human conception: A systematic review**

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**Abstract**

**BACKGROUND:** Despite advances in ART, implantation and pregnancy rates per embryo transfer still remain low. IVF laboratories strive to ensure that the process of handling gametes *in-vitro* closely mimics the *in-vivo* environment. However, there remains a lack of knowledge regarding the *in-vivo* regulation and dynamic variation in biophysical parameters such as oxygen concentration, pH and temperature within the reproductive tract.

**OBJECTIVE AND RATIONALE:** To undertake a systematic review of the current understanding of the physico-chemical parameters of oxygen tension (pO2), pH and temperature within the female reproductive tract, and their potential implications in clinical and pathological processes related to fertility and those pertaining to limited reproductive capacity.

**SEARCH METHODS:** A comprehensive literature search was performed using electronic databases including Medline, Embase, Cochrane Library and Pubmed to identify original and review articles addressing the biophysical parameters (pO2, pH and temperature) in the female reproductive tract of any species. The search included all studies published between 1946 and November 2015. Search terms included ‘oxygen’, ‘pH’, ‘hydrogen ion concentration’, ‘acid base’ and others terms. We also used special features and truncations to identify synonyms and broaden the search. Studies were excluded if they only assessed embryo culture conditions, fetal acid-base status, oxidative stress, outcomes of pregnancy and measurements of these parameters in non-reproductive organs.

**OUTCOMES:** Our search generated 18, 685 records and 60 articles were included. pO2 within the female reproductive tract shows cyclical variation and minute-to-minute oscillations which may be influenced by uterine contractility, hormones, the autonomic system, cardiac pulsatility, and myometrial and smooth muscle integrity. Fine balanced control of pO2 and avoidance of overwhelming oxidative stress is crucial for embryogenesis and implantation. The pH in the female reproductive tract is graduated, with lowest pH in the vagina (~pH 4.42) increasing towards the Fallopian tubes (~pH 7.94), reflecting variation in the site-specific microbiome and acid-base buffering at the tissue/cellular level. The temperature variation in humans is cyclical by day and month. In humans, it is biphasic, increasing in the luteal phase; with the caudal region of the oviduct 1-2 degrees cooler than the cranial portion. Temperature variation is influenced by hormones, density of pelvic/uterine vascular beds and effectiveness of heat exchange locally, crucial for sperm motility and embryo development. We have identified significant deficiencies and inconsistencies in the methods used to assess these biophysical factors within the reproductive tract. We have suggested technological solutions including the development of methods and models for real time, *in-vivo* recordings of biophysical parameters.

**WIDER IMPLICATIONS:** The notion of ‘back to nature’ in assisted conception suggested 20 years ago has yet to be translated into clinical practice. While the findings from this systematic review do not provide evidence to change current *in-vitro* protocols, it highlights our current inability to assess the *in-vivo* reproductive tract environment in real time. Data made available through future development of sensing technology *in-utero* may help provide new insights into how best to optimise the *in-vitro* embryo environment and allow for more precise and personalised fertility treatment.

**Keywords:** Oxygen, pH, temperature, biophysical parameters, female reproductive tract, reproduction, conception, technology, in-vivo, monitoring.

**INTRODUCTION**

Despite advances in ART, IVF success rates have remained static at around 25-30% ([Human Fertilisation and Embryology Authority (HFEA), 2012](#_ENREF_70)). The reasons for this may include the sensitivity of the preimplantation embryo to its environment and the complexities of processes involved in endometrial function and timely implantation.

Preimplantation development is a time of dynamic change and reprogramming, involving extensive modifications of the genome, proteome, metabolome and epigenome, and hence the zygotes and embryos have extreme sensitivities to their external environment ([Marcho et al., 2015](#_ENREF_111)). There is increasing evidence to suggest that IVF culture conditions impact on the period before and after implantation, with lasting effects on future health of the offspring ([Dumoulin et al., 2010](#_ENREF_38); [El Hajj and Haaf, 2013](#_ENREF_42); [Mantikou et al., 2013](#_ENREF_110); [Nelissen et al., 2012](#_ENREF_130)). Suboptimal chemical and physical factors during embryo culture can impair the development of gametes and embryos, leading to a profound developmental impact ([Wale and Gardner, 2016](#_ENREF_171)). The exact composition, variation and interdependent relationships of dissolved oxygen, pH and temperature within the female reproductive tract have not been well interrogated; hence, the relative concentrations of various components ([Gardner, 2008](#_ENREF_50)) and pH ([Swain, 2010](#_ENREF_163)) in commercially available culture media, and the concentration of oxygen ([Bontekoe et al., 2012](#_ENREF_19); [Wale and Gardner, 2016](#_ENREF_171)) and temperature ([Hong et al., 2014](#_ENREF_68); [Wale and Gardner, 2016](#_ENREF_171)) currently used in incubators are based on extrapolated data obtained from mainly animal studies. Technological advancements, including time phase and sensors used in IVF, have been designed to maintain a constant environment, however, in reality, the reproductive tract is likely to be dynamic ([Abrahão-Neto et al., 1996](#_ENREF_1); [Herman, 2016](#_ENREF_66); [Yedwab et al., 1976](#_ENREF_180)).

Elevated and non-physiological oxygen concentrations may have a negative impact through oxidative stress, contributing to contribute to defective embryo development, with higher rates of fragmentation ([Bedaiwy et al., 2004](#_ENREF_15)). Oxygen levels that are too low may impair embryogenesis, a process involving many oxidative metabolic processes.

Other biophysical parameters which could interplay with the levels of dissolved oxygen (DO) are also paramount in the embryo culture environment. pH is important for sperm binding and motility ([Dale et al., 1998](#_ENREF_32); [Emmens, 1947](#_ENREF_44); [Pholpramool and Chaturapanich, 1979](#_ENREF_145)), oocyte maturation ([Bagger et al., 1987](#_ENREF_9); [Downs and Mastropolo, 1997](#_ENREF_36)) and embryo development ([Lane et al., 1999](#_ENREF_95); [Zhao and Baltz, 1996](#_ENREF_183)). *In-vitro*, biologic pH buffers have been introduced into embryo culture media to help stabilise pH ([Will et al., 2011](#_ENREF_177)) and minimize deleterious intracellular changes arising from fluctuations in pH in human pre-implantation embryos ([Phillips et al., 2000](#_ENREF_144)). However, the *in-vivo* regulation of pH in luminal fluids within the reproductive tract is likely to be more complex. Clinically, in humans, conditions associated with an abnormal acidity or alkalinity within the reproductive tract are known to impact on reproductive outcome, although attempts to address this have so far seen limited success ([Guaschino et al., 2006](#_ENREF_61); [Hay et al., 1994](#_ENREF_65); [Huppert et al., 2013](#_ENREF_77); [Locksmith and Duff, 2001](#_ENREF_102)).

The potential involvement of temperature in the myriad of processes that are studied within IVF has somewhat been neglected ([Hunter, 2012b](#_ENREF_72)). There is the acknowledgement that extreme variation in body temperature provokes an immune response, orchestrated through molecular networks of cytokines and microRNAs ([Wong et al., 2016](#_ENREF_179)). Temperature is a variable which impacts significantly on development; and in some animals, sex determination ([Chojnowski and Braun, 2012](#_ENREF_28); [University of Michigan Museum of Zoology, 2014](#_ENREF_165); [Western et al., 2000](#_ENREF_175)). While sex determination serves a more evolutionary adaptive purpose, the role of temperature in the immediate biological systems is evident in its influence on modifications within the transcriptome, proteome, epigenome, and methlylome ([Bedoya-Lopez et al., 2016](#_ENREF_16); [Horowitz, 2014](#_ENREF_69)). Whilst embryos may demonstrate a certain degree of plasticity to small changes in temperature ([Hong et al., 2014](#_ENREF_68)), prolonged exposure in culture to temperatures other than the optimal 37 degrees can reduce fertilization, implantation and successful pregnancy rates ([McCulloh, 2004](#_ENREF_116)).

IVF strives to ensure that the process of handling gametes *in-vitro* closely mimics the *in-vivo* environment. However, the limiting step to such a technological development is the lack of knowledge and data on the actual *in-vivo* oxygen concentration, pH and temperature within the reproductive tract. There is significant variation in how these physiological values are controlled for in the IVF setting.

In this systematic review, we consider the understanding of the physico-chemical parameters of oxygen tension, pH and temperature within the female reproductive tract, and their potential implications in clinical and pathological processes related to implantation, fertility and that pertaining to limited reproductive capacity.

**METHODS**

**(H2) Sources**

Electronic databases, including Medline, Embase, Cochrane Library and Pubmed, were used to conduct a comprehensive literature search of original and review articles addressing the three biophysical parameters (oxygen level, pH and temperature) in the female reproductive tract. The search included studies in all species, and there was no language limitation. The search included studies published between 1946 and November 2015. Search terms included ‘oxygen’, ‘pH’, ‘hydrogen ion concentration’, ‘acid base’, ‘acidosis’, ‘alkalosis’, ‘acid’, ‘temperature’. Search terms used to identify such papers relevant to the female reproductive tract included ‘uterus’, ‘female genital system’, ‘adnexa’, ‘cervix’, ‘fallopian tube’, ‘intrauterine’, ‘ovary’, ‘vagina’, ‘oocyte’, and ‘ovum’. We also used special features and truncations to identify synonyms and broaden the search. The full Medline and Embase search strategies are available in Supplementary Data. Where necessary, we contacted authors of the published studies for specifics. There was no formal attempt to retrieve any unpublished data. References were hand-searched to identify additional references.

**(H2) Eligibility**

Full-text manuscripts were reviewed for relevancy (KYN, RM and YC). We included studies which explored oxygen, pH and temperature within the female reproductive tract. For the term ‘oxygen level’, we included studies which described oxygen tension, concentration, consumption and production. For the term ‘pH’, we included studies which described acid-base status, and hydrogen or bicarbonate ion concentrations. Studies were eligible for the systematic review if they assessed one or more of these parameters within organs of the female reproductive tract, which include vagina, cervix, uterus, Fallopian tube, adnexae, and ovary. We excluded studies which assessed the culture conditions in embryo culture only, studies assessing the parameters from second trimester of pregnancy onwards, studies assessing the method of measurement only, and studies which did not measure the parameters within female reproductive tract organs.

We screened all studies, reviewing full papers where required and disregarding those that did not meet eligibility criteria. This was performed using a double blind approach; clinically related studies were assessed by KYN and YC and technical aspects of the studies relating to the methods of measurement of oxygen, pH or temperature were assessed by RM and KYN. Any disagreement was discussed and a decision was made for inclusion or exclusion. Authorship and data sources were crosschecked to avoid inclusion of duplicate studies. Duplications of studies were excluded.

**RESULTS**

**[H2] Search results**

The PRISMA flow diagram details our search results (Figure 1) ([Liberati et al., 2009](#_ENREF_101)). The systematic search identified a total of 18, 685 records through database searching (Medline and Embase) and an additional 25 records through hand-searching of references. Duplicate studies were removed as well as those that did not meet the initial search criteria. A total of 228 full text articles were assessed for eligibility; the reasons for exclusion are details in Figure 1. Finally, 60 studies were included in the systematic review, including 16 studies relating to oxygen ([Chizhov et al., 1981](#_ENREF_27); [Fischer and Bavister, 1993](#_ENREF_48); [Garris and Mitchell, 1979](#_ENREF_52); [Hammer et al., 1981](#_ENREF_64); [Hofmeyr et al., 1985](#_ENREF_67); [Kaufman and Mitchell, 1994](#_ENREF_86); [Kigawa, 1981](#_ENREF_87); [Maas et al., 1984](#_ENREF_104); [Maas et al., 1976](#_ENREF_105); [Mastroianni and Jones, 1965](#_ENREF_113); [McNamara and Johnson, 1995](#_ENREF_119); [Mitchell et al., 1983](#_ENREF_123); [Mitchell and Van Kainen, 1992](#_ENREF_124); [Mitchell and Yochim, 1968a](#_ENREF_125); [Ottosen et al., 2006](#_ENREF_135); [Yedwab et al., 1976](#_ENREF_180)), 32 studies relating to pH ([Brabin et al., 2005](#_ENREF_20); [Brackett and Mastroianni, 1974](#_ENREF_21); [Caillouette et al., 1997](#_ENREF_24); [Dale et al., 1998](#_ENREF_32); [David et al., 1973](#_ENREF_33); [Eggert-Kruse et al., 1993](#_ENREF_41); [Engle et al., 1968a](#_ENREF_45); [Hunter and Nichol, 1988](#_ENREF_76); [Huppert et al., 2013](#_ENREF_77); [Imoedemhe et al., 1993](#_ENREF_80); [Iritani et al., 1971](#_ENREF_81); [Jenkins et al., 1989](#_ENREF_82); [Lang et al., 1956](#_ENREF_98); [Maas et al., 1984](#_ENREF_104); [Maas et al., 1977](#_ENREF_106); [Macdonald and Lumley, 1970](#_ENREF_107); [Mania-Pramanik et al., 2008](#_ENREF_109); [Mather, 1975](#_ENREF_114); [McLachlan et al., 1970](#_ENREF_118); [Miller et al., 2016](#_ENREF_120); [Nichol et al., 1997](#_ENREF_132); [Obradovic et al., 1982](#_ENREF_134); [Peeters et al., 1972](#_ENREF_141); [Pereira Da Silva et al., 2011](#_ENREF_142); [Ravel et al., 2011](#_ENREF_150); [Shalgi et al., 1972](#_ENREF_155); [Spencer et al., 2013](#_ENREF_159); [Vishwakarma, 1962](#_ENREF_169); [Weber-LaShore et al., 2010](#_ENREF_174); [Yedwab et al., 1976](#_ENREF_180); [Zavos and Cohen, 1980](#_ENREF_182); [Zodzika et al., 2011](#_ENREF_185)), and 12 studies relating to temperature ([Bahat et al., 2005](#_ENREF_10); [Benoit et al., 1976](#_ENREF_17); [David et al., 1972](#_ENREF_34); [Greve et al., 1996](#_ENREF_57); [Grinsted et al., 1980](#_ENREF_58); [Grinsted et al., 1985](#_ENREF_59); [Hunter and Einer-Jensen, 2005](#_ENREF_73); [Hunter et al., 2006](#_ENREF_74); [Kyle et al., 1998](#_ENREF_92); [Samples and Abrams, 1984](#_ENREF_152); [Scolari et al., 2011](#_ENREF_153); [Yedwab et al., 1976](#_ENREF_180)) in the female reproductive tract.

**[H2] Oxygen tension within the female reproductive tract**

The degree of oxygenation within tissue is defined by partial pressure (pO2). In a liquid, this external partial pressure results in the dissolution of oxygen within the liquid. Unlike pO2 which represents partial pressure in mmHg, DO is a measure of the concentration i.e. amount of DO molecules in the liquid (mg/L). DO is dependent on various key physical factors, such as salinity, temperature, and pH, in addition to external pressures. An estimate can be made using the solubility of oxygen in blood (0.0031 mL (L Atm)-1) at atmospheric conditions and body temperature (37.5 0C) (Table 1).

As the oxygenated blood reaches the female reproductive tract, there is a pressure gradient between the blood and tissue. An increase in blood CO2 concentration ensures that oxygen unbinds from haemoglobin. A diffusion flux of oxygen into the tissue and the cellular components of the reproductive tract occurs due to the difference in pO2, as described by Fick’s Law, in one dimension (equation 1); where J is the diffusion flux, D is the diffusion coefficient of oxygen, A is the surface area and d is the distance or thickness of the tissue.

$J=\frac{∆pO2∙D∙A}{d}$ Equation 1

As the distance from the source increases, less oxygen is transported. Therefore, in a healthy individual, pO2 within the tissue of the reproductive tract is, in theory, lower than pO2 of the vascular beds supplying the reproductive organs. It is not known how this will vary in conditions of chronic hypoxia or extreme environmental conditions where arterial pO2 and blood oxygen content can be significantly suppressed ([Grocott et al., 2009](#_ENREF_60)). However, studies in patients who are at high altitude during the period of conception and early pregnancy have higher rates of congenital anomalies ([Jensen and Moore, 1997](#_ENREF_83)) and lower fertility rates ([Bangham and Sacherer, 1980](#_ENREF_13); [Parraguez et al., 2013](#_ENREF_139)). In sheep, high-altitude hypobaric hypoxia has been shown to affect the development of the corpus luteum ([Parraguez et al., 2013](#_ENREF_139)).

A connection between mammalian embryogenesis and oxygen levels was first reported when ([Morriss and New, 1979](#_ENREF_128)) showed that the successful development of the neural fold in *ex-utero* mouse embryos was dependent on culture conditions with low oxygen levels. Oxygen is also required to sustain both spermatozoan motility and fertility. Therefore it is of physiological significance that intraluminal pO2 at the time of insemination is high in comparison to the minimal concentration required to maintain sperm viability ([Nevo, 1965](#_ENREF_131)).

During development, the oocyte and the developing embryo is transported within the Fallopian tubes and the endometrial cavity through oviduct and uterine secretions ([Cheong et al., 2013](#_ENREF_26)). The oocyte travels within the oviduct and, if fertilized, continues until the morula stage within the Fallopian tube. The oocyte is mainly metabolically anaerobic ([Lane and Gardner, 2000](#_ENREF_97)) and metabolism remains relatively quiescent after fertilization, from the cleavage stage embryo right through to the morula stage. As the cleavage stage embryo progresses to a blastocyst, the metabolic activity rises sharply, and the requirement for oxygen increases ([Fridhandler et al., 1957](#_ENREF_49); [Sturmey and Leese, 2003](#_ENREF_162)). If the blastocyst does not rapidly implant, its anaerobic energy stores will be depleted and it will disintegrate.

In theory, *in-vivo*, the oxygenation of the embryo is dependent on DO within the oviduct and uterine secretions. Conditions such as those of a thin endometrium or fluid in the endometrium ([Nejat et al., 2011](#_ENREF_129)), hydrosalpinges ([Eytan et al., 2001](#_ENREF_47); [Johnson et al., 2002](#_ENREF_85)) or ovarian hyperstimulation, when associated with ascites ([Whelan and Vlahos, 2000](#_ENREF_176)) can potentially interrupt the intrauterine cavity environment, interfering with implantation. Pregnancy rates are impaired with a thin endometrium (<7mm), where the functional (surface) layer is thin or absent and the implanting embryo is exposed to much higher uterine pO2 as it is in close proximity to the spiral arteries ([Casper, 2011](#_ENREF_25)). Infection and inflammation of the endometrium can result in blood stained uterine fluid and potentially alter, amongst other factors, the pO2 within it. Figure 2 illustrates oxygen molecules diffusing out of the arteries through various different cell layers to reach the intrauterine cavity.

Historically, atmospheric oxygen tension (~20% or 750 mmHg) (this referring to DO) has been used in tissue culture and in human IVF laboratories for embryo culture. However, the physiological oxygen concentration in the female reproductive tract has been shown to be lower, at between 2 and 8% ([Fischer and Bavister, 1993](#_ENREF_48)), and embryo development is improved when cultured in lower oxygen concentration of 5-7% compared to 20% in mammal studies ([Goto et al., 1993](#_ENREF_56); [McKiernan and Bavister, 1990](#_ENREF_117); [Pabon et al., 1989](#_ENREF_137); [Quinn and Harlow, 1978](#_ENREF_149); [Umaoka et al., 1992](#_ENREF_164)). The clinical outcome and benefits of incubating embryos in varying oxygen concentrations remain controversial. Bontekoe *et al* showed that culturing in a constant level of low oxygen concentration may improve the success rates of IVF and ICSI, and increase live birth rates (odds ratio (OR) 1.39; p= 0.005) ([Bontekoe et al., 2012](#_ENREF_19)).

**(H3) Oxygen tension and estrus/menstrual cycle variation**

***(H4) The estrus and menstrual cycle***

In animals, the menstrual cycle, known as ‘estrus cycle’ is divided into 4 phases, where the follicular phase comprises the proestrous and the estrus phase, and the ‘luteal’ phase comprises the metestrus and diestrus phases. Figure 3 compares the human menstrual cycle and the rat estrus cycle with the relative estrogen and progesterone concentrations.

***(H4) The cyclical variation of oxygen tension***

pO2 throughout the menstrual/estrus cycle was investigated in eight studies, three within the Fallopian tube ([Fischer and Bavister, 1993](#_ENREF_48); [Maas et al., 1976](#_ENREF_105); [Mastroianni and Jones, 1965](#_ENREF_113)), six within the uterus ([Fischer and Bavister, 1993](#_ENREF_48); [Garris and Mitchell, 1979](#_ENREF_52); [Kaufman and Mitchell, 1994](#_ENREF_86); [Kigawa, 1981](#_ENREF_87); [Mitchell and Yochim, 1968a](#_ENREF_125); [Yedwab et al., 1976](#_ENREF_180)), and one within cervix ([Yedwab et al., 1976](#_ENREF_180)). Only two of these studies were with human subjects ([Kigawa, 1981](#_ENREF_87); [Yedwab et al., 1976](#_ENREF_180)).

*(H5) Fallopian tube*

Oviductal pO2 within rhesus monkeys was shown to be lowest in the mid-follicular phase (35 +/- 5 mmHg) compared to either early post-ovulatory or mid luteal phases ([Fischer and Bavister, 1993](#_ENREF_48)).

pO2 within the Fallopian tube prior to and after ovulation was measured in rabbits by ([Mastroianni and Jones, 1965](#_ENREF_113)). pO2 ranged between 40 and 75 mmHg with a mean reading of 60 mmHg, and remained unchanged during ovulation and early gestation. Contrary to the findings of the first two studies, Maas et al reported much lower pO2 during the follicular phase of the cycle (<10 mmHg), and claimed that oviductal pO2 increased following ovulation, but only on the side of ovulation ([Maas et al., 1976](#_ENREF_105)). Whether ovulation stimulates local factors, such as an increase in blood flow, or changes via paracrine or autocrine signals, is open to speculation. The difference in findings of these studies may also result from the use of different sensitivities and calibrations of the polarographic sensors (see later). As an example, Maas et al does not report on the use of a temperature compensation method ([Maas et al., 1984](#_ENREF_104); [Maas et al., 1976](#_ENREF_105)); in contrast to ([Fischer and Bavister, 1993](#_ENREF_48)). Furthermore, there are differences in measurement times, and depth and location of the microelectrode placement in these experiments.

Mass et al examined the physiological properties of the Fallopian tube in the rabbit before and after microsurgery ([Maas et al., 1984](#_ENREF_104)). There was no significant difference in the mean oviductal pO2 between the anastomosed tube and the control tube. This suggests that the pO2 in the Fallopian tubes is dependent on the blood circulation in the sub-epithelial capillary network, and diffusion across the epithelial layers is a process that is not affected by reanastomosis.

*(H5) Uterus*

Six studies assessed pO2 within the uterus ([Fischer and Bavister, 1993](#_ENREF_48); [Garris and Mitchell, 1979](#_ENREF_52); [Kaufman and Mitchell, 1994](#_ENREF_86); [Kigawa, 1981](#_ENREF_87); [Mitchell and Yochim, 1968a](#_ENREF_125); [Yedwab et al., 1976](#_ENREF_180)). All six studies (in humans, monkeys, rabbits and hamsters) have shown that pO2 within the uterus during the reproductive cycle is less than the ambient air pO2 at sea level (~160mmHg). Figure 4a summarises the intrauterine pO2 throughout the estrus cycle in four studies ([Garris and Mitchell, 1979](#_ENREF_52); [Kaufman and Mitchell, 1994](#_ENREF_86); [Mitchell and Yochim, 1968a](#_ENREF_125); [Yedwab et al., 1976](#_ENREF_180)). One study in the rhesus monkeys showed that the pO2 was consistently low with no significant cycle fluctuations ([Fischer and Bavister, 1993](#_ENREF_48)).

A rise in pO2 for a brief duration around the time of ovulation was reported by two studies ([Garris and Mitchell, 1979](#_ENREF_52); [Yedwab et al., 1976](#_ENREF_180)). This may be as a consequence of increased vascularity and hyperemia of the antimesometrial capillary network, together with the rising level of estrogen observed around the time of ovulation. One study in rats showed that pO2 of the uterine lumen during late proestrus increased to 224% above the value for early proestrus ([Yedwab et al., 1976](#_ENREF_180)). The other study in guinea pigs showed a peak in the pO2 during proestrus, similar to the pattern of increase in estrogen and the uterine blood volume ([Garris and Mitchell, 1979](#_ENREF_52)). Further evidence of the role of estrogen in increasing intrauterine pO2 during the ovulatory period is supported by studies showing that exogenous estrogen administration after ovariectomy resulted in significant increases in uterine blood flow, to ten times basal levels and also significant rises in the pO2; however, progesterone was shown to suppress this increase ([Resnik et al., 1974](#_ENREF_151)).

On the contrary, other studies have shown that the intrauterine pO2 is highest during periods of low estrogen and high progesterone, produced by a functioning corpus luteum both in the diestrus phase in animals ([Kaufman and Mitchell, 1994](#_ENREF_86); [Mitchell and Yochim, 1968a](#_ENREF_125)) and in the luteal phase in humans ([Kigawa, 1981](#_ENREF_87)). Kaufman and Mitchell (1994) showed that in hamsters, the maximum pO2 occurred during diestrus, 7-fold greater than at proestrus. Rats which underwent ovariectomy had a markedly increased average intrauterine pO2 above estrus levels during the subsequent 5 days ([Mitchell and Yochim, 1968b](#_ENREF_126)). Daily subcutaneous injection of estrone prevented the post-ovariectomy elevation in pO2, and the administration of progesterone prevented, in part, the increase in pO2 post ovariectomy.

Whilst estrogen is a mediator of vascular response, inducing its effect through endothelial function ([Miller and Duckles, 2008](#_ENREF_121)), the impact of steroid mediators on intrauterine pO2 is likely to be more complex than simple vasodilation and gaseous diffusion. With the increase in vascularity, hyperaemia and oedema ensues ([Bacsich, 1939](#_ENREF_8); [Storment et al., 2000](#_ENREF_160)). Moreover, the increased estrogen levels that begin at late diestrus precede the disappearance of uterine sympathetic nerves at proestrus ([Zoubina et al., 1998](#_ENREF_186); [Zoubina and Smith, 2000](#_ENREF_187)). With the lack of autonomic supply, increase in cellular density and distance for gaseous diffusion, the concentration of pO2 within the uterine cavity is unlikely to be a simple direct correlation with density of the vascular bed around the reproductive tract.

To summarise, the trends in the fluctuations of intrauterine pO2 within the estrous/ menstrual cycle are not consistent amongst the studies, and there are no clear data around the early pregnancy state.

The presence of an embryo further complicates the story. While the oxygen requirement is low when the conceptus resides in the oviduct, the requirement for oxygen increases dramatically as it becomes a blastocyst ([Fridhandler et al., 1957](#_ENREF_49); [Sturmey and Leese, 2003](#_ENREF_162)). ([Garris and Mitchell, 1979](#_ENREF_52)) examined intrauterine pO2 in the guinea pig during the preimplantation period and showed that pO2 is low, but increases to maximum levels at the time of uterine receptivity. ([Fischer and Bavister, 1993](#_ENREF_48)) failed to show any consistent trends in uterine pO2 within the pseudopregnant rhesus monkey or rabbit, however in the hamster there was a rise in uterine pO2 from day 1 to day 3 (highest level reached 60+/-6 mmHg), and then a decrease from day 4. In the guinea pig, intense endometrial hyperemia on the day of oestrus and at the time of implantation ([Bacsich, 1939](#_ENREF_8)) corresponds with the intrauterine pO2 peak.

As well as hormones, there are other mediators of blood flow to the reproductive tissues which may influence pO2. Subjecting pseudopregnant rats to a s.c. injection of nicotine resulted in a marked and prolonged reduction in uterine blood flow, frequency and amplitude of intrauterine pO2 ([Hammer et al., 1981](#_ENREF_64)). This poses implications of smoking for the developing fetus; asymmetric fetal growth restriction was found to be more frequent in heavy smokers ([Delpisheh et al., 2008](#_ENREF_35)). Serotonin administration in rats also disrupted blood flow at the implantation site and reduced intraluminal oxygen availability ([Mitchell et al., 1983](#_ENREF_123)). Alcohol administration to rats increased the mean intrauterine pO2 from 28.3 to 38.7 mmHg (p<0.05, n=10) ([Mitchell and Van Kainen, 1992](#_ENREF_124)). The rise in oxygen tension was accompanied by an increased frequency of fluctuation in the intrauterine pO2.

The evidence around the use of vasodilators, aspirin and prostaglandins to improve IVF success is controversial, with meta-analyses concluding that they were of no clinical benefit ([Akhtar et al., 2013](#_ENREF_3); [Siristatidis et al., 2011](#_ENREF_158)) or possible clinical benefit ([Gutarra-Vilchez et al., 2014](#_ENREF_62)). However, none of the trials were able to triage women based on possible aberrant intrauterine pO2 and their inclusion criteria were broad and heterogeneous, potentially diluting the effect of these therapies.

Figure 4b shows the oxygen tension within the uterus and the Fallopian tube in five studies ([Fischer and Bavister, 1993](#_ENREF_48); [Kaufman and Mitchell, 1994](#_ENREF_86); [Kigawa, 1981](#_ENREF_87); [Mastroianni and Jones, 1965](#_ENREF_113); [Ottosen et al., 2006](#_ENREF_135)). In general, the uterus exhibits lower oxygen tensions (mean of 13-42 mmHg) than the Fallopian tube (mean of 53-60 mmHg) ([Kaufman and Mitchell, 1994](#_ENREF_86)). Within humans, the mean intrauterine pO2 has been shown to vary considerably between individuals, with means of 6.4-32 mmHg ([Ottosen et al., 2006](#_ENREF_135)). Intrauterine pO2 levels were consistently less than the venous blood levels. The functional significance of reduced pO2 in the female reproductive tract, especially in the uterus, is largely unknown. However, there are thoughts that this is a physiological phenomenon to protect the developing blastocyst from oxygen toxicity.

*(H5) Cervix*

([Yedwab et al., 1976](#_ENREF_180)) introduced transducers 2.5cm into the cervix and showed that the mean pO2 of the cervix increases during the ovulatory phase in 86% and 90% of control women and women bearing an intrauterine device (IUD) respectively and speculated that the rise in pO2 is related to the function of these organs as reservoirs for spermatozoa, as spermatozoa retain motility, vitality and higher levels of glycolysis under anaerobic conditions ([Makler et al., 1992](#_ENREF_108)).

**(H3) Oscillatory biorhythm of oxygen tension**

In both pregnant ([Hofmeyr et al., 1985](#_ENREF_67); [McNamara and Johnson, 1995](#_ENREF_119)) and non-pregnant animals, there appears to be fluctuations in the pO2 within the uterus ([Chizhov et al., 1981](#_ENREF_27); [Kaufman and Mitchell, 1994](#_ENREF_86); [Mitchell and Yochim, 1968a](#_ENREF_125); [Ottosen et al., 2006](#_ENREF_135)) and the Fallopian tubes ([Maas et al., 1984](#_ENREF_104)). Table 2 summarises the studies which assess the biorhythm of pO2, in particular the number of cycles per hour and the amplitude of change.

Intrauterine pO2 shows minute-to-minute variation in both animals ([Chizhov et al., 1981](#_ENREF_27); [Kaufman and Mitchell, 1994](#_ENREF_86); [Mitchell and Yochim, 1968a](#_ENREF_125)) and humans ([Ottosen et al., 2006](#_ENREF_135)). ([Ottosen et al., 2006](#_ENREF_135)) using a fibre optic oxygen sensor placed inside the uterus for periods of 5-10 minutes, showed rhythmic oscillations in pO2. Some exhibited rhythmic oscillations with a frequency in the order of 1 min, whereas others did not show any regular patterns. Figure 4c summarises the frequency (peaks/hour) and the mean amplitude of change in pO2 within the uterus throughout the estrus cycle in the hamster ([Kaufman and Mitchell, 1994](#_ENREF_86)) and the rat ([Mitchell and Yochim, 1968a](#_ENREF_125)). There does not seem to be a consistent read-out of the frequency of change in pO2 oscillations measured in the various species; hypoxic cycles were more often observed in non-pregnant versus pregnant rats, with the non-pregnant rats exhibiting a higher frequency of change in pO2 ([Chizhov et al., 1981](#_ENREF_27)). Rhythmic fluctuations in pO2 have also been reported in organs such as the brain, liver and muscle ([Kunze, 1976](#_ENREF_90)). The oscillatory phenomenon may be the result of energy metabolism, in connection with metabolic stress conditions, periods of readjustment, or a part of normal physiology. Physiological hypoxia has been shown to orchestrate cellular differentiation ([Simon and Keith, 2008](#_ENREF_157)), angiogenesis ([Krock et al., 2011](#_ENREF_88)) and organogenesis ([Simon and Keith, 2008](#_ENREF_157)). When cells are in a hypoxic microenvironment, several hypoxia-inducible factors (including HIF-1α and HIF-1β proteins) are stimulated, which in turn co-ordinate the development of vasculature, blood cells, and other organs ([Simon and Keith, 2008](#_ENREF_157)) which are crucial for successful implantation.

Figure 4d summarises the factors which may play a role in influencing the frequency and amplitude of pO2 fluctuations within the uterus.

Where there is a pattern to the fluctuations in pO2, the frequency of change can be, although not exclusively, similar to that of uterine contraction frequencies ([Ottosen et al., 2006](#_ENREF_135)). Therefore, one may speculate that uterine contraction patterns contribute to the variations in oxygen tension within the uterus. From ultrasonographic findings, uterine contractions have been shown to increase in frequency and amplitude from the cervix to the fundus throughout the follicular and periovulatory phases, but remains relatively quiescent around implantation ([Bulletti et al., 2000](#_ENREF_22); [Lyons et al., 1991](#_ENREF_103)). This suggests that estrogen and progesterone exert positive and negative actions on uterine contractility and influence pO2 within the uterus. There have also been various studies demonstrating endometrial wave-like activity, which may affect intrauterine pO2 ([Ayoubi et al., 2003](#_ENREF_7); [Bulletti et al., 2000](#_ENREF_22); [van Gestel et al., 2003](#_ENREF_166)) and may have roles in facilitating the supply of nutrients and oxygen to the preimplantation embryo ([Ijland et al., 1996](#_ENREF_79)). Hyperperistalsis and dysperistalsis in the uterus may contribute to subfertility and the development of endometriosis ([Leyendecker et al., 1996](#_ENREF_100)). The use of oxytocin antagonists, such as Atosiban, may be beneficial before and during embryo transfer to prime the uterus for implantation ([Lan et al., 2012](#_ENREF_93); [Pierzynski et al., 2007](#_ENREF_147)).

Cardiac output (the total amount of blood flow circulating through the organs and all tissues in the body) is influenced by both stroke volume and pulse rate. There is limited literature reporting the presence (or absence) of associations between pulse and its influence on the vasculature or delivery of oxygen to the uterus. However, one may speculate that there is a possible link between pulse rate and ‘peaks’ in pO2 within the uterus. When rabbits experienced bradycardia in the study by Maas et al, there was an immediate decrease in luminal pO2 in the Fallopian tube ([Maas et al., 1984](#_ENREF_104)). However, the pulse of animals was not measured in any of the studies, so it is not possible to deduce any associations.

The amplitude of change in intrauterine pO2 also varies throughout the estrus cycle. In hamsters, the amplitude of pO2 change was maximal during late diestrus (40.7 mmHg) and minimal during pro-oestrus (5.1 mmHg) ([Kaufman and Mitchell, 1994](#_ENREF_86)). In the rat, only slight changes in pO2 amplitude were noted; the maximal amplitude of change was seen in estrus (34.5 mmHg) and minimal change in diestrus 2 (15.7 mmHg) ([Mitchell and Yochim, 1968a](#_ENREF_125)). There seems to be no clear relationship between the frequency and amplitude of fluctuations in pO2 in the uterus. However, in the Fallopian tube of rabbits, fluctuations with small-fast amplitudes (1 mmHg) and additional slow-large oscillations (about 20 mmHg) have been observed ([Maas et al., 1984](#_ENREF_104)), suggesting a possible inverse relationship between frequency and amplitude.

Factors influencing the amplitude of change in pO2 may include hormones, sympathetic and parasympathetic tone, myometrial integrity, and vascular smooth muscle integrity (Figure 4d). Minute-to-minute changes in pO2 of reproductive organs may result from arteriolar vasodilatation and constriction. Synchronous changes in endometrial vascular patency are thought to result in differences in pO2 ([Garris and Mitchell, 1979](#_ENREF_52)). A slower frequency of arteriolar vasodilatation and constriction may allow greater inflow of oxygenated blood through the arteries, and therefore greater amplitudes of pO2 change.

The functional significance of these acute changes in intrauterine pO2 and its relation to gamete viability, conceptus implantation and development is not clear. However, as the amplitude and frequency of minute-to-minute changes in pO2 occur at the time of implantation, one may speculate an important role of fluctuations in pO2. The rhythmical decrease in pO2 in the uterine and fetal tissues may give rise to periodic hypoxia, which may have a role in stimulating metabolic reactions and possibly in preparing the uterus and fetus for various potential insults and pathological mechanisms.

**(H3) Oxygen tension in neoplasia**

([Kigawa, 1981](#_ENREF_87)) measured pO2 and pCO2 using mass spectrometry in 45 cases of uterine myoma, 26 cervical carcinoma, six endometrial carcinoma and 20 normal uterus cases. The intrauterine pO2 was lower and pCO2 was higher in the cases of uterine neoplasm than the control, and in particular, the pCO2 was significantly higher in cervical carcinoma than the control. The intrauterine oxygen tensions in tissue of different pathology are shown in Figure 4e. A solid tumour commonly outgrows its own vasculature beyond the size of several cubic millimetres, resulting in low oxygen levels or ‘hypoxia’.

**(H3) Methods of measuring oxygen tension within the female reproductive tract**

The most well-known and widely used method of sensing oxygen is the non-invasive pulse oximetry method ([Elliott et al., 2012](#_ENREF_43); [Nitzan et al., 2014](#_ENREF_133)). It provides a preliminary reading of the haemoglobin saturation over long periods of time, without the need to analyse blood samples *in-vitro*. However, it cannot determine the amount of oxygen dissolved within plasma or tissue; as is required for measurements in the reproductive tract ([Nitzan et al., 2014](#_ENREF_133)).

Optical sensors rely on the quenching of a fluorescent signal. This can either be based on intensity or decay time ([Gewehr and Delpy, 1993](#_ENREF_54); [Wang and Wolfbeis, 2014](#_ENREF_173); [Wolfbeis, 2015](#_ENREF_178)). An oxygen sensitive fluorescent dye is incorporated in a polymer membrane, which is in contact with the tissue under investigation. A variety of fluorophores are available for sensing oxygen; dyes based on Ruthenium (Ru) compounds are widely used ([Wang and Wolfbeis, 2014](#_ENREF_173)). The intensity of the fluorescent signal is measured upon excitation via a light source. As oxygen interacts with the dye, the fluorescence is quenched. These optical sensors have been incorporated successfully in probes, and inserted within the reproductive tract together with a catheter needle to assess oxygen *in-vivo* ([Ottosen et al., 2006](#_ENREF_135)). The main advantage lies in their high specificity and wide measurement range. Their life-time is determined primarily by the dye ([Gewehr and Delpy, 1993](#_ENREF_54); [Jiang et al., 2008](#_ENREF_84); [Wang and Wolfbeis, 2014](#_ENREF_173); [Wolfbeis, 2015](#_ENREF_178)).

The most widely employed sensors are electrochemical i.e. polarographic and galvanic based sensors. The underlying electrochemical principle is based on a reaction occurring at the interface between a working electrode (WE) and the solution under test. Through the application of a bias potential, either applied (polarographic) or self-polarized (galvanic), oxygen is reduced locally at the electrode surface. Typically, the polarographic sensors consist of three electrodes: an oxygen reducing WE, a current supplying counter electrode, and a biasing reference electrode (RE). An oxygen permeable membrane limits the diffusion of oxygen and prevents interfering ions from reaching the electrode surface. This type of set-up is referred to as a Clark electrode ([YSI](#_ENREF_181)).

As the redox reaction continues, the oxygen near the WE is consumed causing a concentration gradient between the solution under test and an inner electrolyte. Consequently, oxygen diffuses across the membrane until a steady state is reached. The rate of diffusion equals the rate of consumption and the resulting current is related to the flow of oxygen to the WE surface caused by the partial pressure difference between the solution and the inner electrolyte. The major disadvantage of the electrochemical sensors is that the measurement perturbs its own environment. This adds a need for re-equilibration during continuous measurements ([YSI](#_ENREF_181)). Great efforts have been made to miniaturise Clark type sensors for use in *in-vivo* applications. In contrast to macro-scale electrodes, microscale sensors can overcome diffusion limitations by incorporating an ultra-micro electrode design, lowering response time and reducing current requirements ([Aoki, 1993](#_ENREF_4); [Arrigan, 2004](#_ENREF_6)).

Within the female reproductive tract, both optical and electrochemical based sensors can be used. In the aforementioned studies, by far the most widely employed systems rely on the polarographic determination of pO2 ([Fischer and Bavister, 1993](#_ENREF_48); [Hammer et al., 1981](#_ENREF_64); [Kaufman and Mitchell, 1994](#_ENREF_86); [Maas et al., 1976](#_ENREF_105); [Mastroianni and Jones, 1965](#_ENREF_113); [Mitchell and Yochim, 1968a](#_ENREF_125)). The sensors are commonly incorporated into needle type devices, which are inserted into the tissue of interest.

The method of measurement may play a role in discrepancies between the studies. As an example, measurement times vary between experimental methods. ([Mitchell and Yochim, 1968a](#_ENREF_125)) measured average pO2 over a period of 20 minutes; in contrast ([Fischer and Bavister, 1993](#_ENREF_48)) measured over only 1-2 minutes. Looking at the method of calibration prior to measurement, although all studies use at least a two point calibration method using saturated and non-saturated pO2 calibration buffers, reports on temperature compensation are limited. Moreover, temperature recordings during the *in-vivo* measurements may be inaccurate. In some studies, temperature was recorded within the rectum of the anaesthetised animals ([Hammer et al., 1981](#_ENREF_64); [Kaufman and Mitchell, 1994](#_ENREF_86)). This generalised assumption, in which the temperature in different parts of the reproductive tract is considered to be uniform, is not true (we discuss this later in this review).

Finally, the histological effect of the probe placed at the site of implantation is largely unknown. Some studies have concluded that this effect is minimal ([Mastroianni and Jones, 1965](#_ENREF_113); [Ottosen et al., 2006](#_ENREF_135)). In other cases, tissue integrity is affected resulting in an alteration of the pO2 through inflammation. The effect of anaesthesia and ways to minimise its effects have also been discussed by some studies. This includes keeping temperature profiles constant in the surrounding environment and supplying additional oxygen. However, it remains questionable whether the pO2 profile obtained in this manner is accurate.

**(H2) pH in the female reproductive tract**

The pH level within the human body represents the amount of free protons within the aqueous body liquids. It is defined as the negative log of the hydrogen ion activity. Throughout the body, pH is tightly regulated and plays an important role in maintaining homeostasis and cell viability.

The pH in the female reproductive tract has been investigated in numerous studies. Lower pH levels are found within the cervix and vagina compared to the uterus and Fallopian tubes (Figure 5).

(**H3) pH within the female lower genital tract**

The vaginal pH of non-human mammals is never as low as for humans (median vaginal pH in humans is ~4.5, non-human mammals is ~5.4-7.8) ([Miller et al., 2016](#_ENREF_120)). Lactobacilli maintain vaginal health in humans and the relative abundance is typically >70% of the resident bacteria. Estrogen is known to trigger accumulation of glycogen in vaginal epithelial cells, which leads to the production of lactic acid by lactobacilli, lowering the vaginal pH. Recent vaginal microbiome work has shown differences in communities where the lowest median pH (4.0+/-0.3) had vaginal communities dominated by *L. crispatus* ([Ravel et al., 2011](#_ENREF_150)). Loss of lactobacilli dominance has been linked to bacterial vaginosis (BV), which is associated with an overgrowth of anaerobic bacteria and high pH (>4.5). The prevalence of BV in infertile women is high (19%), and an abnormal microflora occurs in 39% of infertile patients ([van Oostrum et al., 2013](#_ENREF_167)).

We identified eight studies which addressed the relationship between the pH of the cervicovaginal region in humans; three studies relating to presence of BV ([Brabin et al., 2005](#_ENREF_20); [Mania-Pramanik et al., 2008](#_ENREF_109); [Zodzika et al., 2011](#_ENREF_185)), eight studies relating to other vaginal infections ([Brabin et al., 2005](#_ENREF_20); [Caillouette et al., 1997](#_ENREF_24); [Eggert-Kruse et al., 1993](#_ENREF_41); [Lang et al., 1956](#_ENREF_98); [Mania-Pramanik et al., 2008](#_ENREF_109); [Peeters et al., 1972](#_ENREF_141); [Weber-LaShore et al., 2010](#_ENREF_174); [Zodzika et al., 2011](#_ENREF_185)). Two studies assessed the relationship between a “high vaginal pH” (pH >4.5) and microorganism status ([Brabin et al., 2005](#_ENREF_20); [Caillouette et al., 1997](#_ENREF_24)). High vaginal pH seems to be associated with aerobic vaginitis, vaginal colonisation including BV, trichomonas vaginalis (TV), mycoplasma genitalium (MG) and Candida infection, and presence of sperm cells in smears ([Caillouette et al., 1997](#_ENREF_24); [Huppert et al., 2013](#_ENREF_77); [Lang et al., 1956](#_ENREF_98); [Mania-Pramanik et al., 2008](#_ENREF_109); [Peeters et al., 1972](#_ENREF_141); [Weber-LaShore et al., 2010](#_ENREF_174); [Zodzika et al., 2011](#_ENREF_185))

There is a link between high vaginal pH (pH>4.5) and the presence of BV ([Brabin et al., 2005](#_ENREF_20); [Mania-Pramanik et al., 2008](#_ENREF_109); [Zodzika et al., 2011](#_ENREF_185)). A third of adolescents and up to 80% of women of reproductive age had BV when the vaginal pH was between 4.5 and 5.5, while a normal pH appears to preclude BV. It is not clear whether high vaginal pH associated with BV is a cause or result of the syndrome. One theory would suggest the host factors cause vaginal pH to increase and lactobacilli to decrease, culminating in an overgrowth of vaginal commensal organisms and BV ([Pybus and Onderdonk, 1999](#_ENREF_148)).

([Lang et al., 1956](#_ENREF_98)) showed that in the non-pregnant woman (n=149), mean pH in women with no infection was 4.79; with candida infection it was 4.89, with trichomonas infection it was 5.42 and with mixed infection the pH was 5.30. A similar association was seen in the pregnant group. In a separate study, the mean intracervical pH for patients with symptoms or signs of candida infection was 6.81; the pH is higher than that observed in the vagina for this group ([Peeters et al., 1972](#_ENREF_141)). A logistic regression model designed to detect MG, showed that pH >4.5 was a predictor of MG (OR 3.1, n=217). The association was strongest for women who were both TV and MG positive ([Weber-LaShore et al., 2010](#_ENREF_174)). Although the authors refer to this as a “point of care test”, this has not been formally validated. Another study showed that of the women without clinical BV or TV, a significantly greater proportion of those with pH>4.5 (25%) had MG, compared to those with pH ≤4.5 (9%) ([Huppert et al., 2013](#_ENREF_77)).

On the contrary, three studies did not find any association between microbial colonization and vaginal pH ([Brabin et al., 2005](#_ENREF_20); [Eggert-Kruse et al., 1993](#_ENREF_41); [Mania-Pramanik et al., 2008](#_ENREF_109)). *C. trachomatis* infection does not seem to lead to a change in the vaginal pH ([Mania-Pramanik et al., 2008](#_ENREF_109)). In a study including adolescents, there was no significant association of human papilloma virus, chlamydia, Herpes Simplex Virus Type 2 (HSV-2) antibodies, gonorrhoea, or presence of mixed infections with a high vaginal pH (>4.5), or of candida with a low vaginal pH (≤4.5) ([Brabin et al., 2005](#_ENREF_20)). ([Eggert-Kruse et al., 1993](#_ENREF_41)) showed no significant relationship between the mucus pH and the microbial colonization of cervix and ejaculates in couples assessed for infertility.

An abnormal vaginal microbiota has been associated with infertility ([van Oostrum et al., 2013](#_ENREF_167)) and a poorer pregnancy rate in IVF patients ([Haahr et al., 2016](#_ENREF_63)). The presence of non-Lactobacillus dominated microbiota in the receptive endometrium (<90% Lactobacillus spp or >10% of other bacteria) has been associated with reduced implantation, pregnancy and live birth rates ([Moreno et al., 2016](#_ENREF_127)). The question remains as to whether patients should be screened and subsequently treated for abnormal microbiota before initiation of fertility treatment. In a prospective study, the vaginal microbiome was analysed in samples taken from infertile patients just before embryo transfer in IVF, and common species identified included *Lactobacillus*, *Staphylococcus* and *Enterobacteriaceae*. Implantation rates were 12.4% for women with one or more bacteria present, versus 14% in those completely negative for bacteria ([Selman et al., 2007](#_ENREF_154)). However, culture-based technology was used in this study, and the major limitation is the under-representation of the presence of the microbiome. Later work used 16S ribosomal RNA sequencing technology to capture the diversity of the microbiome in the cervix and vagina. The diversity of the vaginal microbiome at the day of embryo transfer has been considered a potential influence live birth rate ([Hyman et al., 2012](#_ENREF_78)). A Cochrane review analysed RCTs that investigated the use of antibiotics at embryo transfer but concluded that more evidence was required, with live birth as a primary outcome ([Kroon et al., 2012](#_ENREF_89)).

**(H3) pH and relationship with menstrual cycle and hormone levels**

Four studies assessed cervico-vaginal pH ([Brabin et al., 2005](#_ENREF_20); [Caillouette et al., 1997](#_ENREF_24); [Eggert-Kruse et al., 1993](#_ENREF_41); [Macdonald and Lumley, 1970](#_ENREF_107)); one assessed the intrauterine pH ([Mather, 1975](#_ENREF_114)), and one assessed the pH within the oviduct ([Nichol et al., 1997](#_ENREF_132)) and relationship with the menstrual cycle and hormone levels.

Fluctuations in the endocervical mucus and vaginal pH during the menstrual cycle is controversial ([Brabin et al., 2005](#_ENREF_20); [Macdonald and Lumley, 1970](#_ENREF_107); [Zavos and Cohen, 1980](#_ENREF_182)). Studies have shown an elevated pH in the uterus in bovines just following ovulation (pH 7.35 compared to pH 7.22 prior to ovulation) ([Mather, 1975](#_ENREF_114)) and in human endocervical mucus during ovulation ([Macdonald and Lumley, 1970](#_ENREF_107)).

The lowest vaginal pH is exhibited during periods of highest estrogen, just prior to ovulation when the lactobacilli are most abundant. Estrogen is known to have a positive effect on cervical mucus production ([Gaton et al., 1982](#_ENREF_53)) and increasing estrogen promotes thickening of the vaginal epithelium and glycogen production within cells ([Mirmonsef et al., 2016](#_ENREF_122)). A lower cervico-vaginal pH is associated with the follicular phase and rising estradiol concentrations ([Brabin et al., 2005](#_ENREF_20); [Eggert-Kruse et al., 1993](#_ENREF_41)), and conversely a higher pH of 6.0-7.5, in the absence of abnormal vaginal colonisation, is suggestive of menopause ([Caillouette et al., 1997](#_ENREF_24)) while progesterone does not appear to influence vaginal pH ([Brabin et al., 2005](#_ENREF_20)). The presence of irregular cycles predisposes to higher vaginal pH values. Low endocervical pH levels were significantly more frequent in patients with hyperandrogenemia ([Eggert-Kruse et al., 1993](#_ENREF_41)). The authors proposed that the influence of androgens on pH may be due to relative estrogen deficiency, potentially caused by acting as a competitor with estrogens at the receptor level.

Table 3 summarises the studies for pH within the oviduct. Fluctuations of pH within the pig oviduct composed of low frequency, high amplitude waves superimposed upon small peaks of high frequency and low amplitude; where the frequency of smaller peaks increased in the pre and peri-ovulatory phases, with large variation in pH and a high frequency of large peaks in mid-cycle followed by a stabilised pH post-ovulation, especially in the presence of an ipsilateral embryo ([Nichol et al., 1997](#_ENREF_132)). The exact purpose of the fluctuations in the pH is largely unknown.

**(H3) pH and pathology**

*(H4) pH, infertility and pregnancy outcomes*

Three studies have shown that a low endocervical mucus pH (generally pH ≤ 4.5) level may be associated with infertility ([Eggert-Kruse et al., 1993](#_ENREF_41); [Jenkins et al., 1989](#_ENREF_82); [Spencer et al., 2013](#_ENREF_159)).

In the past, post coital testing (PCT), which examines motility of sperm within postcoital cervical mucus, formed an integral part of basic fertility assessment. Low cervical mucus pH has been correlated with sperm immobility ([Jenkins et al., 1989](#_ENREF_82); [Peek and Matthews, 1986](#_ENREF_140); [Zavos and Cohen, 1980](#_ENREF_182)). Semen raises the low baseline vaginal pH and this is maintained for hours after the sperm enter the cervical canal. For a long time, it had been suspected that the sperm interaction may be impaired by microorganisms colonising the female genital tract, potentially changing the pH. However, the influence of microbial colonisation on sperm penetration was found to be of minor significance and furthermore, sperm mucus interaction could not be significantly improved with antimicrobial therapy ([Eggert-Kruse et al., 1988](#_ENREF_40)). Whilst a bicarbonate vaginal douche may have some impact on sperm motility, such interventions have not been shown to improve pregnancy outcomes ([Jenkins et al., 1989](#_ENREF_82)). Hence PCT is no longer recommended as a component of standard fertility investigation ([Collins et al., 1984](#_ENREF_30); [Glatstein et al., 1995](#_ENREF_55)).

Of interest is that women with cystic fibrosis (CF) often suffer from infertility and their periovulatory pH, bicarbonate secretion and spinnability of the cervical mucus was much lower than controls (pH 7.53 versus. 8.02) ([Spencer et al., 2013](#_ENREF_159)). It is plausible that in CF, the absence of increase in pH and bicarbonate secretion during the ovulatory phase contribute to their reduced fertility, although the exact mechanisms of subfertility with respect to pH and other CF related factors are still unknown.

*(H4) pH and relationship with sexually transmitted infection treatment history, contraception use and sexual activity*

([Brabin et al., 2005](#_ENREF_20)) showed that in a population of adolescents, after controlling for BV and condom use, vaginal pH was positively associated with cervical ectopy (OR=2.5; p=0.05) and sexually transmitted infection (STI) treatment history (OR=2.5; p=0.07), and negatively associated with use of Depo-Provera (OR=0.1; p=0.003) and recent onset (<12 months) of sexual activity (OR =0.2; p=0.004). When looking specifically at the presence of active STIs, no correlations were found with vaginal pH.

Two studies showed that the cervical and uterine luminal pH did not differ in women who have an IUD *in situ* ([Obradovic et al., 1982](#_ENREF_134); [Yedwab et al., 1976](#_ENREF_180)).

**(H3) pH regulation**

*(H4) Intracellular pH*

Intracellular pH (pHi) regulation is a vital component within mammalian cell homeostasis (Figure 5). The main regulatory mechanisms are the: HCO3–/Cl– exchanger; Na+/H+ antiporter; and Na+, HCO3–/Cl– exchanger. Intracellular processes are highly sensitive to pH, e.g. protein synthesis metabolism, mitochondrial activity and cytoskeletal regulation. The mechanism of pHi regulation in the human preimplantation embryo is still largely unknown, however, pHi regulation through these mechanisms has been investigated in the mouse ([Phillips and Baltz, 1999](#_ENREF_143); [Zhao et al., 1995](#_ENREF_184)) and hamster embryo ([Lane et al., 1998](#_ENREF_94), [1999](#_ENREF_95); [Lane and Bavister, 1999](#_ENREF_96)).

*(H4) Extracellular pH*

The extracellular pH (pHe) of the oocyte and the developing embryo during its passage from the ovarian follicle to the uterus is currently not well understood either. The pH of the follicular fluid is reported to be 7.2 to 7.3 ([Imoedemhe et al., 1993](#_ENREF_80); [Shalgi et al., 1972](#_ENREF_155)). However, as we have already explored, the pH of the oviductal fluid is generally more alkaline (pH 7.1 to 8.4, with periovulatory and early pregnancy phases tending to a higher pH) ([David et al., 1973](#_ENREF_33); [Engle et al., 1968b](#_ENREF_46); [Hunter and Nichol, 1988](#_ENREF_76); [Maas et al., 1984](#_ENREF_104); [Maas et al., 1977](#_ENREF_106); [Nichol et al., 1997](#_ENREF_132); [Vishwakarma, 1962](#_ENREF_169)), suggesting that a high pH is required for preimplantation embryo development. In contrast, the uterine environment is generally less alkaline (pH 7.3 to 7.9) ([Brackett and Mastroianni, 1974](#_ENREF_21); [Iritani et al., 1971](#_ENREF_81); [Mather, 1975](#_ENREF_114); [McLachlan et al., 1970](#_ENREF_118)). The mammalian embryo encounters three differing environments; generally the fluid that surrounds the oocyte and the preimplantation embryo is more alkaline than the pHi. The optimal pHe for fertilization has been suggested to be around 7.5 (peak in zona binding and penetration) ([Dale et al., 1998](#_ENREF_32)).

*(H4) Oocytes and the preimplantation embryo pH regulation*

Studies have shown that oocytes and the pre-implantation blastocyst are able to recover from changes in environmental pH and have the ability to stabilize their pH ([Dale et al., 1998](#_ENREF_32); [McLachlan et al., 1970](#_ENREF_118)), although this innate ability for restoration is somewhat perturbed in oocytes which failed to fertilise, those which are immature at the germinal vesicle stage or in early embryos up to the morula stage as opposed to blastocysts. One can infer from these observations that the plasma membrane of oocytes and embryos is highly permeable to H+ ions, and regulation of H+ ion concentration occurs between morula and blastocyst stages, possibly in preparation for the more acidic uterine environment.

([McLachlan et al., 1970](#_ENREF_118)) showed that the 6-day-old blastocyst had a mean pH of 7.62, statistically different to the pH value of the uterine secretion which surrounds the blastocyst before implantation (pH 7.69). There was little variation in the pH, even when the blastocysts were obtained from different rabbits or from different horns from the same rabbit, suggesting that the 6-day pre-implantation blastocyst has mechanisms in place to maintain its own internal environment.

**(H3) Methods of pH measurement**

The studies explored in this systematic review use two main methods of pH sensing; assessment through pH indicator strips (preferred in vaginal studies) and assessment using glass pH microelectrodes (preferred in *in-utero*/oviduct studies).

pH indicator papers rely on the interaction of protons with a colour changing, fixed, chemical (either acid or base) on the paper strip ([Strauss et al., 2005](#_ENREF_161)) They can be good indicators for BV and are hence applied in this field ([Caillouette et al., 1997](#_ENREF_24); [Zodzika et al., 2011](#_ENREF_185)). Their main limitation lies in their inaccuracy and their inability to provide a detailed pH profile.

In contrast, glass pH electrodes have been developed for *in-vivo* applications. These micro-electrodes are 1-4mm in diameter and small enough to access all areas within the female reproductive tract via as incision ([Brabin et al., 2005](#_ENREF_20); [Macdonald and Lumley, 1970](#_ENREF_107); [Nichol et al., 1997](#_ENREF_132)). They use an ion sensitive glass membrane, forming the outside of the probe. The inside of the probe contains a silver-chloride reference electrode (RE) in a solution of fixed pH and saturated KCl to maintain potential stability. The ions within the solution under test are exchanged with the glass, causing a potential difference between it and the internal RE. The potential follows the Nernst-equation and is typically -59 mV pH-1. However, they are fragile and difficult to operate autonomously, so hospitalisation is required for use *in-vivo* ([Vonau and Guth, 2006](#_ENREF_170)).

Alternatives to the aforementioned methods exist but have not been employed extensively for measuring pH within the female reproductive tract. These include ion-sensitive field effect transistors and ion-sensitive metal electrodes. They rely on the principal that ions interacting with the metal surface cause a change in surface potential. Similar to the glass electrode, this potential is referenced against an on-board RE. They are compatible with conventional micro-fabrication techniques, allowing them to be scaled in size. They are also less fragile, can run autonomously and can be in solid-state (no RE is required) ([Bergveld Em, 2003](#_ENREF_18); [Chu et al., 2015](#_ENREF_29); [Kurzweil, 2009](#_ENREF_91)).

Although both glass and paper-based pH indicators can serve a clinically relevant purpose, the inability to assess pH levels continuously is a significant limitation. The sensors most suited for this purpose are the ion-sensitive electrodes.

**(H2) Temperature in the female reproductive tract**

Basal body temperature (BBT) ([Martinez et al., 1992](#_ENREF_112)) is diurnal, with the minimal body temperature occurring in early morning ([Piccione et al., 2003](#_ENREF_146); [Vickers et al., 2010](#_ENREF_168)). It also tends to show a biphasic pattern, increasing in the luteal phase by 0.31 to 0.46ºC compared to the follicular phase ([Baker et al., 2001](#_ENREF_12); [Cagnacci et al., 2002](#_ENREF_23); [Coyne et al., 2000](#_ENREF_31); [Simic and Ravlic, 2013](#_ENREF_156)); the latter is attributed to the thermogenic action of progesterone ([Zuspan and Rao, 1974](#_ENREF_188)). Elevated progesterone levels stimulate norepinephrine release from the hypothalamic temperature control centre, which then increases BBT, reflected in measurements in oral, axillary, vaginal or rectal temperature readings ([Driver and Baker, 1998](#_ENREF_37); [Martinez et al., 1992](#_ENREF_112); [McCreesh et al., 1996](#_ENREF_115); [Zuspan and Rao, 1974](#_ENREF_188)).

In this review, four studies assessed temperature in vagina ([Kyle et al., 1998](#_ENREF_92); [Samples and Abrams, 1984](#_ENREF_152); [Scolari et al., 2011](#_ENREF_153); [Yedwab et al., 1976](#_ENREF_180)), three studies assessed temperature within the oviduct ([Bahat et al., 2005](#_ENREF_10); [David et al., 1972](#_ENREF_34); [Hunter and Nichol, 1986](#_ENREF_75)), and five studies assessed temperature within the ovary ([Benoit et al., 1976](#_ENREF_17); [Greve et al., 1996](#_ENREF_57); [Grinsted et al., 1980](#_ENREF_58); [Grinsted et al., 1985](#_ENREF_59); [Hunter et al., 2006](#_ENREF_74)) (Figure 4f). There were no studies identified which assessed temperature within the uterus.

*(H3) Temperature in the vagina and cervix*

Two studies assessed vaginal temperature within the human ([Samples and Abrams, 1984](#_ENREF_152); [Yedwab et al., 1976](#_ENREF_180)) and two studies assessed it in animals (dairy cattle ([Kyle et al., 1998](#_ENREF_92)), and swine ([Scolari et al., 2011](#_ENREF_153))) (Figure 4f). In a small sample of 66 women, the mean temperature in the posterior fornix of the vagina was 36.7ºC ([Yedwab et al., 1976](#_ENREF_180)), which was not significantly different from the cervical lumen. A mean morning vaginal temperature was found to be 36.48ºC and the mean afternoon vaginal temperature to be 37.20ºC ([Samples and Abrams, 1984](#_ENREF_152)).

Using the vulval or vaginal temperature to predict the onset of estrus has been of interest ([Kyle et al., 1998](#_ENREF_92); [Scolari et al., 2011](#_ENREF_153)); during estrus the cow is sexually attractive to bulls and optimal insemination rates occur at the end of standing estrus.

([Kyle et al., 1998](#_ENREF_92)) showed that the mean maximal increase in vaginal temperature at estrus was 0.9ºC and the mean duration of the estrual peak in vaginal temperature was 6.5 hours. In addition, vaginal temperature was found to be significantly depressed for 3 days prior to estrus and significantly elevated at mid-cycle. The rise and fall in temperatures in cattle and swine as illustrated by these two studies may be associated with detectable events in the estrus cycle, including ovulation.

In humans, commercially available vaginal sensors (e.g. ‘OvuSenseTM’, Warwick, UK) ([Ovusense, 2016](#_ENREF_136)) record vaginal temperature at regular intervals via a vaginal sensor, inserted in a similar way to a tampon. The aim is to detect when ovulation occurs, recognised by a sustained rise in the average core temperature and, hence, when the fertile period is. Although such devices are accurate at detecting ovulation and are widely accepted amongst users ([Papaioannou et al., 2013](#_ENREF_138)), there are no studies assessing whether they improve pregnancy or live birth rates.

**(H3) Temperature gradients within the oviduct**

Temperature gradients within oviducts of ovulated rabbits ([Bahat et al., 2005](#_ENREF_10); [David et al., 1972](#_ENREF_34)) and pigs ([Hunter and Nichol, 1986](#_ENREF_75)) are thought to play a role in directing sperm in the process of fertilisation; capacitated human and rabbit spermatozoa are thermo-tactile and swim up the temperature gradient ([Bahat et al., 2003](#_ENREF_11)). Prior to ovulation, sperm pass from the warmer uterus, through the uterotubal junction into the cooler isthmus which leads to reduced motility ([Hunter and Nichol, 1986](#_ENREF_75)). The caudal region of the isthmus is 1-2°C cooler than the cranial portion of the ampulla of the oviduct ([Bahat et al., 2005](#_ENREF_10); [Hunter, 2012a](#_ENREF_71); [Hunter and Nichol, 1986](#_ENREF_75)). This difference increased to 1.6° C (sperm storage site 34.7°C, fertilisation site 36.3°C) after ovulation (p<0.03). The temperature difference was maintained over a distance of 9.8cm ([Bahat et al., 2005](#_ENREF_10)).

**(H3) Temperature gradients within the ovary**

Preovulatory follicles were 1.3-1.7°C cooler than neighbouring ovarian tissue in the human, rabbit, pig and cow ([Greve et al., 1996](#_ENREF_57); [Grinsted et al., 1980](#_ENREF_58); [Grinsted et al., 1985](#_ENREF_59); [Hunter et al., 2006](#_ENREF_74)) and this temperature gradient increased towards ovulation (maximum difference, 2.3°C) ([Grinsted et al., 1985](#_ENREF_59)). Reversal of this temperature gradient incapacitated the oocyte. The lower temperature of the follicular fluid prior to ovulation may be essential for normal oocyte development and the change in temperature within the ovary at the ovulatory phase may be important for optimal nuclear, cytoplasmic and membranous maturation ([Aroyo et al., 2007](#_ENREF_5); [Wang et al., 2009](#_ENREF_172)).

([Benoit et al., 1976](#_ENREF_17)) showed that in non-ovulating ewes, both mean ovarian temperatures varied at random, whereas in the ovulating ewes, ovarian temperatures decreased by approximately 0.15°C prior to the LH surge. There was a smaller magnitude and later onset of decrease in temperature in the anovulatory compared with the ovulatory ovaries, which may be a result of heat removal through blood flow through the ovary influenced by differing levels of estradiol. An increase in estradiol produced from the maturing follicle increases blood flow to the ovary, which may account for a cooling effect.

**(H3) Factors influencing temperature in the female reproductive tract and their implications on fertility**

The temperature in the reproductive tract depends on the rate of heat production by the metabolically active tissues and the rate of heat loss from that organ. In addition, heat is gained or lost through heat conduction to other tissues, which are in close proximity, for example, bladder and bowel. The differences in vascular beds and contractility of muscles may be important. Many conditions associated with subfertility or implantation failure, such as endometriosis, adenomyosis, leiomyoma, and intrauterine adhesions, present with variation in vasculature, mass and tissue density within the reproductive tract that may alter the temperature gradient profile. Physiological secretions within the reproductive tract or the presence of pathology, for example hydrosalpinges, may support endothermic reactions or regional cooling. A common observation during laparoscopy in women with subfertility and/or pain is that of ‘engorged’ pelvic veins of unknown significance, although in theory larger vasculature can assist in heat lost through convection and provide a cooler *in-situ* environment. The mysterious ‘fluid within the endometrial cavity’ occasionally observed at the time of embryo transfer, associated with poor implantation, may be the natural response of the uterus to achieve cooling. Uterine blood flow is predominately regulated by estrogen ([Abrams et al., 1970](#_ENREF_2)) and progesterone ([Garris and Mitchell, 1979](#_ENREF_52)); conditions such as obesity and polycystic ovary syndrome, where endogenous estradiol is consistently elevated, invite speculation as to the extent of deregulated local temperature being accountable for poorer reproductive outcome.

**(H3) Methods of measuring temperature**

The measurement of temperature in the female reproductive tract is performed using either invasive (implanted), semi-invasive (inserted) or non-invasive (external) temperature sensors. For the invasive methods, either thermistors or thermocouples are used. A thermistor is a temperature-sensitive resistor, where the resistance of a metal-oxide changes with temperature. They are inexpensive and are available in small packages making them ideal for implantation. However, they are limited by their small temperature range (1000C). For *in-vivo* measurements, this drawback is of no concern as body temperature falls within this range.

Thermocouples consist of two different metals that create a potential difference along the metal-metal junction based on the thermo-electric effect. They are used in applications where the temperature range exceeds that of the thermistor. They have a lower accuracy but have a lower cost and faster response time than thermistors.

The semi-invasive temperature sensors employ similar concepts to the invasive devices. Commercially available devices of this kind include ([Ovusense, 2016](#_ENREF_136)), used for measuring the temperature within the vagina. However, these semi-invasive probes cannot be used to determine the *in-vivo* temperature. Alternative methods use radiotelemetry platforms in which the pulse frequency of the radio transmitter is influenced by the temperature (used in the remote monitoring of animals).

Non-invasive methods include thermal imaging using infra-red to examine the thermal properties of tissues. These methods are ideally suited for imaging large surface areas. However, the instrumentation required is expensive and does not provide an accurate reading at a specific site. Other non-invasive devices use temperature sensing elements fixed externally on the skin. An example of such a device is the Duofertility temperature patch ([Duofertility, 2016](#_ENREF_39)).

**DISCUSSION**

The preimplantation embryo is known to be highly sensitive to the external environment as it undergoes a series of dynamic changes and reprogramming in preparation for implantation ([Marcho et al., 2015](#_ENREF_111)). In this systematic review, we have discussed findings from the current literature pertaining to pO2, pH and temperature within the female reproductive tract. pO2 within the uterus and Fallopian tubes fluctuates throughout the estrus and menstrual cycles and tends to show an oscillatory biorhythm. This physiological phenomenon is perhaps important for the survival of the preimplantation embryo and may be influenced by hormones, blood supply, tissue integrity and other external factors. Many studies have identified a link between high pH (>4.5) within the female lower genital tract and the colonisation of micro-organisms (including BV and *Trichomonas vaginalis*, *Mycoplasma genitalium* and candida infections). Endocervical mucus has been shown to increase following ovulation, and an alkaline environment is important for sperm survival. Fluctuations in pH are important for sperm function, oocyte maturation and embryo development, and we know that there are in-built mechanisms for pH regulation in the embryo and the oocyte. An increase in vaginal temperature is a known predictor of ovulation and forms the basis of the ‘OvusenseTM’ system, designed to facilitate conception by predicting the fertile days in the woman. The presence of temperature gradients in the female reproductive tract, including the Fallopian tube and the ovary, are thought to be important, assisting both sperm motility and fertilization, and in normal oocyte development.

There is no simple single solution to bridging the gap between *in-vivo* physiological mechanisms and conditions mimicked in the IVF laboratory. Firstly, the challenge remains in identifying the ‘natural’ environment of the preimplantation embryo. The relative concentrations of components present in, as well as the pH of, commercially available culture media and the concentration of oxygen and temperature in IVF incubators are based on data extrapolated from animal studies. pO2, pH and temperature are only a few of the parameters which may affect embryo development. Many other factors may contribute to the embryo’s environment and these could be explored by studies of the microbiome, genome and metabolome, and proteogenomics. There is a need for the development of probes and devices which will measure *in-vivo* real time information. There is an ongoing challenge in developing devices which can be placed within the reproductive tissue; the main concerns include safety, accuracy of measurement, acceptability, ethics and financial costs. If such devices were available and accepted, not only would we be able to better mimic the physiological environment within IVF, but also identify differences in women with pathology (e.g. endometriosis or subfertility) to potentially make advances in their diagnosis and treatment. Many of the studies identified in this systematic review are dated (prior to 2000), with significant differences among studies in the technology used to measure parameters, and potential inaccuracy in measurement. Owing to heterogeneity of the studies, a valid meta-analysis of the data was not possible within this systematic review.

**CONCLUSION**

The importance of the peri-implantation environment of the embryo has become clearer in recent years. However, while much attention is given to ensuring that the *in-vitro* conditions for embryo development are optimised in the context of IVF treatment, our understanding of the *in-vivo* biophysical environment of the pre-implantation human embryo remains limited. The importance of this goes beyond improving fertility outcomes. What the embryo ‘sees’ is intrinsically linked to developmental programming ([Barker, 1990](#_ENREF_14)). The notion of mimicking the natural *in-vivo* environment for the *in-vitro* embryo has its origins in the earliest days of IVF, and the work of many key figures in embryology such as Leese and Gardiner ([Gardner, 2016](#_ENREF_51); [Leese, 1998](#_ENREF_99)). However, while attention has focused on the constituent factors of culture conditions, the ‘incubator’ function of the uterus has largely been ignored. One possible reason relates to our inability to assess the *in-vivo* environment of the reproductive tract in real time. Technological developments such as time lapse and sensors have been developed to provide a more stable *in-vitro* environment. This systematic review does not identify the evidence to change current protocols. However, it suggests an urgent case for accurate assessment of the *in-vivo* environment, so that future fertility treatment can be better understood and more precisely optimised.

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**Authors’ roles**

Study concept and design was by YC and NM. The literature search was by KYBN, RM, and YC. Data analysis and interpretation was by KYBN, RM, HM, NM and YC. Development of the manuscript and review of the final manuscript was by KYBN, RM, HM and NM and YC.

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**Conflict of interest**

KYBN and RM declare no conflicts of interest. HM is a shareholder of Vivoplex. NM and YC are shareholders of Complete Fertility Ltd and Vivoplex. NM is also a non-executive director of Anecova. NM has received grants, consultancy and speaker fees from Ferring, MSD, Merck Serono, IBSA and SPD. YC has received grants and speaker fees from NIHR, Ferring, Merck Serono and Nordic Pharma.

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**Figure legends**

**Figure 1. PRISMA flow diagram (**[**Liberati et al., 2009**](#_ENREF_101)**).** This flow chart shows the flow of information through the different phases of the systematic review. A total of 18, 685 records were identified by the initial search and 60 studies were included in the systematic review.

**Figure 2. The interface between the human uterus and the developing embryo.** Oxygen molecules diffuse out of the arteries through the different cell layers before reaching the intrauterine cavity.

**Figure 3.**

**Left) 28-day menstrual cycle in humans.** The human menstrual cycle consists of the follicular phase (days 1-14) and the luteal phase (days 15 to 28). At the beginning of the follicular phase, oestrogen and progesterone are lowest, the uterine lining sloughs and menstruation occurs. As ovulation approaches, estrogen increases, causing the uterus to grow or proliferate. LH increases dramatically and briefly, and ovulation occurs. During the luteal phase, oestrogen falls and progesterone increases, produced by the corpus luteum. This makes the endometrium receptive to the blastocyst, by increasing blood flow and uterine secretions and reducing uterine contractility. If no fertilization or implantation occurs, progesterone production will decline, and menstruation occurs.

**Right) The ‘estrus cycle’ in rat.** In animals, the menstrual cycle, known as ‘estrus cycle’ is divided into 4 phases, where the follicular phase comprises of the proestrous and the estrus phase, and the ‘luteal’ phase comprises of metestrus and diestrus phases. In the proestrus phase, the ovarian follicle enlarges and begins to secrete oestrogen under the influence of FSH and LH. Oestrogens increase vascularity and cell growth in the reproductive tract, and at the end of estrus, ovulation occurs. In the ‘metestrus’ postovulation, the corpus luteum secretes progesterone, enabling the thickening of the endometrial lining. And unlike in humans, where endometrial decidualisation occurs monthly, in many animals, decidualisation is only triggered by presence of an embryo (Lee and DeMayo 2004). In the absence of implantation, diestrus follows.

**Figure 4.**

**a. Intrauterine pO2 throughout the estrus cycle.** This graph shows the results from fours studies; **b. Oxygen tension within the uterus and the Fallopian tube (FT).** This shows oxygen tension measured as mm of mercury (mmHg) in the human, hamster, rhesus monkey and rabbit. The means are illustrated by the solid squares and the range illustrated by the error bars; **c. Biorhythm of intrauterine pO2 throughout the estrus cycle.** The frequency of change (peaks per hour) and the mean amplitude of change (mmHg) is shown on the graph**; d. Potential factors influencing oxygen tension (pO2).** The graph shows potential factors influencing the pO2 peaks and amplitude within the uterus**; e. Oxygen tension in the uterus in pathology.** This study assessed pO2 in presence of uterine myoma, cervical carcinoma, endometrial carcinoma and the normal uterus in study by Kigawa et al. The mean values are shown by the solid squares and the values either side are standard error of the mean; f**) Temperature within the female reproductive tract**. This shows results from studies in the vagina (Yedwab et al 1976, Samples and Abram 1984), fallopian tube (Bahat et al 2005) and ovary (Hunter et al 1997). The caudal region of the fallopian tube (isthmus, sperm storage site) is 1-2˚c cooler than the cranial portion (ampulla, fertilisation site).

**Figure 5. pH within the female reproductive tract.** Results from studies are illustrated in the diagram. Zoomed view shows homeostasis of the mammalian cell with the embryo in an uterine environment. The main regulatory mechanisms are (i) HCO3–/Cl– exchanger, which alleviates alkalosis, and (ii) the Na+/H+ antiporter and (iii) Na+,HCO3–/Cl– exchanger, both of which alleviate acidosis. pHi is intracellular pH, and pHe is the extracellular pH.