Characterisation of the physical environment of embryos throughout *in vitro* culture

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Abstract

Characterisation of the physical environment embryos are exposed to throughout *in vitro* culture for treatments involving *in vitro* fertilisation (IVF) has been limited due to measurement difficulties (since the position of an embryo is the point of interest) and the lack of a theoretical framework. Temperature, oxygen concentrations and pH are all important factors in an oocyte's and an embryo's environment which, if away from desired levels, may impact on embryo viability. The development of mathematical models provides a structured approach which helps to overcome measurement difficulties.

The IVF process was broken down into 70 discrete back-to-back steps, from oocyte aspiration to embryo transfer, which could be modelled. Models of heat transfer were developed for a Petri dish, 4-well dish, Pasteur pipette (un-pulled and pulled), plastic pipette tip (two sizes), denuding pipette and transfer catheter. Models of oxygen and carbon dioxide mass transfer were developed for the Petri dish, in which oocytes and embryos spend the majority of their time in culture. The models were solved by the finite element method in the software package COMSOL Multiphysics 3.3a used in conjunction with MATLAB R2006a. Models were then validated against experimental data.

There is considerable variation in the embryology culture process, with respect to the number and timing of steps, both between and within laboratories. Of all the factors in an embryo's environment embryology practice has the greatest impact on temperature. Embryos are cultured in dishes in incubators which maintain the required gaseous and thermal environment. While paraffin oil, which overlays culture media in

a dish, successfully buffers embryos from great changes in oxygen and pH when dishes are removed from an incubator, maintenance of embryo temperature is dependent on numerous factors including the setting of the surface temperature of microscope stages, whether the lid is on or off the dish, the embryo position across the floor of the dish, the dish's foot height, the time out of incubator and the depth of liquid in the dish. For a period of 5 minutes out of an incubator in a standard Petri dish set up, the pH an embryo is exposed to will not likely rise from pH 7.33, as in an incubator, to above pH 7.38. However, the temperature an embryo is exposed to may change by ± < 0.5 °C or may change by ± 1 to 3 °C, depending on embryology practice. Importantly, an increase of 1 °C in embryo temperature may adversely affect embryo viability while a decrease of 1 °C will likely have little impact.

Transfer of an embryo in a pipette is the step identified which subjects embryos to the greatest rate and magnitude of temperature change. While temperature in a dish may change by 1 to 3 °C during 5 minutes out of an incubator, the temperatures within a pulled glass Pasteur pipette can fall by > 10 °C in 10 seconds. Use of plastic pipette tips instead of glass pipettes is beneficial for maintaining embryo temperature as the temperature will fall by approximately 3 °C in 10 seconds, 7 °C less than in the glass pipette under the same conditions. This work identified many simple practical steps, such as the use of plastic pipette tips instead of glass, which minimise temperature changes embryos are exposed to throughout the culture process.

Applying the Model of mass transfer of O_2 in a Petri dish disproved the belief that equilibration of gas in the dish occurs significantly faster without a lid. The model of O_2 transport in a Petri dish demonstrated that it takes ≈1 hour to reach 67 % and ≈4

hours to reach 95 % full equilibration of oxygen between atmospheric and 5 vol % O_2 at 37 □C. Modelling mass transport of CO_2 provided a means to predict pH changes within a media drop in a Petri dish. In equilibration from atmospheric to 6 vol % CO_2, the pH reached within 0.1 unit of the final value in ≈1.5 hours. An important finding of this work was that sufficient equilibration of gas may be achieved in ≈2 hours and therefore the pre-equilibration time for dishes (currently overnight) may be shortened, reducing the degradation of amino acids, which occurs at 37 °C, to ammonium (embryo toxic).

There is considerable variation in embryology practice. This work successfully utilised engineering knowledge and mathematical modelling to describe the physical environment of temperature, oxygen and pH that oocytes and embryos may be exposed to throughout an open embryo culture system, used by the majority of IVF clinics worldwide. The findings here provide a basis for establishing best practice. Further work is needed to quantify the effects on the embryo of fluctuation in the embryo's environment but this work demonstrates that mathematical modelling of the embryo's environment in IVF is a viable tool for improving laboratory practice.

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Nomenclature

α_d	Angle of flaring of the dish walls	°
A	Area	m^2
A_s	Surface area	m^2
β	Thermal expansion coefficient	$°C^{-1}$
Bi	Biot number	-
c	Heat capacity	$J.kg^{-1}K^{-1}$
C	Concentration	$mol.m^{-3}$
C_{O_2}	Concentration of O_2	$mol.m^{-3}$
D	Diffusion coefficient	m^2s^{-1}
ε	Emissivity	-
g	Acceleration due to gravity	$m.s^{-2}$
h	Convective heat transfer coefficient	$W.\ m^{-2}K^{-1}$
H_d	Height of media drop	m
h_{fg}	Latent heat of water	$J.kg^{-1}$
H_{lw}	Height of Petri dish lid wall	m
H_o	Depth of oil	m
H_w	Height of Petri dish wall	m
K	Equilibrium constant	-
k_p	Mass transfer coefficient for a partial pressure driving force	$s.m^{-1}$
M_r	Molar mass	$kg.mol^{-1}$
n	Number	-
P	Partial Pressure	mmHg
P_{v_1}	Partial pressure of water at a surface	mmHg

P_{v_2}	Partial pressure of water above a surface	mmHg
P_{O_2}	Partial pressure of O_2	mmHg
P_{CO_2}	Partial pressure of CO_2	mmHg
P_{aO_2}	Atmospheric O_2 partial pressure	mmHg
P_{iO_2}	Initial partial pressure of O_2	mmHg
P_{eO_2}	Experimentally recorded partial pressure of O_2	mmHg
P_{aCO_2}	Atmospheric CO_2 partial pressure	mmHg
P_{iCO_2}	Initial partial pressure of CO_2	mmHg
pK_a	-log(K)	-
q	Heat flux	$W.m^{-2}$
r	Radial distance	m
R	Thermal resistance	$m^2.K.W^{-1}$
r_a	Radius of air gap beneath Petri dish	m
Ra	Rayleigh number	-
r_d	Radius of media drop	m
Re	Reynolds number	-
R_g	Gas constant	$J.mol^{-1}K^{-1}$
r_i	Internal radius of Petri dish	m
r_l	Radius of Petri dish lid	m
r_o	Radius of the dish at H_o	m
R_x	Cross sectional area/wetted perimeter	m
S	Solubility	$mol.m^{-3}mmHg$
t	Time	s
T	Temperature in Kelvin	K

T_a	Ambient air temperature in Kelvin	K
T_s	Surface temperature in Kelvin	K
U	Apparent heat transfer coefficient	W. $m^{-2}K^{-1}$
v	Velocity	$m.s^{-1}$
V_d	Volume of media drop	m^3
V_{oil}	Volume of paraffin oil	m^3
x	Thickness	m
x_a	Width of the Petri dish foot	m
x_b	Thickness of the Petri dish floor + air gap	m
x_f	Thickness of Petri dish floor	m
x_l	Thickness of the Petri dish lid	m
x_{la}	Thickness of the air gap between the Petri dish and its lid	m
x_{lw}	Thickness of the Petri dish lid wall	m
x_w	Thickness of the Petri dish wall	m
y	Vertical distance	m
θ	Temperature in degrees Celsius	°C
θ_a	Ambient air temperature in degrees Celsius	°C
θ_b	Temperature of base boundary	°C
θ_e	Experimentally recorded temperature	°C
θ_i	Initial temperature	°C
θ_s	Surface temperature in degrees Celsius	°C
λ_{eff}	Effective thermal conductivity	$W.m^{-1}K^{-1}$
ρ	Density	$kg.m^{-3}$
λ	Thermal conductivity	$W.m^{-1}K^{-1}$

μ	Viscosity (kinematic)	m^2s^{-1}
α	Thermal diffusivity	m^2s^{-1}
σ	Stefan-Boltzmann constant	$W.m^{-2}K^{-4}$
Ω	Denotes the subdomain	-

Subscripts

a	Air
g	Glass
m	Media
o	Paraffin oil
p	Polystyrene
pp	Polypropylene