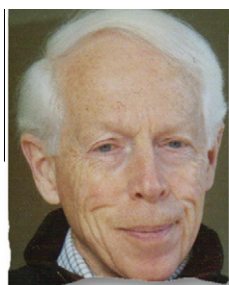## REVIEW

Temperature gradients in female reproductive tissues

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Abstract Deep body temperature in mammals is generally but incorrectly regarded as uniform. Alterations of temperature in oviducts and preovulatory Graafian follicles may play a vital role in gamete maturation, fertilization and early embryonic development. At a molecular level, the conformation of regulatory proteins is susceptible to changes in temperature. Deviation from physiological temperature during IVF procedures could thereby exert a profound influence on patterns of gene expression as the embryonic genome unfolds during early cleavage stages and act to generate specific anomalies. Systematic studies are urgently required.

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Introduction

Most reproductive biologists have not reflected seriously on a potential involvement of temperature in the diverse processes that they study. Indeed, in the present author's experience, asking an audience of reproductive biologists to suggest spheres in which considerations of temperature might be relevant, there are usually just two responses: the cooling potential of scrotal testes and the mid-cycle elevation of body temperature in women. Seldom do such biologists recall the influence of ambient temperature during incubation of an embryo on sex determination in turtles, alligators, crocodiles and some lizards. This apparent lack of information or reflection is all the more surprising if one accepts that reproductive studies are now firmly established on a molecular stage.

A second reason for concern, especially in the case of domestic animal research, is the widespread assumption that deep rectal temperature is an accurate reflection of deep body temperature and that such temperature is uniform in and across the abdominal organs. As one consequence, studies performed in systems of culture, such as IVF and early development of the embryo, invariably use incubator settings that match deep rectal temperature and are held constant. Nonetheless, perusal of the literature on measurements of temperature in mammalian reproductive tissues gives guidance that should not be overlooked, not least in a context of gamete maturation and molecular embryology.

Major questions remain concerning male mammals, such as the temperature of intra-abdominal testes and that of succeeding regions of the epididymal duct in scrotal mammals, but this short essay will focus on female mammals and their reproductive tissues. Temperatures have been measured in genital ducts and gonads by means of thermistor probes and/or infra-red sensing.

Temperature gradients in the oviduct

Regional differences in temperature have been reported within the genital tract of three species and these are greatest in the oviduct of oestrous animals in the hours before ovulation. The caudal region of the isthmus may be 1−2°C cooler than the cranial portion of the ampulla (reviewed in Hunter (2009); Table 1). Sperm passage from warmer uterus through the uterotubal junction into cooler isthmus would contribute to reduced motility and a sperm storage function of the caudal isthmus during the preovulatory interval (Hunter, 2011; Hunter and Nichol, 1986; Suarez, 2007, 2008). The magnitude of the temperature gradient changes according to the stage of the oestrous cycle and, in particular, close to the time of ovulation. A periovulatory increase in isthmus temperature would influence sperm activation and sperm surface architecture, not least by prompting the transmembrane migration of protein molecules. Even though spermatozoa may swim up an oviduct temperature gradient from cooler to warmer, this does not necessarily infer thermotaxis (Bahat et al., 2003). Indeed, such a putative role of thermotaxis has been challenged with a series of experimental observations (Hunter, 2009).

Temperature gradients in the ovary

Temperature gradients have also been noted across ovarian tissues (Table 1). These may be accentuated at specific stages of an oestrous or menstrual cycle. In brief, preovulatory follicles were 1−2°C cooler than neighbouring ovarian tissues in rabbits and women (Grinsted et al., 1980, 1985). Preovulatory follicles in pigs were 1.3−1.7°C cooler than adjoining ovarian tissues (Hunter et al., 1997, 2000) and

both ovarian compartments were cooler than rectal and jugular temperatures. There were no exceptions in a total of 73 observations. In cattle, Graafian follicles of 15−18 mm diameter were 1.5°C cooler than neighbouring ovarian tissues (Greve et al., 1996).

Despite shortcomings in the techniques employed to make the above observations, gradients in temperature clearly exist between different regions of the same oviduct and different regions of the same ovary. Their magnitude remains to be measured with precision, bearing in mind that the extent of such gradients may vary with the stage of the oestrous or menstrual cycle. As with other cyclic reproductive phenomena, variations in temperature in genital tissues are influenced by the pattern of ovarian steroid secretion (Hunter et al., 2006).

Generation of gradients in temperature

Factors involved in generating temperature gradients deep within the abdomen include the relative distribution of blood flow, regional contractile activity of the myosalpinx and specific endothermic reactions (Hunter, 2009). Compared with the oviduct isthmus, the ampulla has an extensive capillary bed with marked dilatation of blood vessels late in the follicular phase as ovarian oestradiol secretion peaks. At this time, contractile activity of the ampulla exceeds that of the isthmus. Differences in the vascular bed and contractile activity could therefore contribute to temperature differences between the isthmus and ampulla. Countercurrent heat exchange mechanisms would underlie their maintenance (Einer-Jensen and Hunter, 2005).

More significant, however, could be the involvement of macromolecular secretions in the duct. As preovulatory follicles grow and mature and secrete increasing titres of oestradiol, viscous glycoproteins derived from the endosalpinx accumulate in the lumen of the caudal isthmus. If such secretions support endothermic reactions, as discussed below for ovarian macromolecules, they would offer a specific means of regional cooling. Such secretions are dissipated early in the luteal phase of the cycle, as oedema of the mucosa diminishes, the myosalpinx relaxes and the embryo progresses along the isthmus to the uterus.

Table 1   The magnitude of temperature gradients recorded in female reproductive tissues around the time of ovulation.

Organ and location	Measurement method	Reference	Species	Overall difference in temperature (°C)	Comment
Oviduct: isthmus versus ampulla	Indwelling probes	Bahat et al. (2003)	Rabbit	0.8−1.6	Isthmus always cooler than ampulla
		Hunter and Nichol (1986)	Pig	0.2−1.6	
Ovary: preovulatory follicles versus ovarian stroma	Microelectrodes and/or acute thermosensing	Grinsted et al. (1980)	Rabbit	1.4	Mature follicles always cooler than stroma
		Grinsted et al. (1985)	Human	2.3	
		Greve et al. (1996)	Cow	1.5	
		Hunter et al. (1997, 2000)	Pig	1.3−1.7	

As for ovarian temperature gradients, physicochemical evidence for endothermic reactions in follicular fluid comes from in-vitro studies (Luck and Griffiths, 1998; Luck et al., 1999). Hydration of large extracellular matrix molecules in ovarian follicular fluid can depress temperature (Luck and Gregson, 2000; Luck et al., 2001). Extensive mucification and expansion of the cumulus oophorus in preovulatory follicles would facilitate hydration of proteoglycans and a localized cooling of the oocyte−cumulus−complex (Hunter, 2003). In addition, the ratio of antral fluid volume to that of granulosa cell mass increases markedly as the follicle expands whereas other ovarian tissues remain largely cellular. Together, these features may explain cooler maturing follicles compared with other ovarian tissues. A countercurrent transfer of heat in the vascular system of individual maturing follicles would permit maintenance of temperature differentials (see figures in Hunter (2003) and Hunter et al. (2006)).

Significance of changing temperature gradients

Because temperature gradients in oviduct and ovarian tissues are prominent during final maturation of male and female gametes, respectively, the potential significance of such gradients needs consideration. Changing temperatures could play a role in stabilizing spermatozoa with suppressed motility and intact surface membranes during preovulatory storage in the oviduct isthmus and then in peri-ovulatory reactivation and completion of capacitation. Similarly, oocytes may require exposure to shifts in temperature for optimal nuclear, cytoplasmic and membranous maturation shortly before and after ovulation (Aroyo et al., 2007; Shi et al., 1998; Wang et al., 2009). Proteases secreted by the oocyte could respond to temperature changes, as may remodelling of the zona pellucida (Coy et al., 2008). The preceding remarks extend to the process of fertilization itself, not least an influence of temperature on activation of acrosomal enzymes during the final phases of capacitation and changes in the cytoskeleton of the oocyte upon sperm penetration. In addition to changes in the gametes themselves, endosalpingeal secretion and cilial activity would both respond to pre- and post-ovulatory modifications of temperature.

Implications at the molecular level cannot be overlooked (Lonergan et al., 2006; Wrenzycki et al., 2005). Even small changes in temperature influence the degree of folding and conformation of nuclear proteins and could thereby perturb the sequence and extent of gene expression in young embryos. In a practical context, failure to mimic changing physiological temperatures during IVF procedures and culture of the zygote offers one explanation for wayward development of the mammalian embryo or fetus as seen, for example, in the large offspring syndrome in ruminants (Duranthon et al., 2008; McEvoy et al., 2000; Thompson et al., 2007).

Reflecting more broadly on questions of phenotypic sex, exposure of a mammalian embryo to inappropriate temperature or changes in temperature might influence the sequence of expression of sex differentiation genes and related downstream events; these would include gonadal development, sexual orientation and patterns of sexual behaviour (reviewed in Hunter (1995)). Anomalous differentiation might be prompted in vivo if the genital tract were exposed to elevated temperatures in neighbouring abdominal organs or systemically during febrile episodes. Labile ancestral gene programmes for temperature-dependent phenotypic plasticity might thereby be activated, despite the presence of heat shock proteins in both oviduct and oocyte.

Concluding proposals

In order to take the field forward and to establish the full physiological impact of temperature gradients in vivo, the following approaches would be helpful. (i) Using a non-invasive imaging technology, make precise measurements of temperature in fully-conscious animals at different stages of a spontaneous oestrous or menstrual cycle. Such an approach needs to distinguish temperatures in different compartments of the same ovary and on either side of the uterotubal junction. (ii) Examine the influence of culturing gametes and zygotes at different temperatures or ranges of temperature on the pattern of gene expression in preimplantation embryos. (iii) Model the influence of small changes in temperature on the conformation of specific nuclear, cytoplasmic and membrane proteins. Taken together, the results of these approaches should enlarge understanding of molecular perturbations and their genesis in mammalian embryos.

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