

LIVING LEGEND ADDRESS

Signals from inner space

GK Barrell

Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, Christchurch, New Zealand

Email: graham.barrell@lincoln.ac.nz

Abstract

Advances in knowledge of mammalian physiology have been achieved through endocrine studies of sheep and deer. Such advances include the role of the pineal gland as the mediator between changes in daily photoperiod and melatonin secretion, plus the role of thyroid hormones, for controlling reproduction in seasonally breeding livestock. Antlers provide an example of post-maturity bone formation in a mammal and we have shown that additional hardening (mineralisation) of the antlers results from activation of nearby oestradiol receptors. Based on studies of sheep, C-type natriuretic peptide has emerged in spite of its name as an important adaptive signal for fetal well-being and may be an important regulator of brain function.

Keywords: seasonal breeding; pineal gland; melatonin; antlers; C-type natriuretic hormone; cerebrospinal fluid

Introduction

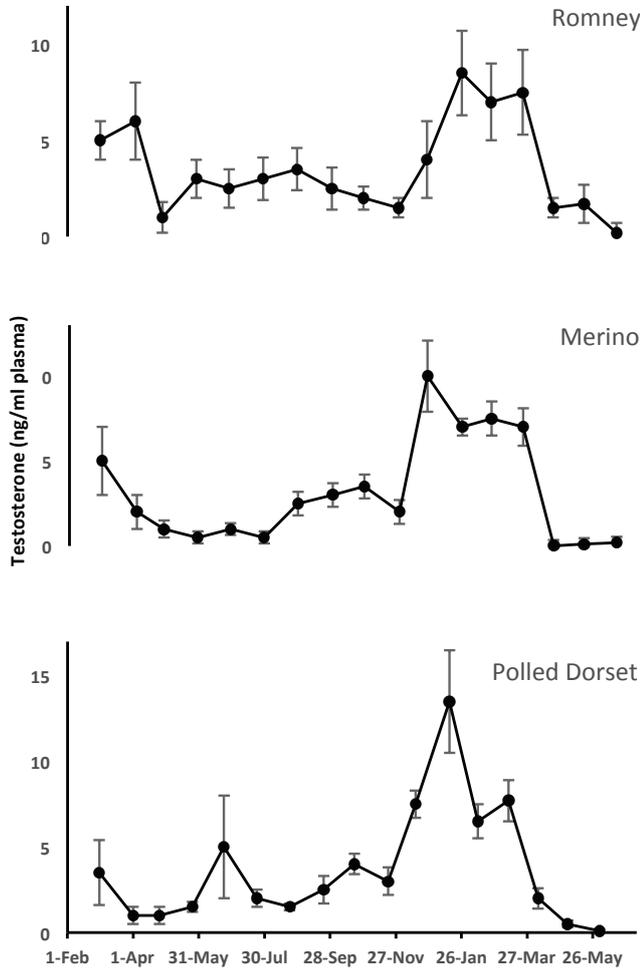
For some of us, how animals function – their physiology – is a constant fascination. This is because of the fact that despite the massive fund of knowledge that has been accumulated under the heading of mammalian physiology, we are still a long way from any complete understanding of the mysteries of the body. My childhood preoccupation with the inner mechanisms of man-made machines has shaped my scientific career where I have pursued the notion that if you look inside an animal carefully enough you can determine how it works. I can report now that there is nothing wrong with this notion – it's just that a lot more 'looking inside' will be required before we have a complete picture. My interest has been in the information pathways that coordinate function among body organs, in particular the signalling molecules that are often called hormones. The study of these 'inner signals' involves a branch of science known as endocrinology and, where it involves hormonal signalling to, from or within the brain, has the name – neuroendocrinology. Although the record of my scientific research appears to be a jumble of unrelated projects with a variety of animal species, they are all mammals and there is a single underlying question – How does it function? Some examples follow.

Seasonality of reproduction

We all know that sheep are seasonal animals. They have annual cycles of appetite, wool growth and reproduction, being so-called 'short-day breeders'. We also know that these cycles are somehow driven by changes in daylight. However, when pressed for detail most people answer that it is something to do with 'daylength'. Considering that the length of a day is 24 h – give or take whether it is a solar or sidereal day – this answer is obviously unsatisfactory. What they really mean is that the controlling factor underlying seasonality is the number of hours of daylight – i.e., the daily photoperiod – which changes in a predictable manner that increases in amplitude as one moves further from the Equator.

My first foray into viewing this process in sheep was a study of New Zealand Romney, Polled Dorset and Merino rams conducted at Massey University, New Zealand. The rams showed marked seasonal variation in semen characteristics and in levels of circulating reproductive hormones (Figure 1, Barrell & Lapwood 1979a) but they were probably always fertile to some extent throughout the year. To determine the regulatory pathway between photoperiodic signals and reproduction in sheep, the nervous link between the eyes and the pineal gland was interrupted by removing the cranial cervical sympathetic ganglia from rams (Barrell & Lapwood 1979b; Barrell et al. 1987). This interfered with their reproductive seasonality and further evidence for this mechanism was revealed soon after by removal of the pineal gland itself (Figure 2, Barrell & Lapwood 1978; 1979c; d). These studies were the first for any livestock species to demonstrate the role of the pineal gland as the mediator of photoperiodic signalling and paved the way for showing that the hormonal signal arising from the pineal gland – melatonin – could be administered to deer (Adam & Atkinson 1984; Webster & Barrell 1985; Asher et al. 1987) and sheep (Knight et al. 1992) for successful advancement of seasonal breeding, as used by some farmers today. Years later, I took this work further during a series of sabbatical visits with Professor Fred Karsch at the University of Michigan, United States of America. His laboratory had established that the annual cycle of reproduction in sheep was driven solely by the increasing daily photoperiod each spring. (Strictly speaking, the cycle is 'entrained' by the spring photoperiods, rather than 'driven' by them.) They had shown that only 70 nights of administration of melatonin to pinealectomised Suffolk ewes during spring was sufficient to maintain the strict annual (i.e., 12 monthly) cycle of reproductive activity for a whole year (Woodfill et al. 1991). We took this a step further by showing that the melatonin signal delivered in spring also had to be a 'spring' signal: a 'winter' signal of nightly melatonin delivered at this time was unable to

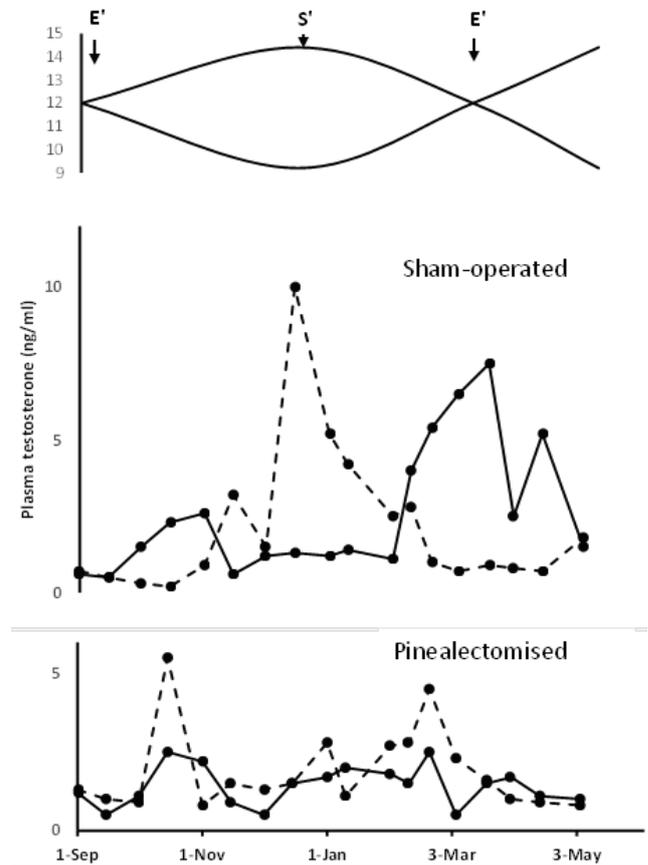
Figure 1 Seasonal variations in plasma testosterone concentrations recorded from rams of three breeds (NZ Romney, n = 6; Merino, n = 5; Polled Dorset, n = 4) throughout a single year. Data are means with vertical lines indicating \pm s.e.m. (Reproduced from Barrell & Lapwood, 1979, with permission from the publisher)



maintain the annual cycle of reproductive activity (Barrell et al. 2000). It is possible that this requirement for the correct ‘quality’ of the signal (‘spring’), as well as the correct timing (springtime), is common to all seasonal mammals. However, we may never know for sure because experiments of this nature in long-lived species are costly and require several years of study. The present research climate probably means that such studies are never likely to be carried out again.

My knowledge of seasonality meant that when I read about Weddell seals having a daily peak of circulating melatonin concentration during summer (Griffiths et al. 1986) I experienced frank disbelief. At that time of the year there are no hours of darkness available to provoke secretion of melatonin from the pineal gland. With help from Dr Grant Montgomery of AgResearch, New Zealand a short but substantial study of our own in McMurdo Sound, Antarctica put this apparent anomaly to rest by showing that there is a complete absence of melatonin in the blood of Weddell seals during the constant daylight of summer

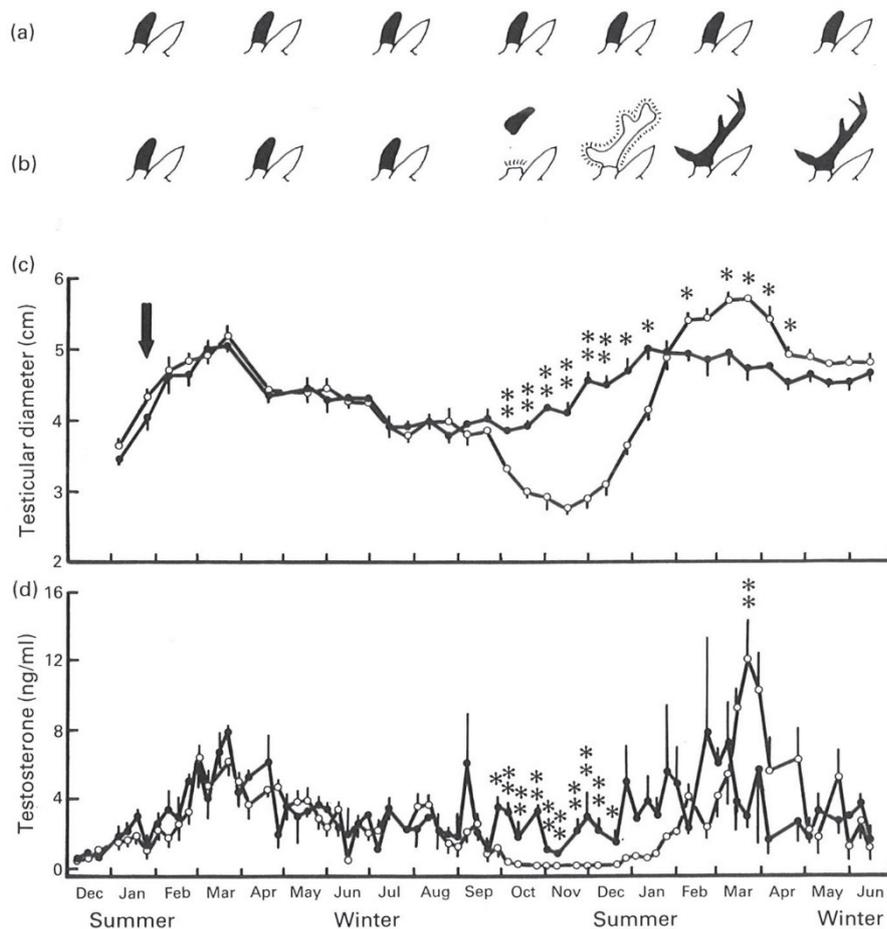
Figure 2 Variations in plasma testosterone concentration of pinealectomized or sham-operated rams (n = 4) exposed to normal (solid lines) or reversed (broken lines) lighting regimes for 9 months. Upper graph shows the changes in daily photoperiod. E’, equinoctial photoperiod; S’, solstitial photoperiod. (Adapted from Barrell & Lapwood, 1979, with permission from the publisher)



(Barrell & Montgomery 1989). This accords with the now well-established view that melatonin secretion from the pineal gland during the hours of darkness provides animals with an internal chemical signal that gives a measure of the changing daily photoperiod. This internal signal is read by cells in the brain, particularly in regions that house internal biological ‘clocks’, e.g. the suprachiasmatic nuclei, and at other regulatory sites in the hypothalamus and pituitary gland in order to manage cyclical events such as seasonal breeding.

Another aspect of regulation of seasonality was revealed from work on the Lincoln University Deer Unit following a visit by Professor Tony Care from Leeds University, United Kingdom. His interest in a possible role of the parathyroid glands in antler bone formation led us to remove all the parathyroid tissue from some of our red deer, which entailed removing both thyroid glands as well. This increased the bone density of antlers (Care et al. 1985) and we found that the stags survived in full health, even after we had stopped providing thyroxine to compensate for the

Figure 3 Changes in the antlers of (a) thyroidectomized and (b) intact red deer stags (i.e. dead bone, casting, regrowth with velvet, cleaned antler) recorded for 18 months. Variations in (c) testicular diameter and (d) plasma testosterone concentration of thyroidectomized (●, n = 3) and intact (○, n = 4 in c and n = 5 in d) red deer stags recorded for 18 months. Arrow indicates date of thyroidectomy. Data are means with vertical lines indicating \pm s.e.m. Asterisks indicate significant (*P < 0.05, **P < 0.01) differences between means. (Reproduced from Shi & Barrell, 1992, with permission from the publisher)



loss of the thyroid glands. However, to our surprise, the stags remained in a state of permanent rut. We conducted further studies on red deer which showed that, as found in British ewes at about the same time (Nicholls et al. 1988), thyroidectomy prevents cessation of the breeding season in stags (Figure 3, Shi & Barrell 1992; 1994) and hinds (Anderson & Barrell 1998). This work was extended in sheep by Professor Fred Karsch at the University of Michigan (Moenter et al. 1991) with Dr James Webster from AgResearch, New Zealand and myself as occasional participants (Webster et al. 1991). Although this knowledge about the role of thyroid hormones revealed a whole new component in the regulation of seasonal breeding, we have not been able to find any applications for its use in livestock (Maurenbrecher & Barrell 2003).

Antlers are bones

Antlers are unique bones that are grown each year, starting as cartilaginous structures attached to prominences (pedicles) of the frontal bones of the stag's skull. The

structures grow in length from their tips and the cartilage becomes progressively replaced by bone tissue until full size is reached. Towards this final stage of elongation there is a rapid increase in bone density, sometimes termed 'terminal mineralisation', followed by death so that all skin and associated tissues slough off ('velvet stripping') and the stag has two dead antlers (bones) attached to his live pedicles. Some months later the antlers fall off ('casting') and the cycle of growth repeats itself. Because there is a direct link between these events and the annual reproductive cycle, the physiology of antlers has captured my interest (Muir et al. 1987; 1988). In particular, I was intrigued by the potency of oestradiol, an oestrogen, for stimulating the terminal bone mineralisation process in antlers. Oestradiol is much more potent than testosterone for this response (Goss 1968) and it has encouraged us to investigate antlers for the presence of oestradiol receptors (Table 1, Lewis & Barrell 1994). A series of studies led us to the finding that oestradiol receptors are

present in the fibrous membranes surrounding the antler shaft, i.e., the perichondrium around cartilage near the antler tips and periosteum surrounding bony regions of the shaft, but not within the bone tissue itself (Barrell et al. 1999). This was the first *in vivo* evidence for the presence of oestradiol receptors associated with bony tissue in any mammal and it should have implications for research directed at understanding the occurrence of osteoporosis in

Table 1

	Binding capacity (fmol/mg protein)		
	n	mean	SEM
periosteum	10	73	17
tip	7	47	10
cartilage	6	23	11
calcifying cartilage	5	12	2
bone	0	nd	

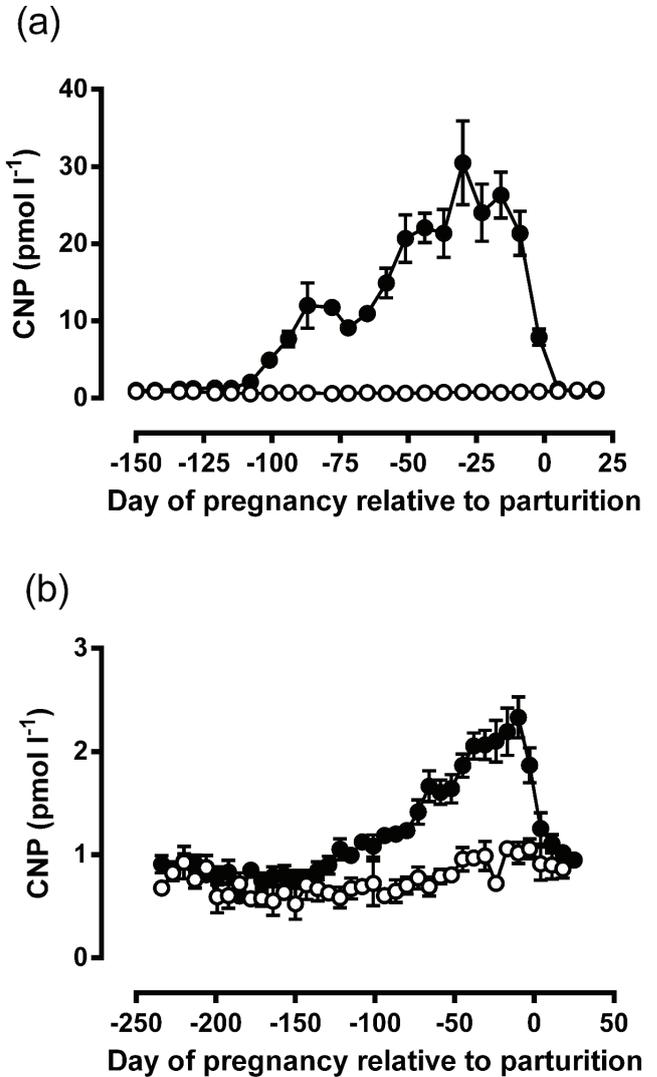
humans. Typically, about the only situation in which adult mammals form new bone is after a fracture. Otherwise, humans for instance, reach peak bone mass in their late 20s and, thereafter, it is all downhill; the progressive loss of bone affecting every adult human. This makes the phenomenon of whole new bones (antlers) forming each year in adult stags a remarkable, unique process that deserves much further attention.

What is C-type natriuretic hormone?

The natriuretic hormones, atrial natriuretic hormone (ANP) and brain natriuretic hormone (BNP), provide signals that increase urinary sodium excretion – natriuresis – hence their names. A third compound that is very similar in structure has earned by default the name C-type natriuretic peptide (CNP) but it does not appear to have much potency as a natriuretic signal. However, we found that its levels in the blood of female sheep (McNeill et al. 2009), deer (McNeill et al. 2010) and cattle, soar during pregnancy, peaking just prior to term and rapidly diminishing to background values at parturition (Figure 4). Our discovery that the source of CNP during pregnancy in sheep is the placenta – specifically the binucleate cells of the trophoblast (McNeill et al. 2010) – has led to the hypothesis that CNP serves as part of an adaptive response to any event that might restrict development of the fetus. Evidence for this hypothesis has come from studies we have conducted in collaboration with Professor Eric Espiner and his group at the University of Otago, Christchurch, New Zealand. Nutrient deficiency of ewes during early or late stages of gestation causes an increase in the maternal circulating concentration of CNP (Prickett et al. 2007; McNeill et al. 2012; Madhavan et al. 2016) and a similar change is seen in women with complicated pregnancies just prior to the occurrence of adverse events (Reid et al. 2014). CNP, thus, may have potential as a marker of fetal well-being, and may prove to be as useful to the veterinarian as to the obstetrician.

Apart from the changes associated with pregnancy, concentrations of CNP in blood are generally very low. In stark contrast, cerebrospinal fluid contains CNP at concentrations far in excess of those in peripheral plasma (Schouten et al. 2011). Regulation of CNP levels in cerebrospinal fluid appears to be independent of plasma concentrations (Schouten et al. 2011) and is not influenced by the dramatic rise in peripheral levels during ovine pregnancy (Wilson et al. 2015). In fact, we have found that the levels of CNP in cerebrospinal fluid of sheep are rigidly maintained even during anaesthesia and there is no evidence of any diurnal change (Wilson et al. 2015). We postulate that the high CNP levels and their apparently tight regulation in cerebrospinal fluid indicate an important action for this signal in the maintenance of brain function, and we are pressing on with work to determine the role of CNP in the central nervous system – using sheep as the experimental model. The possibility of being associated with making a substantial discovery here provides a strong incentive for me to remain in the workforce.

Figure 4 Plasma concentrations of C-type natriuretic peptide (CNP) in (a) ewes (●, pregnant; ○, non pregnant; n = 8) and (b) red deer hinds (●, pregnant, n = 8; ○, non pregnant, n = 4) recorded throughout the gestation period. Parturition occurred at day 0. Data are means with vertical lines indicating ± s.e.m. (Adapted from McNeill et al., 2009 and 2010, with permission from the publishers)



Concluding remarks

I have selected a few topics here to demonstrate that endocrinology provides a platform from which it is possible to engage farm animals in the quest for new knowledge about basic mammalian biology that is beneficial for both livestock production and medical science. In the quest for scientific progress, laboratory animals have their place, but I maintain that *in vivo* studies with larger domesticated species are essential for providing the full picture of mammalian physiology that is required for improved livestock production and for better welfare of animals and humans. Most body processes are regulated by to-and-fro messaging with the central nervous system and whether the chemicals that transmit these messages are called hormones, neurotransmitters, cytokines or some other name – they are certainly signals from inner space.

Acknowledgements

Many of the studies described in this paper have depended on the input of my colleagues at Massey, Lincoln and Otago Universities, University of Michigan, AgResearch and the following postgraduate students at Lincoln University: Jim Webster, Paul Muir, Geoff Asher, Zhendan Shi, Lynley Lewis, Greg Anderson, Bryony McNeill, Sengodi Madhavan, and Michele Wilson.

References

- Adam CL, Atkinson T 1984. Effect of feeding melatonin to red deer (*Cervus elaphus*) on the onset of the breeding season. *Journal of Reproduction and Fertility* 72: 463-466.
- Anderson, GM, Barrell GK 1998. Effects of thyroidectomy and thyroxine replacement on seasonal reproduction in the red deer hind. *Journal of Reproduction and Fertility* 113: 239-250.
- Asher GW, Day AM, Barrell GK 1987. Annual cycle of liveweight and reproductive changes of farmed male fallow deer (*Dama dama*) and the effect of daily oral administration of melatonin in summer on the attainment of seasonal fertility. *Journal of Reproduction and Fertility* 79: 353-362.
- Barrell GK, Davies, R, Bailey C 1999. Immunocytochemical localisation of oestrogen receptors in perichondrium of deer antlers (*Cervus elaphus*). *Reproduction, Fertility and Development* 11: 189-191.
- Barrell GK, Lapwood KR 1978. Effects of pinealectomy of rams on secretory profiles of luteinizing hormone, testosterone, prolactin and cortisol. *Neuroendocrinology* 27: 216-227.
- Barrell GK, Lapwood KR 1979a. Seasonality of semen production and plasma luteinizing hormone, testosterone and prolactin levels in Romney, Merino and Polled Dorset rams. *Animal Reproduction Science* 1: 213-228.
- Barrell GK, Lapwood KR 1979b. Effects of modifying olfactory and pineal gland function on the seasonality of semen production and plasma luteinizing hormone, testosterone and prolactin levels in rams. *Animal Reproduction Science* 1: 229-243.
- Barrell GK, Lapwood KR 1979c. Effects of various lighting regimens and pinealectomy on semen production in Romney rams. *Journal of Reproduction and Fertility* 57: 273-279.
- Barrell GK, Lapwood KR 1979d. Effects of pinealectomy on the secretion of luteinizing hormone, testosterone and prolactin in rams exposed to various lighting regimes. *Journal of Endocrinology* 80: 397-405.
- Barrell GK, Lapwood KR, Elgar HJ 1987. Seasonality of secretion of follicle stimulating hormone in New Zealand Romney rams and effects of cranial sympathetic ganglionectomy. *New Zealand Journal of Agricultural Research* 30: 439-442.
- Barrell GK, Montgomery GW 1989. Absence of circadian patterns of secretion of melatonin or cortisol in Weddell seals under continuous natural daylight. *Journal of Endocrinology* 122: 445-449.
- Barrell GK, Thrun L, Brown ME, Vigiú C, Karsch FJ 2000. Importance of photoperiodic signal quality to entrainment of the circannual reproductive rhythm of the ewe. *Biology of Reproduction* 63: 769-774.
- Care AD, Ross R, Barrell GK, Muir PD, Aaron J, Oxby C 1985. Effects of long-term thyroparathyroidectomy on antler growth in red deer. In: *Biology of deer production: Bulletin 22*, The Royal Society of New Zealand: 255-256.
- Goss RJ 1968. Inhibition of growth and shedding of antlers by sex hormones. *Nature* 220: 83-85.
- Griffiths DJ, Bryden MM, Kennaway DJ 1986. A fluctuation in plasma melatonin level in the Weddell seal during constant natural light. *Journal of Pineal Research* 3:127-134.
- Knight TW, Muir PD, Smith JF, Scales GH, Reid TC, McPhee SR, Staples LD 1992. Effects of long-acting melatonin implants on the reproductive performance of Corriedale, Borderdale, Romney, Coopworth and Perendale ewes in New Zealand. *New Zealand Journal of Agricultural Research* 35: 185-193.
- Lewis LK, Barrell GK 1994. Regional distribution of estradiol receptors in growing antlers. *Steroids* 59: 490-492.
- Madhavan S, Prickett TCR, Espiner EA, Barrell GK 2016. Nutrient restriction in early ovine pregnancy stimulates C-type natriuretic peptide production. *Reproduction, Fertility and Development*. In press.
- Maurenbrecher S, Barrell GK 2003. Suppression of thyroid gland function and its effects on the breeding season of Coopworth ewes. *New Zealand Journal of Agricultural Research* 46: 1-7.
- McNeill BA, Barrell GK, Ridgway MJ, Wellby, MP, Prickett TCR, Espiner EA 2012. Caloric restriction, but not caloric loading, affects circulating fetal and maternal C-type natriuretic peptide concentrations in late ovine gestation. *Reproduction, Fertility and Development* 24: 1063-1070.
- McNeill BA, Barrell GK, Wellby, MP, Prickett TCR, Yandle TG, Espiner 2009. C-type natriuretic peptide forms in pregnancy: maternal plasma profiles during ovine gestation correlate with placental and fetal maturation. *Endocrinology* 150: 4777-4783.
- McNeill BA, Barrell GK, Wooding FPW, Prickett TCR, Espiner EA 2011. The trophoblast binucleate cell is the source of maternal circulating C-type natriuretic peptide during ovine pregnancy. *Placenta* 32: 645-650.

- McNeill BA, Prickett TCR, Wellby M, Ridgway MJ, Espiner EA, Barrell GK 2010. Circulating levels of C-type natriuretic peptide (CNP) are strongly linked to pregnancy but not liveweight change in ruminants. *Proceedings of the New Zealand Society of Animal Production* 70: 13-18.
- Moenter SM, Woodfill CJI, Karsch FJ 1991. Role of the thyroid gland in seasonal reproduction: thyroidectomy blocks seasonal suppression of reproductive neuroendocrine activity in ewes. *Endocrinology* 128: 1337-1344.
- Muir PD, Sykes AR, Barrell GK 1987. Growth and mineralisation of antlers in red deer (*Cervus elaphus*). *New Zealand Journal of Agricultural Research* 30: 305-315.
- Muir PD, Sykes AR, Barrell GK 1988. Changes in blood content and histology during growth of antlers in red deer (*Cervus elaphus*) and their relationship to plasma testosterone levels. *Journal of Anatomy* 158: 31-42.
- Nicholls TJ, Follett AR, Goldsmith AR, Pearson H 1988. Possible homologies between photorefractoriness in sheep and birds: the effect of thyroidectomy on the length of the ewe's breeding season. *Reproduction, Nutrition, Development* 28: 375-385.
- Prickett, TC, Rumball CW, Buckley AJ, Bloomfield FH, Yandle TG, Harding JE, Espiner, EA 2007. C-type natriuretic peptide forms in ovine fetal and maternal circulations: evidence for independent regulation and reciprocal response to undernutrition. *Endocrinology* 148: 4015-4022.
- Reid RA, Prickett TCR, Pullar BE, Darlow BA, Gullam JE, Espiner EA 2014. C-type natriuretic peptide in complicated pregnancy: increased secretion precedes adverse events. *Journal of Clinical Endocrinology and Metabolism* 99: 1470-1478.
- Schouten BJ, Prickett TCR, Hooper AA, Hooper GJ, Yandle TG, Richards AM, Espiner EA 2011. Central and peripheral forms of C-type natriuretic peptide (CNP): evidence for differential regulation in plasma and cerebrospinal fluid. *Peptides* 32: 797-804.
- Shi ZD, Barrell GK 1992. Requirement of thyroid function for the expression of seasonal reproductive and related changes in red deer (*Cervus elaphus*) stags. *Journal of Reproduction and Fertility* 94: 251-259.
- Shi ZD, Barrell GK 1994. Thyroid hormones are required for the expression of seasonal changes in red deer (*Cervus elaphus*) stags. *Reproduction, Fertility and Development* 6: 187-192.
- Webster JR, Barrell GK 1985. Advancement of reproductive activity, seasonal reduction in prolactin secretion and seasonal pelage changes in pubertal red deer hinds (*Cervus elaphus*) subjected to artificially shortened daily photoperiod or daily melatonin treatments. *Journal of Reproduction and Fertility* 73: 255-260.
- Webster, JR, Moenter SM, Barrell GK, Lehman MN, Karsch FJ 1991. Role of the thyroid gland in seasonal reproduction. III. Thyroidectomy blocks seasonal suppression of gonadotropin-releasing hormone secretion in sheep. *Endocrinology* 129: 1635-1643.
- Wilson MO, Barrell, GK, Prickett TCR, Espiner EA 2015. Sustained increases in plasma C-type natriuretic peptides fail to increase concentrations in cerebrospinal fluid: evidence from pregnant sheep. *Peptides* 69: 103-108.
- Woodfill CJI, Robinson JE, Malpoux B, Karsch FJ 1991. Synchronization of the circannual reproductive rhythm of the ewe by discrete photoperiodic signals. *Biology of Reproduction* 45: 110-121.