sheep during postmortem examination at Glasgow university veterinary school. The aetiology of this condition remains unknown.

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References


Seasonal variation in development of in vitro produced bovine embryos

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In hot climates there is a large decrease in the fertility of lactating dairy cows during summer months (Hansen 1997). At least some of this infertility may reflect damage to the oocyte, since heat stress before breeding was associated with decreased 90-day non-return rate to first service in lactating dairy cows in southern USA (Al-Katanani and others 1999). While such an effect would imply that oocytes collected during the summer would be less likely to give rise to blastocysts when subjected to in vitro fertilisation, experiments are equivocal as to whether this is so. The proportion of oocytes that developed into blastocysts was lower in July and August when compared to other months, in Louisiana (Rocha and others 1998) and Wisconsin (Rutledge and others 1999). However, in Florida, development to the blastocyst stage was not affected by season (Rivera and others 2000). The objective of this study was to re-evaluate the effect of seasonal variation on in vitro embryo development under subtropical conditions. Given that exposure of preimplantation embryos to elevated temperature (heat shock) can affect subsequent development (Rivera and Hansen 2001), the second objective was to determine whether the deleterious effect of heat shock at the two-cell stage is affected by seasonal variation.

Oocytes were obtained from ovaries collected at a local abattoir that processed both beef cattle (65 to 75 per cent; Bos indicus, Bos taurus and crossbreds) and dairy cattle (25 to 35 per cent; almost exclusively Holstein). Procedures for in vitro production of embryos have been reported previously (Paula-Lopes and others 1998). Embryos at the two-cell stage (29 to 32 hours postinsemination) were collected and transferred to a separate 50 μl drop of CR1aa medium (six to 24 embryos per drop). Embryos were either maintained continuously at 38-5°C and 5-0 per cent carbon dioxide as controls or were exposed to a heat shock by being cultured at 41°C and 7-0 per cent carbon dioxide for six hours and then returned to 38-5°C. Fetal calf serum (10 per cent [v/v]) was added on day 5 postinsemination. The proportion of two-cell embryos that developed to the blastocyst stage was determined on day 8 postinsemination. Data, collected from 32 replicates over a period of five years (n=423 embryos) and 494 control embryos, were transformed using an arcsine transformation and analysed by least-squares analysis of variance using the general linear models procedure of the statistical software SAS (SAS/STAT 1989). The number of embryos per day was used as a covariate. The model included the main effect of year, month, treatment and month x treatment.

There was no effect of month on cleavage rate, which varied from 68-8 (6-7) per cent to 86-0 (10-6) per cent. In contrast, the proportion of two-cell embryos that developed to the blastocyst stage decreased (P<0-001) during the summer months (July to August) when compared with other months of the year (Fig 1). Being exposed to a heat shock reduced development in all months (P<0-01) but the magnitude of the reduction in development caused by a heat shock was not affected by season.

The fact that fewer two-cell embryos produced in summer (July and August) could develop to the blastocyst stage is consistent with previous experiments suggesting reduced oocyte competence in summer in Louisiana (Rocha and others 1998) and Wisconsin (Rutledge and others 1999). Such an effect was not seen in an earlier study in Florida (Rivera and others 2000). The discrepancy between results is likely to represent genotype effects. Rocha and others (1998) demonstrated seasonal variation of oocyte competence in Holstein cows but not in more thermostolerant Brahman cows. One might expect then that the effect of season on in vitro production of embryos would depend not only on geographical location, which determines the degree of heat stress that cows experience, but also on the predominant cattle type from which oocytes were collected.
Previous studies in vivo (Ealy and others 1993) and in vitro (Edwards and Hansen 1997, Rivera and Hansen 2001) have shown that embryos are sensitive to elevated temperature at the two-cell stage. The current finding confirmed this adverse effect of elevated temperature. However, while embryos produced in summer have reduced developmental competence, there was no evidence to suggest that they were more susceptible to damage caused by heat shock after fertilisation.

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Short Communications

Isolation of Staphylococcus schleiferi subspecies coagulans from two cases of canine pyoderma

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Staphylococcus schleiferi was first described by Freney and others (1988). Isolates were recovered from various clinical human specimens and this species is now well recognised as an opportunistic nosocomial pathogen in infections following the implantation of pacemakers, or in postsurgical wound infections (Celard and others 1997, Kliuytmans and others 1998). In 1990, S. schleiferi was divided into two subspecies as a result of the description of S. schleiferi subspecies coagulans by Igimi and others (1990). All the studied strains had been cultured from discharges of the external ears of dogs suffering from external otitis. To the author’s knowledge, this subspecies was the object of only one subsequent report which included one strain isolated from a finger pulp infection in a human patient (Vandenbosch and others 1994). This short communication describes, to the authors’ knowledge, the first isolation of S. schleiferi subspecies coagulans from two cases of canine pyoderma, a commonly encountered problem in small animal practice.

The isolate ENV99.034 was recovered from a seven-year-old male collie with a deep pyoderma secondary to pemphigus foliaceus; the strain ENV99.0047 originated from a nine-year-old male German shepherd dog with extensive superficial folliculitis secondary to flea allergy dermatitis. Cutaneous swabs were plated on 5 per cent sheep blood agar and incubated for 24 hours at 35°C. Strain ENV99.034 was obtained as a pure culture while strain ENV99.0047 was isolated along with a coagulase-negative staphylococcal strain after enrichment in brain heart infusion. The two strains were identified as S. schleiferi subspecies coagulans as a result of the production of free coagulase with rabbit plasma (bioMérieux), various metabolic characteristics principally studied by using ID32 Staph gallery (bioMérieux) listed in Table 1, and the negative results given by a Staphylococcus aureus culture identification test (Accuprobe; bioMérieux). S. schleiferi subspecies coagulans and S. schleiferi subspecies coagulans differ in that S. schleiferi subspecies coagulans produces a free coagulase as well as a urosepe. Both subspecies differ from S. aureus in their inability to produce acid from maltose, and from Staphylococcus hyicus and Staphylococcus intermedius in their acetoin production.

Simple identification systems are currently not available to correctly identify isolates of S. schleiferi subspecies coagulans, and the ID32 Staph profiles of the two canine isolates were interpreted as unacceptable by the identification software. As the two canine strains produced a free coagulase and a thermonuclease, they could have belonged to a species not included in the API database, or they could have been atypical isolates of S. aureus, S. intermedius or S. hyicus (Honea and others 2000). Simple tests such as the coagulase test with rabbit plasma and latex agglutination tests detecting the clumping factor, protein A and specific S. aureus antigens, are not sufficiently specific; for example, protein A and coagulase were detected in strains belonging to S. schleiferi subspecies coagulans (Vandenbosch and others 1994).

Consequently, the two canine isolates were characterised by PCR analysis of the 16S to 23S intergenic spacer region (ITS-PCR). Mendoza and others (1998) demonstrated that this technique was able to identify all staphylococcal species. ITS-PCR patterns were identical for the two canine strains and were similar to the specific ITS-PCR pattern of the two S. schleiferi subspecies-type strains, thus confirming phenotypical species diagnosis. However, as for many other species, ITS-PCR did not allow identification at a subspecies level. Moreover, it is necessary to have a phenotypical presumptive diagnosis before performing ITS-PCR, as numerous patterns have been observed for S. intermedius and S. aureus (Dolzani and others 1994, M. Bes, personal observations).

Three coagulase-positive staphylococcal species – S. intermedius, S. aureus and S. schleiferi subspecies coagulans – can be isolated from dog skin, especially from suppulsive lesions (Hajek 1976, Biberstein and others 1984, Woldehiwet and Jones 1990, Ihreu 1996). S. intermedius is reported to be the most common cause of canine skin infections; of a total of 52 coagulase-positive staphylococci successfully isolated from canine cutaneous specimens submitted to the laboratory between 1999 and 2000, 48 strains were identified as S.