FOOD/FARMED ANIMALS

Closantel toxicity in a pregnant ewe at mid gestation: the pathological evaluation of the ewe and lamb nine months later

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SUMMARY

In February 2013, a flock of 20 pastured ewes was treated with oral closantel. Within 10 days of dosing, one 2-year-old Texel-cross ewe that was approximately three months in lamb was noticed to be spending a prolonged time in recumbency. On the 6th of March, the animal was referred to Scottish Centre for Production Animal Health and Food Safety (SCPAHFS). At admission, the ewe appeared blind, pupils were dilated with a negative menace response and pupillary light reflex bilaterally. Closantel toxicity was suspected. Following admission, this ewe was housed and was stable despite remaining blind. On April 21, a single male lamb was born. This lamb was found to have normal clinical and neurological parameters over five months of observation following birth. On postmortem examination closantel toxicity was confirmed in the ewe while following detailed gross and histological examination of all tissues of the lamb there was no indication of the toxicity suffered by its mother.

BACKGROUND

Closantel is a salicylanide derivative widely used in the UK for the treatment of *Fasciola hepatica* and *Haemonchus contortus* in sheep. Five products containing closantel are currently licensed in the UK, all of which carry a licence for use in pregnant ewes not producing milk for human consumption. This includes products for which closantel is the sole active ingredient as well as combination products containing ivermectin or mebendazole (DEFRA 2014).

As a flukicide, closantel is effective against both mature and late immature stages. It has also been shown to delay the resumption of egg shedding by up to 13 weeks post-treatment in experimentally infected sheep (Maes and others 1990), a characteristic referenced within the product information for Supaverm oral Suspension (Eli Lilly& Company Ltd) and Mebadown Super Oral suspension (Eli Lilly & Company Ltd) (DEFRA 2014). This gives closantel efficacy in control of pasture contamination comparable with triclabendazole (Maes and others 1990). These properties, combined with a proven efficacy against triclabendazole-resistant fluke (Coles and others 2000, Thomas and others 2000, Olaechea and others 2011), mean that while triclabendazole remains the drug of choice in instances of acute fasciolosis, closantel may play an increasing role in fluke control in the future.

There are numerous reports of closantel toxicity in both sheep and goats (Obwolo and others 1989, Gill and others 1999, Barlow and others 2002, Tiwari and others 2007). However, the effects of closantel toxicity on mid gestation pregnant ewes and, furthermore, the lamb in utero have not been previously reported. Previous embryo toxicity studies carried out in ewes included the delivery of 20 or 40 mg/kg once on day 11, 17 or 23 of gestation (Van Cauteren and others 1985). These ewes were observed to have equal distribution of repeat breedings and perinatal death between treated and untreated control groups. This study did not document lamb survival and production following birth.

The use of flukicides with efficacy against immature fluke has traditionally been reserved for autumn, targeting the period of greatest risk of acute fasciolosis. However, recent studies have suggested a change in the occurrence of acute fasciolosis in the UK with the majority of cases submitted to regional labs during the months of November, December and January (Bartram and Taylor 2013). These data are likely biased by the fact that many flocks will be treated for acute fasciolosis during autumn, thereby reducing morbidity and mortality at this time. The likelihood that sudden deaths occurring in the autumn are attributed by vets and shepherds to acute fasciolosis and as such not submitted to regional labs for postmortem examination must also be considered as a possible bias in these figures. However, the increased number of cases documented during November, December and January, between 2002 and 2011, does highlight the continued threat of acute fasciolosis in late winter and early spring.

This trend is likely to lead to the increased use of flukicides with efficacy against immature fluke during the gestation period and in the case of closantel an increased risk of toxicity in pregnant ewes. Recently, Sustainable Control of Parasites in Sheep (SCOPS), a group representing the sheep industry in the UK, reported increasing cases of 'Downer ewe syndrome' and blindness in pregnant ewes following fluke treatment (SCOPS 2014). This prompted the issue of an alert to all sheep farmers urging careful use of flukicides containing closantel, oxyclozanide or nitroxynil.

To our knowledge, no previous reports have documented the findings in a lamb carried to term following closantel toxicity in the mother. This case report documents the clinical and pathological

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findings in a ewe and her offspring following closantel toxicity at three months' gestation.

CASE PRESENTATION

In February 2013, a flock of 20 pastured ewes was found to be suffering from acute fasciolosis that had led to four fatalities. Following veterinary advice, the flock was gathered and treated with an oral suspension containing 50 mg/ml closantel, 75 mg/ml mebendazole (Supaverm Oral Suspension, Elanco Animal Health). Within 10 days of dosing, one 2-year-old Texel-cross ewe that was approximately three months in lamb was noticed to be spending a prolonged time in recumbency.

On examination the ewe was noted to be blind with an absence of a menace or pupillary light reflex bilaterally. No signs of weakness were noted and vital parameters were within normal limits. B and C vitamins (Combivit, Norbrook) and amoxicillin (Noroclav, Norbrook) were administered. The blindness persisted and circling behaviour developed.

On the 6th of March, this animal was referred to the teaching hospital at the Scottish Centre for Production Animal Health and Food Safety (SCPAHFS) University of Glasgow. At admission, the ewe appeared bright and alert, all vital parameters were within normal limits. Intermittent circling without a preference for one direction and bilaterally horizontal nystagmus was observed. The pupils were dilated with a negative menace response and pupillary light reflex (PLR) in both eyes and the animal appeared blind. All other parameters on neurological exam were within normal limits.

The history revealed a dosing gun was used to deliver an intended dose of 16 ml of Supaverm Oral Suspension (Eli Lilly & Company Ltd) per os, suitable for a ewe weighing 80 kg. However, as the dosing gun had not been calibrated, no accurate assessment of the true quantity delivered could be made. Estimation of weight on admission placed the ewe at approximately 60 kg.

Following admission, the ewe was housed along with three other sheep in a straw pen isolated from routine activity within the byre. Ad-lib food and water was provided in static troughs to which the ewe became quickly accustomed. Handling of the ewe was kept to a minimum, and she was seen to thrive under these conditions.

DIFFERENTIAL DIAGNOSIS

The presentation of blindness with an absent PLR is indicative of pathology associated with the retina or optic nerves and warrants consideration of a number of differential diagnoses. Bright blindness due to chronic ingestion of bracken fern (Hirono and others 1993), hypovitaminosis A and toxicity associated with rafoxanide or closantel (Odiawo 1991) were considered as possible diagnoses.

OUTCOME AND FOLLOW-UP

On the 21st of April, one male lamb was born. This lamb weighed 7 kg and was healthy at birth. For the following five months, the ewe and her lamb were housed together with three more ewes and monitored with bi-weekly clinical and monthly neurological exams. Over this time no clinical, neurological or ocular abnormalities were detected in the lamb though the ewe reminded blind. This ewe did not appear distressed by her condition, and she was housed until euthanasia of her and her lamb by intravenous barbiturate on the 8th and 13th of November 2013 respectively (nine months postclosantel administration). To allow comparison of gross findings in the ewe, a breed and age-matched ewe that had not been exposed to closantel with normal clinical and neurological parameters was submitted for postmortem on the same day.

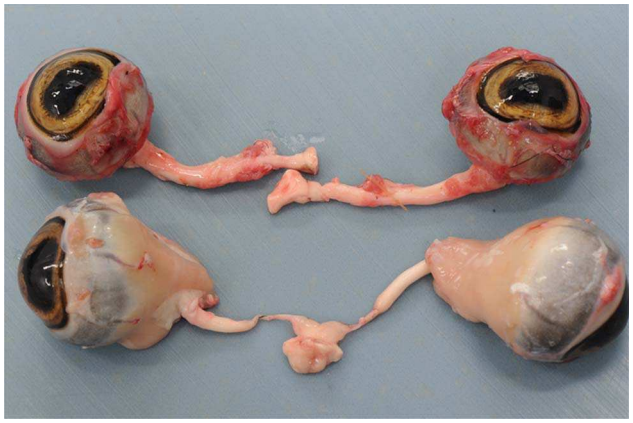

FIG 1: Image of the eyes, optic nerve and optic chiasm of the affected ewe (bottom) highlighting marked thinning of the intracanalicular portion of the optic nerves and mild-to–moderate thinning of the extracanalicular portion of the optic nerves compared with an age-matched control (top)

Postmortem examination of the ewe confirmed findings consistent with closantel toxicity (Borges and others 1999, Gill and others 1999, Barlow and others 2002, van der Lugt and Venter 2007, Crilly and others 2014). The intracanalicular portions (approximately 1 cm) of both optic nerves were markedly thinned (approximately 2 mm diameter) with only a very fine band of fibrous tissue remaining (Fig 1). The sections of the optic nerves between the intracanalicular portion and the globe were also thinned (approximately 1/2 to 1/3 of normal diameter). No other gross abnormalities were detected in the carcase.

The eyes, optic nerves and brain were fixed in 10 per cent neutral buffered formol saline, processed routinely, sectioned at 5 µm and stained with H&E and Masson's trichrome stain. Histologically, within the extracanalicular portion of the optic nerve, there were mildly reduced numbers of axons, occasional vacuolation of remaining axons and gliosis. The intracanalicular portion of the optic nerve showed marked axonal loss and replacement with fibrovascular tissue (Fig 2, H&E and Masson's trichrome) along with moderate thickening of the leptomeninges. Within the eyes, changes within the retina included marked loss of both the inner and the outer nuclear layers, which in places were reduced to a single layer of cells or totally absent. There was also marked thinning of the inner and outer plexiform layers, and diffuse loss of the retinal photoreceptor cells and also a marked reduction in the number of retinal ganglion cells with complete absence in places. There were scattered isolated or small clusters of pigment-laden cells multifocally detectable within the disrupted and depleted outer retinal layers (consistent with pigment cell migration from the retinal pigmented epithelium).

The history of recent closantel administration in addition to the clinical signs and pathological findings observed in this ewe confirmed the suspected diagnosis of closantel toxicity. Bright blindness secondary to bracken poisoning and hypovitaminosis A in adult animals were ruled out as both would cause retinal degeneration alone without changes within the optic nerves. Hypovitaminosis A in growing animals will cause thinning of the optic nerve in addition to retinal degeneration; however, this occurs as a result of narrowing of the optic foraminae due to altered bone remodelling (Van Donkersgoed and Clark

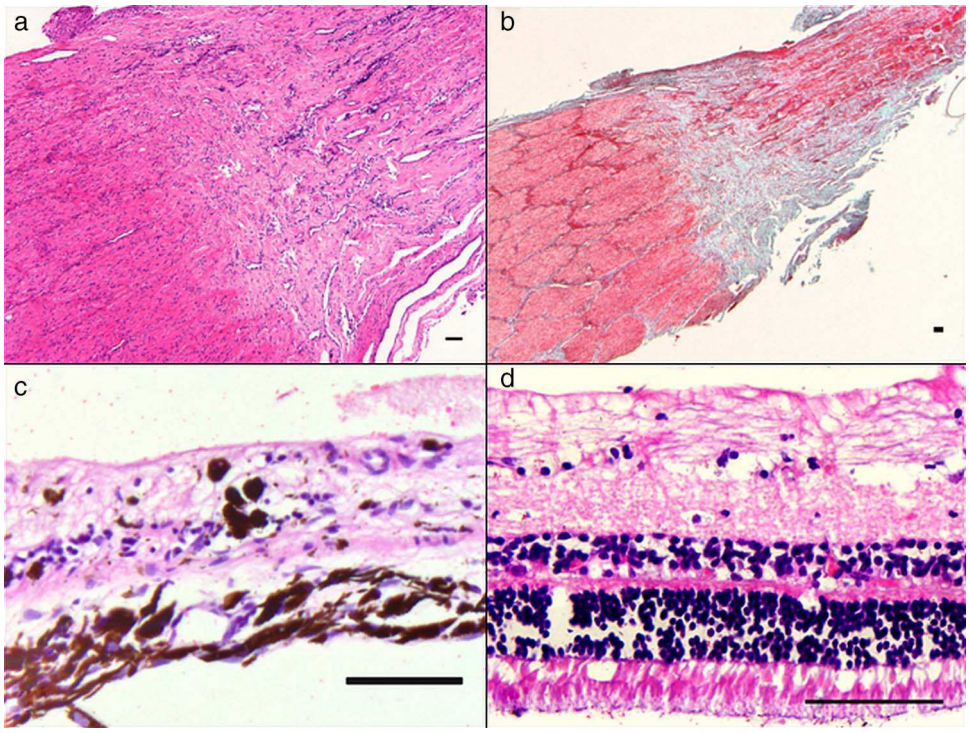

FIG 2: Histopathology of the optic nerve and retina. Black scale bars=100 μm. (a) Optic nerve (H&E) with marked axonal loss and replacement with fibrovascular tissue within the intracanalicular portion and with mildly reduced numbers of axons and mild-to-moderate gliosis in the extracanalicular portion. (b) Optic nerve (Masson's trichrome) highlighting increased collagen (green staining) within the markedly thinned intracanalicular portion of the nerve. (c) and (d) Retina (H&E) of affected ewe (c) and labelled age-matched control (d); compared with the normal retina, in the affected animal there is marked loss of both the inner (4) and the outer (2) nuclear layers, marked thinning of the inner (5) and outer (3) plexiform layers, loss of the retinal photoreceptor cells (1) and reduction in the number of retinal ganglion cells (6)

1988), a change that was not present in this adult ewe. Changes similar to those described have resulted from rafoxanide administration; however, there is no history of rafoxanide use in this animal (Van der Lugt and others 1996).

Postmortem examination of the lamb revealed no gross changes, with the brain, eyes and optic nerves within normal limits. Extensive histological examination of all tissues, including brain, optic nerves, eyes and peripheral nerves (femoral, sciatic and radial), did not reveal any significant findings.

DISCUSSION

This case report documents a case of closantel toxicity in a pregnant ewe, although the exact dose administered could not be determined. The therapeutic dose of closantel is 10 mg/kg. In studies carried out by Janssen Pharmaceuticals, the first fatalities following oral overdose are reported at 70 mg/kg when tested on healthy sheep (Van Cauteren and others 1985). This is in contrast to reported toxicities in the field that have documented adverse effects including death at 30 mg/kg (Gill and others 1999) and more recent reports of blindness at levels as low as 10–14 mg/kg (Crilly and others 2014). Reports of adverse effects concerning closantel made to the Veterinary Medicines Directorate (VMD) in the three years from 2011 to 2013 listed death, blindness and ataxia as the three most commonly observed clinical signs (personal communication V.M.D. 2013) though weakness, recumbency and diarrhoea have also been reported (Borges and others 1999, Gill and others 1999). An apparent individual susceptibility has been documented with numbers as low as 0.9 per cent of those treated showing clinical signs despite receiving similar milligrams per kilogram of closantel (Gill and others 1999). This may complicate the diagnosis of toxicity in instances where a small percentage of those treated are affected or where there is confidence in the dose delivered being at recommended levels.

It should be noted that although reports of closantel toxicity at doses close to the therapeutic dose exist, the majority of reports involve gross overdosing (Obwolo and others 1989, Borges and others 1999, Gill and others 1999, Barlow and others 2002). Thus, while individual weighing of sheep is optimal, accurate individual weight estimation, gun calibration and avoidance of accidental double dosing remain the key factors in the safe use of this drug.

No evidence of closantel toxicity was seen in the lamb in this case despite clinical and histopathological changes consistent with closantel toxicity in the mother. This may be due to lower fetal sensitivity to the toxic effects of closantel, lower closantel concentrations reaching fetal circulation or a greater plasticity in the fetal and ocular nervous systems allowing repair of any toxic damage or a combination of these factors.

Transplacental drug transfer is a dynamic process changing over the course of gestation, and while some factors may be considered to be relatively constant, such as the molecular characteristics of the drug, others such as umbilical blood flow, level of protein binding and the susceptibility of the fetus to toxicity are dependent on the timing of exposure (Mirkin 1975). While no studies exist regarding the ability of closantel to cross the placenta, it is reasonable to assume that this drug will enter the fetal circulation following maternal administration (Villee 1965, Mihaly and others 1983). Placental blood flow has been linked with drug clearance in sheep (Wilkening and others 1982) and is known to increase with

fetal maturation (Rudolph and Heymann 1970), leading to varying concentration of drugs being delivered and removed from fetal circulation though gestation.

The level of plasma protein binding by any drug significantly affects its ability to traverse cell membranes and diffuse within tissues. Following administration to adult animals, over 99 per cent of closantel is protein bound, almost exclusively to albumin. This leaves only a small percentage of free drug that will be available for transplacental equilibration. Given that albumin has an extremely limited capacity to cross the placenta (Boyd and others 1983), this high degree of protein binding likely limits the exposure of the fetus to closantel where maternal blood albumin levels are not diminished. This level of protein binding within maternal circulation however cannot be used as a template for binding in the fetal circulation as they often differ (Kumar and others 2000, Shoeman and others 1972) and vary throughout gestation (Kumar and others 2000). These differences are likely to influence the distribution of the drug and may result in differing localisation within fetal and maternal tissues (Mirkin 1975).

Finally, the toxic effects drugs in mature and immature animals vary and are extremely compound specific even within drug classes (Yeary and others 1966). Indeed, a study of the toxic effect of lidocaine in adult, newborn and fetal sheep found that dosages necessary to produce signs of toxicity were highest in the fetus and lowest in adults (Morishima and others 1981). In the specific case of closantel toxicity, which causes vacuolation of the myelin sheath throughout the CNS (van der Lugt and Venter 2007), the timing of exposure in relation to fetal maturation will likely be an important factor in determining the effects seen. The initiation of myelination of the CNS in the sheep varies between 60 and 100 days of gestation depending on anatomical location with the completion of myelin maturation occurring between 90 and 140 of gestation (Barlow 1969). These variables will likely alter the effect of closantel toxicity on the fetus depending on the stage of myelin development at exposure.

The costs associated with closantel toxicity in pregnant ewes stem from deaths on farm as well as veterinary costs and increased husbandry requirements for ewes that have lost their sight. In the case described here, the lamb was found to have normal clinical and neurological parameters over the five months of observation following birth. Following detailed gross and histological examination of all tissues, there was no indication that this lamb was affected by the toxicity suffered by its mother.

Given these findings, it is likely that, where adequate husbandry can be provided to ewes that have survived toxicity, losses may be mitigated by the production of healthy productive offspring.

However, it must be emphasised that the welfare of the ewe in these instances is paramount. In the case reported here, the ewe's temperament, the provision of a quiet, stress-free environment as well as pen-mates was key to the maintenance of this ewe on our holding. Thus, in instances where sheep unaccustomed to housing are affected or where correct housing requirements cannot be met, it would be inadvisable for blind sheep to remain on farm. The attending veterinarian is best positioned to advise shepherds as to the appropriate course of action in each individual set of circumstances.

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Contributors All authors were instrumental in the conception and development of the report. JG drafted the neurological aspect of the report as well as carrying out repeated neurological assessments of both animals. As part of the clinical team TG, KAE, FPH and JDGL were involved in the clinical care and evaluation of both animals documented in this report. Dr Francesco Marchesi and JDGL carried out the postmortem and histological examination of both animals as well as authoring the pathological interpretation section of the case report. All authors have critically reviewed the report for intellectual content and given final approval before submission.

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REFERENCES

BARLOW A., SHARPE J., KINCAID E. (2002) Blindness in lambs due to inadvertent closantel overdose. *Veterinary Record* 151, 25–26

BARLOW R. (1969) The foetal sheep: morphogenesis of the nervous system and histochemical aspects of myelination. *Journal of Comparative Neurology* 135, 249–261

BARTRAM D., TAYLOR M. (2013) Immature liver fluke infection in winter and spring. *Veterinary Record* 172, 82

BORGES A. S., MENDES L. C., DE ANDRADE A. L., MACHADO G. F., PEIRO J. (1999) Optic neuropathy in sheep associated with overdosage of closantel. *Veterinary and Human Toxicology* 41:378–380

BOYD R., CANNING J. F., STACEY T., WARD R., WEEDON A. (1983) Volumes of distribution of sodium and albumin in the sheep placenta. *The Journal of Physiology* 336, 13–26

COLES G. C., RHODES A. C., STAFFORD K. A. (2000) Activity of closantel against adult triclabendazole-resistant Fasciola hepatica. *Veterinary Record* 146, 504–507

CRILLY J. P., DEL POZO J., SCOTT P. R., WILSON D. J., SARGISON N. D., TRUYERS I. (2014) Retinopathy and optic neuropathy following closantel treatment of ewes. *Veterinary Record Case Reports* 2;e000044.

DEFRA V. M. D. (2014) http://www.vmd.defra.gov.uk/ProductInformationDatabase/ [Online]

GILL P. A., COOK R. W., BOULTON J. G., KELLY W. R., VANSELOW B., REDDACLIFF L. A. (1999) Optic neuropathy and retinopathy in closantel toxicosis of sheep and goats. *Australian Veterinary Journal* 77:259–261

HIRONO I., ITO M., YAGYU S., HAGA M., WAKAMATSU K., KISHIKAWA T., NISHIKAWA O., YAMADA K., OJIKA M., KIGOSHI H. (1993) Reproduction of progressive retinal degeneration (bright blindness) in sheep by administration of ptaquiloside contained in bracken. *The Journal of Veterinary Medical Science* 55, 979–983

KUMAR S., TONN G. R., RIGGS K. W., RURAK D. W. (2000) Diphenhydramine disposition in the sheep maternal-placental-fetal unit: Gestational age, plasma drug protein binding, and umbilical blood flow effects on clearance. *Drug metabolism and Disposition* 28, 279–285

MAES L., VANPARIJS O., LAUWERS H., DECKERS W. (1990) Comparative efficacy of closantel and triclabendazole against Fasciola hepatica in experimentally infected sheep. *Veterinary Record* 18, 450–452

MIHALY G., JONES D., MORGAN D., CHING M., WEBSTER L., SMALLWOOD R., HARDY K. (1983) Placental transfer and renal elimination of cimetidine in maternal and fetal sheep. *Journal of Pharmacology and Experimental Therapeutics* 227, 441–445

MIRKIN B. L. (1975) Perinatal pharmacology: placental transfer, fetal localization, and neonatal disposition of drugs. *Anesthesiology* 43, 156–170

MORISHIMA H., PEDERSEN H., FINSTER M., SAKUMA K., BRUCE S., GUTSCHE B., STARK R., COVINO B. (1981) Toxicity of lidocaine in adult, newborn, and fetal sheep. *Anesthesiology* 55, 57–61

OBWOLO M., ODIAWO G., OGAA J. (1989) Toxicity of a closantel-albendazole mixture in a flock of sheep and goats. *Australian Veterinary Journal* 66, 229–230

ODIAWO G. (1991). Acute amaurosis in sheep due to rafoxanide neurotoxicity. *Zimbabwe Veterinary Journal (Zimbabwe)*22,64–69

OLAECHEA F., LOVERA V., LARROZA M., RAFFO F., CABRERA R. (2011) Resistance of Fasciola hepatica against triclabendazole in cattle in Patagonia (Argentina). *Veterinary Parasitology* 178, 364–366

RUDOLPH A. M., HEYMANN M. A. (1970) Circulatory changes during growth in the fetal lamb. *Circulation Research* 26, 289–299

SCOPS (2014) http://www.scops.org.uk/alerts-detail.php?AlertID=25 [Online]

SHOEMAN D. W., KAUFFMAN R. E., AZARNOFF D. L., BOULOS B. M. (1972) Placental transfer of diphenylhydantoin in the goat. *Biochemical Pharmacology* 21, 1237–1243

THOMAS O., COLES G., DUFFUS K. (2000) Triclabendazole resistant Fasciola hepatica in south-west Wales. *Veterinary Record* 146:200

TIWARI H., KUMAR A., VIHAN V. (2007) An accidental closantel toxicity in goats-A clinical report. *The Indian Journal of Small Ruminants* 13, 219–220

VAN CAUTEREN H., VANDENBERGHE J., HÉRIN V., VANPARYS P., MARSBOOM R. (1985) Toxicological properties of closantel. *Drug and Chemical Toxicology* 8:101–123

VAN DER LUGT J., OLIVIER J., JORDAIN P. (1996) Status spongiosis, optic neuropathy, and retinal degeneration in Helichrysum argyrosphaerum poisoning in sheep and a goat. *Veterinary Pathology Online* 33, 495–502
VAN DER LUGT J. J., VENTER I. (2007) Myelin Vacuolation, Optic Neuropathy and Retinal Degeneration after Closantel Overdosage in Sheep and in a Goat. *Journal of Comparative Pathology* 136, 87–95
VAN DONKERSGOED J., CLARK E. G. (1988) Blindness caused by hypovitaminosis A in feedlot cattle. *The Canadian Veterinary Journal* 29, 925
VILLEE C. A. (1965) Placental transfer of drugs. *Annals of the New York Academy of Sciences* 123, 237–244
WILKENING R. B., ANDERSON S., MARTENSSON L., MESCHIA G. (1982) Placental transfer as a function of uterine blood flow. *American Journal of Physiology* 242, H429–H436
YEARY R., BENISH R., FINKELSTEIN M. (1966) Acute toxicity of drugs in newborn animals. *The Journal of Pediatrics* 69, 663–667