Short Note

Re-mating in otter *(Lutra lutra)*

Sim Broekhuizen, Dennis R. Lammertsma & Gerard J.D.M. Müskens

Alterra, Centre for Ecosystem Studies,
Droevendaalsesteeg 3, P.O.Box 47, NL-6700 AA Wageningen, e-mail: sim.broekhuizen@wur.nl

Abstract: After the first observation of three morulas in the uterus of an otter (*Lutra lutra*) (Broekhuizen et al. 2004) a second observation of three morulas, together with three early-stage blastocysts, was made in April 2005. In the light of the short period of delayed implantation of blastocysts in the mucous membrane of the uterus we discuss the probability of this indicating re-mating and compared the evidence with the more frequent occurrence of superfoetation in the American mink. (*Mustela vison*).

Keywords: otter, Eurasian otter, *Lutra lutra*, re-mating, delayed implantation, superfoetation, morula, blastocyst.

Introduction

The female otter (*Lutra lutra*) is known to be polyoestrous, having a succession of oestrous periods and no specific breeding season. This mustelid does not have the several months long delayed implantation of ova in the mucous membrane of the uterus that is found in the martens (*Martes spp.*), the badger (*Meles meles*), the American mink (*Mustela vison*), the stoat (*Mustela erminea*), the American long-tailed weasel (*Mustela frenata*) or the American river otter (*Lontra canadensis*). In those species fertilised ova develop into morulas during their passage in the oviduct, the stage in which the lump of cells becomes surrounded by a thick layer of protective mucus, the zona pellucida. At around the time that the morula enters the uterus, a cavity is formed within the morula and the zona pellucida disappears. The morula then becomes a blastocyst, which consists of one layer of wall cells around the cavity and the remaining lump of inner cell mass from which the embryo (the embryoblast) will develop. During delayed implantation the blastocyst remains in the uterus, where it is unattached to the uterus wall and grows slowly by enlarging the central cavity.

We might generalise from the discovery of three morulas in the uterus of an otter (Broekhuizen et al. 2004) that the ova of this species develop more slowly during their passage in the oviduct than is the case with other related species (in which the ova have already developed to the blastula stage upon entering the uterus). In the case described here, the morulas still had a thick zona pellucida after entering the uterus.

Although the otter does not have a delayed implantation of several months, this species belongs to a group of mustelids in which the pre-implantation period is longer than in many other mammal species. In otters this pre-implantation period lasts 12 days or more after mating (Mead & Wright 1983), in the weasel (*Mustela nivalis*) it lasts about 11 days (Heidt 1970), in the ferret (*Mustela putorius furo*) it occurs after 10 days (Strahl 1906, cited in Hansson 1947) and in the American mink after about 16 days (Hansson 1947). This means the chances of finding blastocysts in the uterus of these species is greater than in mammal species with a 'normal' pre-implantation time of about three days.

After the first finding of morulas in the uterus of a female otter, checking the uterus of otters for morulas has became part of the routine autopsy procedure for females found dead.

Observation

Since 2002 otters have been reintroduced into the Netherlands (Lammertsma et al. 2006), but the species remains rare. Several cases of reproduction have been established, but the number of otters is being reduced by road traffic victims. On April 30, 2005 a female otter was killed on the road close to where she was released on June 25, 2004, after having been caught along the river Tirza in Latvia on May 24 2004. At that time her body weight was 5.9 kg and her age was estimated (based on the tooth wear) as 3-4 years old. When she died her condition was quite good and her body weight was 6.45 kg. Both uterus horns had a placental scar, indicating that she had littered before. As the nipples were visible but not very pronounced, her last litter had already been weaned.

When the uterus horns were flushed three morulas, with a thick zona pellucida, were found, as well as three blastulas with a still thin zona pellucida (figure 1). Two morulas and one blastula were found in the left horn and one morula and two blastulas in the right one. Only three corpora lutea were visible, two in the left ovary and one in the right one.

Discussion

For otters we have no information about how long after entering the uterus the morulas lose the zona pellucida. The thin remains of the zona pellucida still present around the blastulas found in the uterus suggest that these blastulas did not stay there for long before the morulas arrived, although this is not certain. The question arises

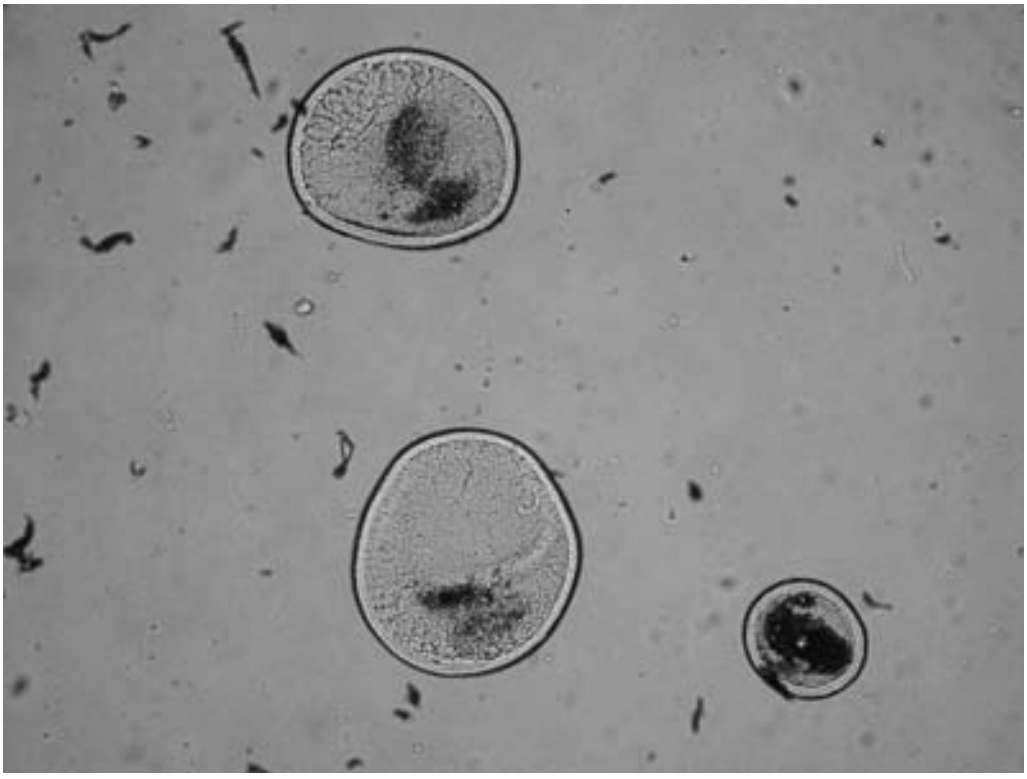

Figure 1. One morula and two blastocysts found in one of the uterus horns of an otter. *Photograph: Wim Dimmers, Alterra.*

of the possible cause of the simultaneous presence of these two stages of development of ova. Several possibilities can be hypothesised.

One hypothesis is that both stages of development resulted from the same bout of ovulation, but there was some variation in the rate of development of the ova. In the mink it takes the ova about 6.5 days after ovulation until the stage of morula has been reached (Hansson 1947). Normally the litter size of the otter is small, mostly two or three and rarely four (Wayer 1979, Kruuk 2006). Occasionally, however, litters of five have been reported (Van Wijngaarden & Van de Peppel 1970, Hauer et al. 2002). Harris (1968) and Baruš & Zejda (1981) mention rare cases of six cubs. An argument against the possibility of one ovulation and a variation in the rate of development of the ova is that all three morulas and all of the three blastulas were in almost exactly the same stages of development.

Another possibility is the fertilisation of ova from two different ovulations within the same period of oestrus. Female otters come into oestrus every 30-45 days (Gorman et al. 1978, Wayer 1979) and remain so for about two weeks (Wayer 1979). Within these periods of oestrus the female appears to be an induced ovulator (Jorga et al. 2004). Reuter (1993) found that among female captive otters successful impregnation was nearly always achieved as soon as a male was admitted to the female.

We have no information about the occurrence of repeated ovulation after a successful mating during the likely period of two weeks of oestrus of the female or of re-mating during that period. Green et al. (1984) found in a pair of radio-monitored free ranging otters that after copulation the male appeared to continue to attempt further matings, but that the female's interest had waned.

Hansson (1947) found that female American minks allow several matings at intervals of between 1-2 and 7-10 days. Ovulation occurs 36-37 hours after mating (Dunstone (1993) mentions a variation of 36-42 hours). Hansson (1947) also found that female minks frequently ovulate for a second time after re-mating. This observation was based on the autopsies of females that had been mated twice, among which two sets of corpora lutea were found which histologically appeared to be of different ages. If this also holds true for the otter, the different stages of development of the ova that we found could be the result of two matings. Among mink Shackleford (1952) found consecutive matings, usually seven days apart. In nearly 15% of the females that he allowed to mate twice within this period the mating resulted in a litter containing offspring from different males, based on the different colourings of the fathers. More recently Yamaguchi et al. (2004), using microsatellite markers, confirmed the occurrence of multi-paternity in free-ranging American minks.

Shackleford (1952) considered mink litters resulting from fertilisation of ova of different ovulations as cases of superfoetation, as distinct from superfecundation, i.e. the fertilisation by successive matings of ova from the same period of ovulation. Superfoetation is known among the badger, where females sometimes become impregnated for a second time during the, less intensive, autumn mating-season, after having already been impregnated during the main mating-period in late winter or early spring (Harris & Neal 1956, Cresswell et al. 1992). As the growth of the blastocysts is more or less asymptotical, the blastocysts of the second impregnation may be similar in size at the moment of implantation, resulting in a mixed litter, possibly sired by different males.

It is not known whether the simultaneous presence of morulas and blastocysts within the uterus of the female otter originated from one and the same ovulation (superfecundation) or from two different ovulations (superfoetation). The repeated impregnation might be the result of mixed paternity, as otters are probably both polygynous and polyandrous (Kruuk 2006). However, it is unknown whether litters with cubs sired by different fathers occur in nature.

Although a litter size of six is exceptional for otters, this does not imply that ovulation of six ova is equally rare. Hauer et al. (2002) found prenatal losses of foetuses, e.g. three foetuses in a litter of five among otters in Eastern Germany. In the American mink superfoetation results in a larger litter size. Possibly, some of the larger litters in otters result from superfoetation. In cases of mixed paternity among minks only a small proportion of

the ova from the first ovulation succeed in implantation in the uterus wall (Shackleford 1952).

As an incidental observation, when slicing the ovaria by hand, we only found three thriving corpora lutea. According to Hauer et al. (2002), thriving corpora lutea will fill about 75% of the ovaria, so it is unlikely that we missed three corpora lutea. We have no real explanation for this discrepancy. Possibly, it was a consequence of multi-oocytic follicles (MOFs), characterised by two or more oocytes surrounded by a common follicular envelope. MOFs have been reported at low but variable frequencies in mammal species from different orders, with the frequency increasing with oestrogenic contaminants in the environment (Guillette & Moore 2006). It is speculative whether this is a factor in this case.

Acknowledgements: We are very indebted to the two anonymous referees who commented on an earlier version of this paper. The very detailed comments of one of them inspired many improvements in this text. Hans Kruuk and Nick Parrott kindly corrected the English of this paper.

References

Baruš, Š. & J. Zejda 1981. The European otter (*Lutra lutra*) in the Czech Socialist Republic. Acta Scientiarum Naturalium Academiae Scientiarum Bohemoslovacae, Brno 15: 1-41.

Broekhuizen, S., H. Jansman & G.J.D.M. Müskens 2004. Morula's van een otter (*Lutra lutra*): een toevallige waarneming. Lutra 47 (2): 123-126.

Dunstone, N. 1993. The mink. T & A D Poyser, London, UK.

Cresswell, W.J., S. Harris, C.L. Cheeseman & P.J. Mallinson 1992. To breed or not to breed: an analysis of the social and density-dependent constraints on the fecundity of female badgers (*Meles meles*). Philosophical Transactions of the Royal Society of London B. 338: 393-407.

Gorman M.L., D. Jenkins & R.J. Harper 1978. The anal scent sacs of the otter (*Lutra lutra*). Journal of Zoology, London 186: 463-474.

Green, J., R. Green & D.J. Jefferies 1984. A radio-tracking survey of otters *Lutra lutra* on a Perthshire river system. Lutra 27: 85-145.

Guillette, L.J. Jr. & B.C. Moore 2006. Environmental contaminants, fertility, and multioocytic follicles: a lesson from wildlife? Seminars in reproductive medicine 24 (3): 134-141.

Hansson, A. 1947. The physiology of reproduction in mink (*Mustela vison*, Schreb.) with special reference to delayed implantation. Acta Zoologica 28: 1-136.

Harris, C.J. 1968. Otters. A study of the recent Lutrinae. Weidendeld and Nicolson, London, UK.

Harris, R.J. & E.G. Neal 1956. Ovulation during delayed implantation and other reproductive phenomena in the badger (*Meles meles* L.). Nature 4517: 977-979.

Hauer, S., H. Ansorge & O. Zinke 2002. Reproductive performance of otters *Lutra lutra* (Linnaeus, 1758) in Eastern Germany: low reproduction in a long-term strategy. Biological Journal of the Linnaean Society 77: 329-340.

Heidt, G.A. 1970. The least weasel *Mustela nivalis* Linnaeus. Developmental biology in comparison with other North American *Mustela*. Publications of the Museum, Michigan State University. Biological Series 2, 7: 227-282.

Jorga, W., W. Tschirch & S. Eichhorn 2004. Bemerkungen zur Tragezeit des Eurasischen Fischotters (*Lutra lutra*). Beiträge zur Jagd- und Wildforschung 29: 297-406.

Kruuk, H. 2006. Otters: ecology, behaviour and conservation. Oxford University Press, Oxford, UK.

Mead, R.A. & P.L. Wright 1983. Reproductive cycles of Mustelidae. Acta Zoologica Fennica 174: 169-172.

Lammertsma D.R., F.J.J. Niewold, H.A.H Jansman, A.T. Kuiters, H.P. Koelewijn, M.I. Perez Haro, M. van Adrichem, M.C. Boerwinkel & J. Bovenschen 2006. Herintroductie van de otter: een succesverhaal? De Levende Natuur 107 (2): 42-46.

Reuter, C. 1993. *Lutra lutra* (Linnaeus, 1758) – Fischotter. In: J. Niethammer & F. Krapp (eds.). Handbuch der Säugetiere Europas, Band 5: 907-961. Aula Verlag, Wiesbaden, Germany.

Shackleford, R.M. 1952. Superfoetation in ranch mink. American Naturalist 86 (830): 311-319.

Van Wijngaarden, A. & J. Van de Peppel, 1970. De otter, *Lutra lutra* (L.), in Nederland. Lutra 12: 1-72.

Wayer, P. 1979. The private life of the otter. Batsford, London, UK.

Yamaguchi, N., R.J. Sarno, W.E. Johnson, S. O'Brien & D.W. Macdonald 2004. Multiple paternity and reproductive tactics of free-ranging American minks (*Mustela vison*). Journal of Mammalogy 85: 432-439.

Samenvatting

Dubbele bevruchting bij een otter (*Lutra lutra*)

Na de eerste waarneming van de aanwezigheid van drie morula's in de baarmoederhoorns van een otter (Broekhuizen et al. 2004), werden bij een in mei 2005 doodgereden otter (*Lutra lutra*) naast drie morula's ook drie blastocysten in de baarmoeder aangetroffen. Dit gelijktijdig voorkomen van verschillende ontwikkelingsstadia van bevruchte eicellen duidt op herhaalde bevruchting in verschillende perioden van oestrus (superfoetatie). Het optreden van superfoetatie is mogelijk doordat otters een, zij het korte (ruim 12 dagen), periode van uitgestelde implantatie van de blastocysten hebben. Het voorkomen van superfoetatie bij otters wordt vergeleken met dat bij Amerikaanse nertsen (*Mustela vison*), waarbij de aanwezigheid van gemengde worpen afkomstig van opeenvolgende paringen met verschillende mannetjes ook in het wild is vastgesteld.

Received: 30 November 2006
Accepted: 15 October 2007