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Assisted Reproduction in Parrots

Michael Lierz
Clinic for Birds, Reptiles, Amphibians and Fish,
Faculty of Veterinary Medicine, Justus Liebig University,
Frankfurter Straße 91-93
35392 Gießen, Germany
michael.lierz@vetmed.uni-giessen.de

Introduction

The IUCN (International Union for the Conservation of Nature) has currently listed about 13% of the bird species worldwide as endangered or even critically endangered (IUCN, 2013). This especially counts for many parrot species. Probably the most famous example is the Spix's macaw (*Cyanopsitta spixii*), which only remains in captivity and is said to be extinct in nature (IUCN, 2013). Many parrot species are threatened by loss of habitat, illegal trade or hunting as well as environmental risks. Therefore, breeding programs play an important role in species conservation programs for threatened species. They are to preserve genetically the species and gain time in order to be able to eliminate the causes of the threat, so that animals can be reintroduced later on (Reinschmidt et al., 2008; ICMBio, 2012). Furthermore, breeding programs can satisfy the commercial demand and, in doing so, make illegal trade less attractive. Breeding programs for highly endangered species, however, are not always successful. This is primarily due to the lack of suitable breeding animals, especially if, for example, one sex is over-represented in the breeding population. Furthermore, infertility, disorders or health problems play a great role and can lead to the exclusion of an animal from breeding. Especially monogamous parrots are affected, because unsuitable partners cannot easily be replaced. The lack of suitable animals leads to forced pairings, which may result in no eggs or unfertilized clutches. Especially in case of unfertilized clutches, assisted reproduction may help to produce offspring (Saar et al., 1983; Blanco et al., 2009). A basic requirement for the integration of assisted reproduction techniques into an existing breeding program is the possibility of semen collection from the male animal. This facilitates the evaluation of breeding males on the basis of their semen quality and either a possible exclusion from the breeding program or the use as a genetically valuable reserve. Furthermore, this semen can be used for artificial insemination.

Semen Collection:

The possibility of semen collection varies greatly between different bird species. Although already described for the first time in the 1930s for commercial poultry (Burrows and Quinn, 1935), a routine technique could not be established for every group of birds. Semen collection is very well established for birds of prey, using tools where imprinted animals copulate on (for example a hat) or alternatively massages for semen collection (Temple, 1972; Grier, 1973; Boyd et al., 1977; Blanco et al., 2002; Fischer et al., 2011). Comparable massage techniques are used for cranes (Guojun et al., 1998), geese (Varga et al., 2004), emus (Malecki et al., 2011), ostriches (Hemberger et al., 2001), penguins (O'Brien et al., 1999; Waldoch et al., 2007), tragopans (Zhang, 2006) and parakeets (Samour et al., 1986; Anderson et al., 2002; Behncke, 2002; Stelzer, 2004; Stelzer et al., 2005; Neumann et al., 2013). Despite of these possibilities, the massage technique for semen collection could not be successfully adapted to large psittacines; it only worked anecdotally (Brock, 1991; DellaVolpe et al., 2011; Behncke und Stelzer, 2003). It was only the development of a new technique based on electrostimulation which allowed a regular and reproducible semen collection from large parrots; in the pilot study it was possible to collect semen from 109 different parrot species and subspecies, including rare species like the Spix's macaw (Lierz et al., 2013). The collected semen was also used for artificial insemination. 64 inseminations were accomplished, 36 of them followed by egg-laying. In 24 (69.4) of those eggs from 10 different species embryos developed, including cockatoos, amazons, macaws, Eclectus and other species. Importantly, in this study the production of a macaw and a cockatoo chick through an assisted reproduction technique was successful for the first time worldwide. The ejaculates gained in

the study were investigated also spermatologically. The investigation demonstrated that the spermatological parameters within a family are comparable, whereas there are significant differences between families. Eclectus parrots had a much higher sperm density and total number of spermatozoa than, for example, macaws. This difference may result from the breeding biology of the species. Female Eclectus parrots mate with several males, so their semen competes with the others' for the ovum. The more semen a male can enter into the female's genital tract, the higher the likelihood that his sperm fertilizes the ovum. Monogamous species like macaws for example, do not have this kind of selection, resulting in that even a male with a low semen concentration can father offspring. The study also indicated that the success of the semen collection technique, of the semen quality and the success of the artificial insemination depends on the reproductive status of the pair, the season and the point of time of the insemination either before or after the egg-laying.

The seasonality of the semen quality was therefore evaluated in a further study with single males from different psittacine families, examining them weekly during one year (Bublat et al., 2014). The study proved the differences between the various parrot families already indicated by the first study. The success rate of the semen collection and the semen concentration of macaws and amazons showed a large seasonal influence with a significant increase a few days before and around the egg-laying of the partner animal. Outside of the breeding season, no semen could be obtained from these groups, whereas this was possible for Eclectus parrots throughout the whole year with relatively equal parameters. Cockatoos also showed almost no strong seasonality.

Spermatological Examination:

The significance of the spermatological examination in breeding programs cannot be overestimated. It primarily serves to judge the male breeding animals and allows determination of the reasons for unfertilized clutches. Furthermore, it is a basic requirement for artificial insemination, because this can only be done with high-quality semen to guarantee an insemination success as high as possible. In addition, the semen can be evaluated in a way that the high-quality semen can possibly be diluted in order to use one portion of semen for several inseminations. The spermatological examination of birds is comparable to that of humans and domestic mammals (Weitze, 2001; Waberski und Petrunkina, 2007; WHO, 2010). According to Fischer et al. (2011), volume, colour, consistency and pH-value of the ejaculates as well as their level of contamination (for example with faeces, uric acid and/or blood) are evaluated. Furthermore the motility, vitality (via Intra Vital Staining) and the morphology of the spermatozoa are assessed. Herewith, it is important to establish specific reference values for each species, as already different parrot families varies significantly as shown before. Cutting edge examination methods, like the Computer Assisted Semen Analysis (CASA), are able to determine different parameters for the movement and the speed of individual spermatozoa. Hereby it is of great importance to adapt the software of the system to the specialities of each species under examination (Schneider et al., 2013; Fischer und Lierz, 2013). Last but not least, function tests of the semen are especially important in order to finally assess the ability of the viable semen to fertilize. The perivitelline membrane penetration test, which has recently been modified for various bird species, plays an important role in this process (Krohn et al., 2012). In this test, semen is placed onto a perivitelline membrane of an egg in order to assess whether the sperm is able to penetrate the membrane, which is a basic requirement for the fertilization of an egg (Robertson et al., 1997).

Artificial Insemination:

The obtained semen can be used for artificial insemination. This has been done various times for birds and already succeeded exemplarily for cockatiels (Neumann et al., 2013) as well as for large parrots (Brock, 1991; Lierz et al., 2013). According to the positioning of the semen in the genital tract of the female, the placement is done intracloacal, intravaginal (opening of the oviduct) and intramagnal insemination (Blanco et al., 2002). The intravaginal insemination has clear practical advantages and promises the most success (Lierz et al., 2013). In order for an artificial insemination to lead to a fertilized egg, a minimum amount of semen with a certain number of life motile spermatozoa is necessary. This minimum requirement varies greatly between the bird species and is nearly unknown for large parrots and only experimentally known for cockatiels (Lierz et al., 2010). The artificial insemination allows single genetically valuable males to be more often represented within the breeding population (Lierz et al., 2013), because their sperm might be divided and inseminated into several females. Furthermore, surplus females in a population can me paired with sterilized males from other related species and inseminated with semen

from males of their own species. In the end, even isolated or socially incompatible animals (Lierz et al., 2013), as well as handicapped males unable to reproduce naturally, can be genetically integrated into the population, as recently proven in large parrots (Fischer et al., 2013). Apart from the insemination, the availability of an ovum ready to be fertilized at the moment of semen transmission is a basic requirement for the production of a chick. Therefore, the point of time of insemination is highly important. This point of time is not clearly known in parrots. Since semen is able to survive longer in the female genital tract, the first insemination should be performed 4-5 days before the first egg is laid. Another insemination should follow at least after every further egg-laying. For this purpose, close observations of the breeding animals using cameras in nest boxes are of advantage for increasing the success of such techniques. However, especially in species conservation programs, the semen donor and the female are not always kept close together or an alternative male is lacking in the case that semen collection is unsuccessful at the point of time of the expected egg-laying. The availability of semen at the point of time of insemination is the limiting factor of these techniques. Even though, the vitality of the semen can be preserved up to 24h with various buffer solutions; the cryopreservation of parrot semen, however, would solve most of the problems.

Cryopreservation:

The cryopreservation of parrot semen is a solution independent from time and place for the long-term conservation of gene reserves (Schneider et al., 2013). Only for a few bird species protocols for semen- cryopreservation are described, but most of them are anecdotal (for example budgerigars (Samour, 2002)). Due to the large physiological differences of the semen of different bird species, such protocols are not transferable to other parrot species and have to be newly established every time. In order to do so, first the basic physiological data of the semen of a bird species have to be determined and investigation protocols needs to be established accordingly, for example for electron microscopy. Finally, adequate diluents and cryoprotectants as well as a freeze protocol have to be established in series of tests in order to guarantee the survival of the semen during the freezing process. According to investigations of Schneider et al. (2014), an adequate diluent and cryoprotectant was developed for the semen of the cockatiel serving as a model.

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For more detailed information please see:

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References:

Anderson, S.J.; Bird, D.M.; Hagen, M.D.: 2002. Semen characteristics of the Quaker Parakeet (*Myiopsitta monachus*). Zoo Biol., 21, 507-512

Behncke, H. 2002. Spermagewinnung unduntersuchung sowie endoskopische Beurteilung des Geschlechtsapparats in Abhängigkeit von der Spermaproduktion bei Psittaziden am Beispiel des Wellensittichs (Melopsittacus undulatus). Dissertation, Veterinärmedizinische Fakultät Universität Leipzig.

Behncke, H.; Stelzer, G.: 2003. Case report: Semen collection, analysis and first attempt of artificial insemination (AI) in red-tailed cockatoos (*Calyptorhynchus magnificus*). In: 7th EAAV Conference and 5th ECAMS Scientific Meeting, Litografia Romero Puerto de la Cruz, Teneriffa, Spanien: pp. 367-369.

Blanco, J.; Wildt, D.; Hofle, U.; Voelker, W.; Donoghue, A. 2009. Implementing artificial insemination as an effective tool for ex situ conservation of endangered avian species. Theriogenology, 71, 200-213

Blanco, J.M.; Gee, G.F.; Wildt, D.E.; Donoghue, A.M. 2002. Producing progeny from endangered birds of prey, urine contamination, intramagnal insemination. Journal of Zoo and Wildlife Medicine 33, 1-7,

Boyd, L.L.; Boyd, N.S.; Dobler, F.C.: 1977. Reproduction of prairie falcons by artificial insemination. Journal of Wildlife Management 41, 266-271.

Brock, M.: 1991. Semen collection and artificial insemination in the Hispaniolan parrot (*Amazona ventralis*). Journal of Zoo and Wildlife Medicine. 22, 107-114.

Bublat, A., S. Bruslund, D. Fischer, H. Schneider, S. Meinecke-Tillmann,A. Wehrend, M. Lierz. 2013. Spermagewinnung und spermatologischeParamter bei Großpapageien im Jahresverlauf. Proceedings der 1. Jahrestagung der DVG-Fachgruppe Vögel-und Reptilien, 6-8.03. München

Burrows, W.H.; Quinn, J.P. 1935. A method of obtaining spermatozoa from the domestic fowl. Poultry Science. 14, 251-254.

DellaVolpe, A.; Schmidt, V.; Krautwald-Junghanns, M.-E. 2011. Attempted semen collection using the massage technique in blue-fronted amazon parrots (*Amazona aestiva aestiva*). Journal of Avian Medicine and Surgery. 25, 1-7.

Fischer, D.; Lierz, M. 2013. Assistierte Reproduktion beim Vogel Neue Chancen für den Artenschutz. Tagungsband der 34. Tagung über tropische Vögel der Gesellschaft für Tropenornithologie und der Estrilda, Ehlscheid.

Fischer, D.; Garcia de la Fuente, J.; Wehrend, A.; Lierz, M. 2011. Semen quality and semen characteristics of large falcons with special emphasis on fertility rate after artificial insemination. Reproduction in Domestic Animals. 46: 14.

Fischer, D.; Neumann, D.; Schneider, H.; Giersher, K.; Bouts, T.; Purchase, C.; Hebel, C.; Reinschmidt, M.; Bruslund, S.; Bublat, A.; Meinecke-Tillmann, S.; Wehrend, A.; Lierz, M. 2013. Assisted reproduction in two rare psittacine species - The Spix's macaw and the St. Vincent amazon. In: Proceedings of the 1st International Conference on Avian, Herpetological and Exotic Mammal Medicine, VVB LaufersweilerVerlag Wiesbaden: 295-296.

Grier, J.W.: 1973. Techniques and results of artificial insemination with golden eagles. Journal of Raptor Research. 7: 1-12.

Guojun, C.; Gee, G.F.; Nicolich, J.M.; Taylor, J.A. 1998. Investigation into semen characteristics of naturally-fertile pairs in vitro and their correlation with fertility in Florida sandhill crane. Journal of Forestry Research. 9: 71-76.

Hemberger, M.Y.; Hospes, R.; Bostedt, H. 2001. Semen collection, examination and spermiogram in ostriches. Reproduction in Domestic Animals. 36, 241-243.

ICMBio: 2012. Executive summary of the national action plan for the Spix's macaw conservation (Projeto-ArarinhanaNatureza). Chico Mendes Institute for the Conservation of Biodiversity Brazil.Aufrufbarunter: http://www.icmbio.gov.br/portal/images/stories/docs-plano-de-acao/pan-ararinha-azul/sumario-ararinhaazulingles.pdf, 2012.

IUCN . 2013. International Union for Conservation of Nature Red List of Threatened Species. Aufrufbarunter: http://www.iucnredlist.org 2013.

Krohn, J.; Meinecke-Tillmann, S.; Schneider, H.; Lierz, M.; Wehrend, A. 2013. Technical Note: Investigations on the accomplishment of the avian perivitelline membrane penetration assay. Reproduction in Domestic Animals, Wiley-Blackwell Malden, MA, USA: 33.

Lierz, M. A. Wehrend, E.F. Kaleta und D. Neumann 2010. Artificial Insemination in Parrots. Proceedings of the 31st Annual Conference and Expo of the Association of Avian Veterinarians, 2. 5. August 2010, San Diego, USA, 295-296.

Lierz, M.; Reinschmidt, M.; Müller, H.; Wink, M.; Neumann, D. 2013. A novel method for semen collection and artificial insemination in large parrots (Psittaciformes). Scientific Reports. 3, 2066.

Lüpold, S.; Calhim, S.; Immler, S.; Birkhead, T.R. 2009. Sperm morphology and sperm velocity in passerine birds. Proceedings of the Royal Society B. 276, no. 1659: 1157-1181.

Malecki, I.A.; Sood, S.; Tawang, A.; Martin, G.B. 2011. Artificial insemination technology for the emu - improving sperm survival. Reproductive Biology. 11, 43-49.

Neumann, D.; Kaleta, E.F.; Lierz, M.W. 2013. Semen collection and artificial insemination in Cockatiels (Nymphicus hollandicus) – A potential model for Psittacines.Tierärztliche Praxis., 41, 101-105.

O'Brien, J.K.; Oehler, D.A.; Malowski, S.P.; Roth, T.L.. 1999. Semen collection, characterization, and cryopreservation in Magellanic penguin (*Spheniscus magellanicus*). Zoo Biology. 18, 199-214.

Reinschmidt, M.; Waugh, D.; de la Cruz, P.; Spanien, T. 2008. Haltung und Zucht des Spix-Aras (*Cyanopsitta spixii*) in der Loro Parque Fundación auf Teneriffa. Der Zoologische Garten. 77, 134-156.

Robertson, L.; Brown, H.L.; Staines, H.J.; Wishart, G.J. 1997. Characterization and application of an avian in vitro spermatozoa–egg interaction assay using the inner perivitelline layer from laid chicken eggs. Journal of reproduction and fertility. 110, 205-211.

Saar, C.;Gerriet, S.; Paasch, B.; Spaeter, C. 1983. Die künstliche Besamung beim Wanderfalken (*Falco peregrinus*). Ein Beitrag zur Rettung einer vom Aussterben bedrohten Art. Kleintierpraxis, 28, 163-176.

Samour, J.H. 2002. The reproductive biology of the budgerigar (Melopsittacus undulatus): Semen preservation techniques and artificial insemination procedures. Journal of Avian Medicine and Surgery. 16, 39-49.

Samour, J.H.; Smith, C.A.; Moore, H.D.; Markham, J.A. 1986. Semen collection and spermatozoa characteristics in budgerigars (*Melopsittacus undulatus*). Veterinary Record 118, 397-399.

Schneider, H.; Fischer, D.; Bergmann, M.; Ehling, C.; Meinecke-Tillmann, S.; Wehrend, A.; Lierz, M. 2013. Investigations on cryopreservation of psittacine semen. Proceedings of the 1st International Conference on Avian, Herpetological and Exotic Mammal Medicine, VVB LaufersweilerVerlag Wiesbaden: 386-387.

Schneider, H. D. Fischer, M. Bergmann, C. Ehling, S. Meinecke-Tillmann, A. Wehrend, M. Lierz. 2013. Untersuchungen zur Kryokonservierung von Psittazidensperma. Proceedings der 1. Jahrestagung der DVG-Fachgruppe Vögel-und Reptilien, 6-8.03. München

Stelzer, G. 2004. Spermagewinnung, -untersuchung und -flüssigkonservierung bei verschiedenen Papageienvögeln (Psittaciformes). Dissertation der Veterinärmedizinischen Fakultät der Universität Leipzig.

Stelzer, G.; Crosta, L.; Bürkle, M. 2005. Krautwald-Junghanns, M.-E.: Attempted semen collection using the massage technique and semen analysis in various psittacine species. Journal of Avian Medicine and Surgery. 19, 7-13.

Temple, S.A. 1972. Artificial insemination with imprinted birds of prey. Nature. 237-238.

Varga, A.; Barna, J.; Almási, A. 2004. Sperm analysis and sexual characteristics of frizzled hungarian ganders (preliminary study).
Waberski, D.; Petrunkina, A.M. 2007. Spermatologie, In: Busch, W.; Waberski, D. (Eds.), Künstliche Besamung bei Haus- und Nutztieren, Schattauer GmbH Stuttgart.

Waldoch, J.; Root, T.; Ramer, J.; Proudfoot, J. 2007. Semen collection and characterization in rockhopper penguin. Journal of Zoo and Wildlife Medicine. 38, 13-17.

Weitze, K.-F. 2001. Prinzipien der andrologischen Untersuchung, Spermatologische Untersuchung, In: Busch, W.; Holzmann, A. (Eds.), Veterinärmedizinische Andrologie, Schattauer GmbH Stuttgart.

WHO (Ed.) 2010. WHO laboratory manual for the examination and processing of human semen.World Health Organization Press Genf, Schweitz.

Zhang, Y.Y. 2006. Semen characterization and sperm storage in Cabot's tragopan. Poultry Science. 85: 892-898.