ULTRASONOGRAPHY AS A TOOL IN PROPAGATION OF ZOO ANIMALS

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Reproductive disorders are a major problem in wild animals held in captivity and have a significant impact on many captive breeding programs. Basic scientific information regarding reproduction is missing or incomplete for most animal species. Questions regarding sex determination, sexual maturity, cycle length, sperm viability in the female genital tract, embryonic development and gestation length remain unanswered for the majority of zoo maintained animals and this is especially true for those species under the threat of extinction. As the maintenance of viable populations of these animal species in captivity becomes increasingly important, knowledge of the unique, reproductive biological features of a species and information concerning the reproductive status of the individual members of a breeding group is essential for successful captive breeding. The development of endocrine monitoring within the last years is an important first step toward increasing our knowledge of basic reproductive physiology in different animal species. Furthermore, the introduction of non-invasive techniques for measuring sex hormones in urine and faeces has been of considerable practical value in the reproductive management and treatment of diverse zoo species by avoiding the need for capture or restraint conforms with animal protection efforts.[17,29]

The potential of real time ultrasound as an alternative approach for non-invasive exploration of reproductive biology and pathology of zoo and wild animals has not been fully utilised. Despite tremendous progress in the development of sonography and its incorporation into human medicine during the 1960's and veterinary medicine in agricultural and laboratory animals at the end of the 1970's, until now, ultrasound has only played a limited role in wildlife medicine.[2,3,7,8,9,15,31,33,41,43,44]

The general advantages of ultrasonography as opposed to other imaging techniques are: i) it is a non-invasive technique, ii) provides real time, visual and reproducible information regarding organs, iii) produces cross-sectional images of tissues and organ structures, as well as organs in motion which is difficult or not feasible with other techniques, iv) enables morphometric measurements of examined objects to be obtained, v) facilitates documentation and preservation of primary data on storable cassettes, vi) can be easily integrated with other examination methods.

The use of ultrasound imaging in reproductive biology provides a better understanding of early embryonic development and contributes essential, new knowledge regarding the function of the uterus and ovaries. As a non-invasive imaging technique, ultrasound delivers new information concerning reproductive biology, which has not been accessible to the researcher using classical methods of experimental embryology and endocrinology. However application of ultrasonography to zoo and wild animals does have a number of specific difficulties which do not exist, or at least are less problematic, in human or classic veterinary medicine. Furthermore, knowledge obtained in humans or domestic animals, although useful, is usually not directly transferable to wild or zoo animals. In Table 1, the principle types of difficulties in applying ultrasound technology to zoo animals and wildlife are shown together with some specific examples.

Table 1. Problems in applying ultrasound technique in zoo and wild animals

Problem No.	Problem	Example
1	Physical or chemical restraint of subject	Most species; some birds may be held by the wings and legs in dorsal recumbency; investigation of cold-blooded animals at 0˚ C; tranquilization necessary for carnivores.
2a	Acoustic coupling of the ultrasound probe on the skin	Shells, plates, feathers, fur
2b	or insufficient penetration of ultrasound waves through the tissue investigated	Avian air sacs, thick skin, large subcutaneous fat pads, long distances in megavertebrates
3	Modification of ultrasound system components for zoo and wild animals	Portable system, accumulator pack, cable extension for probes, specifically designed scan heads
4	A minimum of reference data on ultrasonographic appearance of different organs	Gonads and kidneys in birds, ovaries of elephants, etc.
5	Limited experience of investigator in a large number of species	About 5,000 mammalian species
6	High cost of ultrasound equipment	Approximately $55,000

In order to find some solutions to these problems in applying ultrasound to zoo and wild animals, the ultrasound research group of the Institute for Zoo Biology and Wildlife Research (IZW) has, during the last three years, performed approximately 1,100 individual ultrasound exams in over 100 animal species including amphibians, reptiles, birds and mammals. As part of this effort, ultrasound technology has been integrated into our studies on reproductive disturbances in zoo and wild animals to address the following issues.

1. Sex determination of monomorphic species

For many animal species, it is not possible for an animal keeper to make appropriate management decisions regarding breeding due to the fact that monomorphic species show no, or only slight, phenotypic differences which can be used to distinguish between the sexes. In addition, differences in behaviour based on gender are frequently not evident in captivity. There are many species of monomorphic birds and, until now, sex determination has mainly been achieved with invasive techniques such as laparoscopy or by chromosomal evaluation, which requires capture of the bird to obtain a blood sample. Ultrasonography is a non-invasive and accurate technique which can be used to detect gonadal or intragenital structures at various topographical locations. The unique anatomical characteristics of birds, however, may initially pose problems for successful sex determination using ultrasonography. The air sacs and the compact intestinal convolution prevent transmission of the ultrasound waves. In our studies, these difficulties have been overcome by the development of specialised probes allowing trans-intestinal or trans-cloacal examination. The gonads can be visualized by inserting a miniaturised probe with a frequency of 7.5 to 15.0 MHz and definitive sex determinations can be made based on the appearance of the structures seen.[13,19,23,24,26]

Sonographic sex determination can also be applied to several mammalian species such as the beaver, sloth and spotted hyena. Sex determination in the two-toed sloth *(Choloepus didactylus, Choloepus hoffmanni)* and in the spotted hyena *(Crocuta crocuta)* have been successful using trans-rectal ultrasound.[10,40]

2. Determination of sexual cycle in females

Detailed information regarding the female sexual cycle of most animals is limited. Repeated examinations performed throughout the cycle are necessary to make statements regarding the length of the sexual cycle and its component phases. Procedures in zoo animals are accomplished in most cases by physical or chemical restraint, although methods for urinary or faecal hormone analysis provide a useful non-invasive alternative. Invasive manipulations are not without risk and can cause stress which may harm the individual animal or interfere with its reproduction. We repeatedly examined chemically immobilised lowland anoa *(Bubalus depressicornis)*[11] and were able to determine for the first time that the length of the estrus cycle is 22 days. In some species, it is preferable to perform an ultrasound examination without physical or chemical restraint. For example, we have performed trans-rectal ultrasound examinations in trained elephants at the Hagenbeck Animal Park in Hamburg, Germany without any means of chemical restraint. It is possible to visualize structures on the ovaries with a high-frequency probe and a specially designed ultrasound configuration system.[22] A single examination of the female genital tract does not offer any indication of cycle length, but may provide information on the phase of the cycle[29,38] since in many species it is possible to distinguish corpora lutea from the various follicular stages using a high frequency ultrasound probe.

3. Monitoring of gestation with possible prediction of day of parturition or laying term

For a long time, biologists, zoo keepers and veterinarians have relied on observations of sexual behaviour, mating and birth to determine the average gestation length for different animal species. With these methods, the day of delivery can be estimated and management and feeding of the gravid animal can be adjusted accordingly. The introduction of sonography to veterinary medicine allows monitoring of the early stages of gestation and fetal development. Measurement of fetal structures and comparison of these findings with reference data[20,30,32,39] allow the stage of gestation to be estimated and, therefore, prediction of approximate date of delivery. Indirect methods of pregnancy determination, such as progesterone and/or oestrogen metabolites in faeces or urine can be highly informative but are often species-specific due to differences in hormone metabolism and physiology. Pregnancy diagnosis is possible in a large number of wild and zoo animals (amphibians, reptiles and mammals including elephant and rhinoceros) with a one time trans-intestinal or trans-cutaneous ultrasound examination. Another possible option is fetal sex determination by ultrasonography.[6] Sexing requires a definite age, position of the fetal and sufficient amniotic fluid. Therfore, there are only few opportunities to perform foetus sex determination successfully. With this method, statements regarding number, vitality and developmental stage of the embryo may also be possible. Trans-cloacal and trans-intestinal sonography [23,24] can be employed to image the yolk and ovary in birds or reptiles and based on the extent of calcification, a prediction regarding the laying term is possible. Since ultrasound is a non-invasive, direct method which allows imaging of embryonic/fetal morphologic structures, it has therefore many advantages as a method for pregnancy diagnosis in zoo animals.

4. Identification of pathological alterations to the inner genital tract

Knowledge concerning the appearance of healthy genital structures is necessary for clear recognition of pathological alterations. Given the wide variability of genital tract structures within mammals, it is understandable that this is not always possible.

Some pathological changes are relatively easy to identify such as cysts or tumors.[3,10,11,22,25,28,36,37,41] For example, cystic degeneration of the ovaries and endometrium in old carnivora[42] and especially in great cats is frequently reported.[4,5,35] Alterations in the testes, such as calcification or intra-parenchymal cysts, enables healthy and pathological structures to be relatively easily distinguished. It is more difficult to distinguish between physiologic uterine fluid and a pathological condition. For example, purulent endometritis in the monkey is not easily distinguished from the monthly menstruation.[18]

Assessment of the reproductive capacity of the individual is based on the health of the internal genital tract. It is therefore important to detect pathological alterations of the inner genital tract and to determine the meaning or influence it may have on reproductive performance before forming breeding groups.

5. Identification of pathological disorders of embryo genesis

Information regarding the viability of the early embryo is relatively quickly obtained with trans-cutaneous or trans-intestinal sonographic examination. In some species, the time frame in which pathological changes are detected is mainly in the first trimester of gestation in mammals, although this may extend until the end of the second trimester. In most cases, it is not possible to obtain detailed diagnostic images with a high-frequency ultrasound probe late in gestation due to the formation of fetal fur and increased calcification of the skeleton.

However, it is possible to assess heart activity, volume of embryonic fluid and condition of the placenta during mid-to-late pregnancy. In addition, some abnormalities such as schistosoma reflexa can be detected by the appearance of coils of intestine floating in the embryonic fluid. Growth disorders (e.g., severe retardation) are also apparent with sonography during this stage of gestation.

Most pathologic disorders of embryogenesis occur during the time of implantation. Early embryonic death is a frequent occurrence which can be retrospectively detected with ultrasound, and structural changes within the endometrium of the slightly enlarged uterus can be visualized. Changes indicating early embryonic death include the detection of an approximately 1 mm large undifferentiated echogenic form in the uterine lumen and a corpus luteum on at least one ovary. Embryonic membranes and fluid are frequently still present in the uterus.
In some cases, identification of fetal malformations can be problematic. With ultrasound, we detected an abnormality in a Przewalski horse fetus.[21] Only after examination of the isolated uterus could we clearly see that it was a symmetrical malformation of the fetus. Malformations causing accumulation of fluid such as ascites, cystic kidneys, hydrocephalus, etc. are clearly imaged with ultrasonography. As previously indicated in section 4, knowledge regarding the normal appearance of the different gestational stages is absolutely necessary to detect pathological changes.

6. Identification of maternal pathological processes with potential effects on structure and function of genital organs or embryo genesis

Sonographic examination of the major abdominal organs, such as liver, spleen, kidney, adrenal and pancreas may provide useful criteria to appraise the fitness or breeding potential of an animal. Detection of subclinical changes in these organs indicates that a clinically apparent metabolic disorder may occur under the physiological burden of pregnancy which could have possible lethal consequences for the mother and/or fetus. Pathological changes of the liver are most frequently found in the form of cysts or nodes (e.g., lowland anoa, dhole). Tumors in the kidney, spleen, intestine and greater omentum have also been discovered (generalised sarcoma in great cats). Excessive intra-abdominal accumulation of fluid was detected in two elephants by sonographic investigation of the genital tract, which proved to be secondary to a subclinical cardiac irregularity. Findings regarding the general health of the animal and those specific to the genital tract should be considered in order to make appropriate decisions regarding which animals to breed.

7. Support of diagnostic procedures, therapy or assisted reproduction

Ultrasound as a non-invasive imaging technique in real-time mode can provide visualization of diverse diagnostic procedures and treatments. Direct visualisation provided by ultrasound aids the investigator in performing insertion or manipulation of instruments in patients, such as flushing of the urinary bladder and uterus, collection of salivary fluid [12] or obtaining diagnostic biopsy specimens.[16,34] Ultrasonography can also be of assistance by providing intra-operative orientation for the castration of elephant bulls. Sonographic assisted follow-up evaluations make it easier to monitor the recovery of patients or to optimize ongoing treatment. Ultrasonographic supported embryo collection in *Macaca fascicularis* [20] is a non-surgical way to collect blastocysts from the uterus by trans-cervical insertion of a two-way catheter. Equivalent methods are useful for intrauterine artificial insemination [14] or non-surgical embryo transfer in small sized mammals.

8. Control of health status of reproductively inactive individuals

A stable hierarchy is the basis for establishing a successful breeding group in animals with a highly developed social structure. Especially in species like elephants or great apes, old individuals not employed in reproduction take up a special social position within the group. These animals fulfil special tasks in the raising of infants. They play an essential role in passing on social behaviour. The loss of high ranking animals leads to a stressful reorganisation of the social structure of the whole breeding group. In these phases of social instability, reproduction decreases or tapers off[1]. Therefore, we believe that it is essential to integrate individuals not employed in reproduction in to the ultrasound supported health control program along with the fertile members of the group. Pathological alterations in organs can immediately be detected and adequately treated when sonographic examination series are established.

Summary

Ultrasonography is an alternative approach for non-invasive exploration of reproduction and reproductive pathology of zoo and wild animals which is still under-utilized in wildlife medicine. It is a non-invasive technique, which provides reproducible real time images, cross-sectional imaging of tissues, organ structures and motions, morphometric measurements, documentation and preservation of data. The advantages of this technique favour its more intensive use. Ultrasound imaging in reproductive biology delivers new information regarding embryonic development, function of the uterus and ovaries where experimental embryology and endocrinology fail. In order to solve the problems which occur with the application of ultrasonography in zoo and wild animals, we performed 1100 individual examinations in over 100 species. Our interest was focused on :

1. Sex determination of monomorphic species to make appropriate breeding management decisions and to avoid invasive sexing techniques. We performed successful examinations in reptiles, birds and in mammals such as beaver, sloth and spotted hyena.

2. Ultrasonography is an alternative or supportive method to urinary or faecal analysis in the determination of sexual cycle in females. We determined the cycle length in lowland anoa (22 d) and performed trans-rectal examinations on trained elephants without sedation to determine the phase of the reproductive cycle.

3. Pregnancy diagnosis is possible in many species using ultrasound. Monitoring early stages of gestation, fetal development and degree of maturation of the placenta allow a prediction of the date of delivery. Estimation of laying term is possible in reptiles and birds through trans-cloacal and trans-intestinal ultrasound as well.

4. Understanding pathological alterations within the inner genital tract and their influence on reproductive performance is important in order to consider reproductive capacity of the individual.

5. Of interest in embryogenesis is the detection of heart activity, volume of embryonic fluid and condition of the placenta. Pathological disorders in embryogenesis such as growth disorders, malformations and early embryonic death can be visualized.

6. Another criteria for appraisal of the fitness or breeding potential of an animal is the investigation of the major abdominal organs. Pathological alterations sonographically detected such as cysts, nodes, tumors or intra-abdominal fluid can be a secondary cause for infertility.

7. Sonography can be used as a supportive technique for the insertion or manipulation of instruments in the patient, as an additive help for orientation in the operating area or in the field of assisted reproduction like artificial insemination and embryo collection. Therapies can be optimised by sonographical follow-ups.

8. The control of the health status of socially high-ranking individuals not employed in reproduction is of significance in successful breeding. Non-reproductive animals play a responsible role in the socialisation of infants. Early diagnosis of diseases by ultrasound can, therefore, prevent social instability or stress in reproduction.

Application of ultrasound to the systematic examination of selected animal groups as opposed to case-related investigations is necessary in the frame of zoo and wild animal research to offer new solutions to manage reproductive problems. Improvements in ultrasound technology, as well as the development of new types of application techniques for wild animals will support this process.

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