8. Summary

Comparative investigations on the reproduction biology of bears (Ursidae)

Six of eight existing bear species are close to extinction. Amongst the approaches to preserve these animals, their management and breeding in captivity play an important role. The aim of this study is to present the potential of modern assisted reproduction techniques and further develop selected approaches. Some techniques were applied to examine the suitability of non-endangered bear species as models for endangered bears. These model species can later be used to improve and test techniques for assisted reproduction without utilizing the already weak populations of the endangered species.

The bear family consists of three sub-families. Within the Ursinae the Brown bear and the Polar bear as well as the Black bear and the Asiatic Black bear are the closest related. The Spectacled bear is the link between the Ursinae and the Giant panda, which has lately been approved as a member of the Ursidae.
The reproduction biology in most bears is very similar. Bears are seasonal breeders whose pregnancy is characterized by a long embryonic diapause which lasts until the implantation of the blastocyst. The Malayan Sun bear is an exception: it is aseasonal and has a very short diapause, if at all. Several breeding seasons are reported for the Spectacled bear and the Sloth bear leading to the hypothesis that the cycle might be regulated by light or food availability. The pregnancy in bears varies largely and is mainly influenced by the length of the diapause. Implantation in most bears takes place in late autumn; its regulation mechanism is so far unknown. The time of embryonic development is genetically fixed and is equally long in every bear species. Thus most births take place in winter. Exceptionally the Giant panda delivers its cubs in late summer and in captive Malayan Sun bear births can be observed at any time of the year.

In this study male and female bears were morphologically and sono-morphologically examined. Moreover in males a spermatological and in females an endocrinological assesment was undertaken.
It has to be pointed out that some of the results of the Spectacled are described for the first time, so comparisons with other authors are missing.

The size of the testes in Malayan Sun bears, Sloth bears, Spectacled bears, Brown bears and Polar bears depends on the individual body size. If the volume of the testes are put into relation with body-size, smaller bears have comparatively larger organs. Apart from that, the Giant panda owns relatively as well as actually the largest gonads. In male Brown bears, Spectacled bears and a Giant panda a seasonal change in testes size can be observed. During the breeding season the testes reach their maximum dimensions. In contrast the sizes of the accessory sex glands *Ampulla ductus deferentis* and *Glandula prostatica* are linear to the body size and their seasonal changes in dimension are very limited. Again the Giant panda is an exception. Its prostate almost doubles its size during the breeding season. In a castrated Asiatic Black bear the accessory sex glands were atrophied; a hint on their hormone induced growth and regulation. Semen gained from Giant panda and Spectacled

bears using electroejaculation is of best quality during the estrous of the female. The active stage of spermatogenesis is mirrored by the increase of the testis dimensions. The quality and quantity of the Giant panda's semen is outstanding compared to the Spectacled bear's and the descriptions of other bears in the literature. But in the Spectacled bear the question about the seasonality has not been finally answered and this might have a strong influence on these parameters.
In the female Malayan Sun bear, American Black bear, Brown bear and Polar bear the length of the uterus correlates with the size of the animals. In the American Black bear it also correlates with seasonality. The comparison of the uterine body and the uterine horns is reciprocal depending upon body size. The uterine diameter in all eight bear species was assassed using transrectal ultrasonography. The differences in diameter of the uters body and the uterus horns are negligible. The weight-related diameter of the uterus is decreasing with increasing body mass. Again the Giant panda is an exception: during the active reproduction stages estrous, diapause and pregnancy it has the largest weight-related diameter of of all bears. The weight-related diameter of the uterus varies during the different reproduction stages. But there is no seasonally related change with a maximum during pregnancy as was expected. There is no relation between the proliferation of the endometrium and the reproduction stage. The detection of pregnancies using the conterlateral diameter of the uterus horn or the uterine corpus is not possible. Also free floating oocytes or blastocysts before implantation are impossible to demonstrate. Pregnancies can be positively proven after implantation. The implantation site is characterized by a local enlargement of the uterus horn and the proliferation of the endometrium. The remaining uterus stays unchanged and remains in appearance as that of a non-pregnant animal. In the ovaries a reciprocal trend of the weight-related size of can only be observed during anestrous and pregnancy. During any other reproduction stage the size of the ovaries correlates with the body size. Apart from this the Giant panda owns the largest gonads, up to three to four times larger than those of the other bears. The volume of the ovaries changes on a seasonal base and is influenced be the growth of functional bodies. Functional bodies, follicles and *corpora lutea*, can be well assessed and differentiated by using ultrasonography. Thus a diagnosis of the reproduction status is possible using an ovarian diagnostic in combination with the interpretation of the functional bodies.

Endocrinological investigations have been performed on members of all three bear sub-species. They are based upon the experience of the Institute for Zoo and Wildlife Research, Berlin, studying the Giant panda. As mentioned above, the reproduction biology of most members of the ursine family is very similar (the Malayan Sun bear is an exception). The brown bear was included into this study because it is the most popular bear in European zoos. The Spectacled bear was chosen as the third species because on the one hand it links the Giant panda and the Brown bear genetically and on the other is also an endangered bear.
In the Giant panda urinary estradiol is used for ovulation detection. A gradual increase of about eight days in the estradiol secretion precedes the preovulatory estradiol peak followed by a sharp drop to base level at the time of ovulation. This method does not reveal clear results in either the Brown nor Spectacled bear. Also another immune-assay for epiandrosterone, which is used in Malayan Sun bears to trail the follicular phase (Schwarzenberger *et al.* 1998) as well as the analysis of saliva did not lead to any

interpretable results. An alternative method for estrous detection in bears is the determination of urinary volatile substances (volatiles). In the Giant panda a two to three days lasting peak in the secretion of urinary fatty acids occurrs at the same time as the increase in estrogens and in the behavioral changes announcing estrous. These fatty acids can also be detected in Brown bears during heat. They have also been distinguished in Spectacled bears, but there were no clear estrous signs in these animals. So far the origin of these substances is a matter of speculation. They can be detected outside a specialized laboratory in close proximity of the animals using a commercial colorimtric test kit for fatty acids.
To monitor pregnancies in the Giant panda urinary pregnandiol is routinely used to demonstrate the luteal activity. This method does not function in Brown or Spectacled bears. But the luteal phase can be assessed in both species using progesterone in faeces or urine. Saliva again shows no results.

In conclusion the reproductive tracts of bears basically have similar structures from an anatomical and topographical point of view. Their size relies on the size of the animal. Only the Giant panda has the largest gonads and uterus diameter both actually and in relation to its body size. For the development of instruments for electroejaculation, ovum pick-up or micro-invasive surgery model species of similar body size to the target species can be used. Also the techniques for electroejaculation can be directly transferred between species. Depending upon the localization of research projects one can recommend different model species. In Europe the Brown bear is advised because it is the most common bear in this region. In South-East Asia the Asiatic Black bear is preferred. These bears are bred on farms and are therefore easily obtained for any examination. Extensive studies have already been carried out in North America on American Black bears. Johnston *et al.* (1994) recommended the use of these bears as model species.
From an endocrinological point of view the bears differ significantly. Each species needs its own method for estrous detection and pregnancy monitoring. A differentiation between pregnancy and pseudo-pregnancy is still not possible. For the Giant panda the assessment of urinary estradiol for estrous detection and urinary pregnandiol for pregnancy monitoring has already been established. The luteal phase of the Brown bear can be determined by faecal and urinary progesterone; in the Spectacled bear by urinary progesterone. For Malayan Sun bears the follicular activity can be displayed using faecal epiandosterone. The measurement of urinary volatiles is a promising investigation for estrous detection in the future. Due to the limited number of samples in this study more research is need to back-up the results.