7. Summary

Experimental investigations of reproduction biology and embryo transfer in the European Roe Deer (Capreolus capreolus)

The aim of this study was to characterise embryonic diapause (eD) in the roe deer using modern techniques of assisted reproduction, especially embryo transfer.

For this purpose techniques established in domestic cattle such as synchronisation and superovulation of embryo donors and recipients, recovery and transfer of embryos and techniques for the genetic allocation of parentage were applied to this wildlife species for the very first time.

The results of synchronisation, superovulation, recovery and transfer of embryos and their meaning for the embryonic diapause in roe deer are summarised as follows:

1. The application of intravaginal gestagene sponges, developed for the use in sheep and goats just before the beginning of the natural rutting season, resulted in the synchronisation of all embryo donors. After removal of the sponges, all animals came into oestrus and mated.

2. Under protection of the artificial luteal phase created by the vaginal sponges, it was possible to superovulate the females by injection of ECG followed by subsequent antagonisation with anti-ECG. Transrectal adaptersonography made it possible to monitor ovarian dynamics from the inactive ovary, the development of follicles to the prime of the corpora lutea and to quantify the number of dominant follicles, respectively the number of corpora lutea. Significantly higher numbers of corpora lutea and significantly higher numbers of recovered embryos in combination with higher values of progesterone in the superovulated animals compared to the non-superovulated animals as well as satisfying rates of ovulation of 70 % on average lead to the conclusion that like in domestic cattle and like in other Cervidae hormone regimes with ECG/anti-ECG are suitable for the superovulation of roe deer. The number of corpora lutea per animal superovulated with ECG was 7.1 (2.8 in the non-superovulated females) on average. The number of recovered embryos, oocytes an non-classifiable objects was 3.4 (1.0 in the non-superovulated females). The number of corpora lutea correlated positively with the amount of progesterone measured in the feces.

3. Due to the anatomy of the reproductive tract of female roe deer, embryos could not be recovered non-surgically although insertion of the catheter into the uterine lumen was possible. The results of the surgical flushing of each uterine horn resulted in recovery rates of 41 % on average (relation of the number of embryos to the number of corpora lutea), which is satisfying and comparable to the results reported from other *Cervidae*. Compared to the recovery rate of embryos reported for domestic species like goat and sheep (on average 75 %) the surgical flushing of embryos in roe deer was less successful.

4. Flushing of embryos on estimated days 7 and 9 of cycle brought up embryos in a wide variety of developmental stages, these embryos being classified as 2-cell, 4-cell, 8-cell, 16-cell stages and as morulae and early blastocysts. In contrast to what is reported for other animal species, all of those early developmental stages were found in the uterine horns and not in the oviducts. None of the embryos showed signs of degeneration or retardation. From these results it was concluded that embryonic development in roe deer is delayed immediately after conception and not, as previously reported, only after the formation of the blastocyst.

5. Application of $PGF_{2\alpha}$ to synchronise cycles in six of eight recipients during the diapause in September and October resulted in a measurable decrease of progesterone in the faeces to a quarter followed by a return back to the starting point. Parallel to these findings it was possible to monitor the regression and the development of new corpora lutea, being at their prime on the day of embryo transfer.

6. After embryo transfer in autumn, three of eight females became pregnant (one of them carrying twins). Two pregnancies could be diagnosed in January shortly after implantation using transrectal ultrasonography. All of the pregnant females had been synchronised with the age of the embryo transferred, there was no pregnancy in the non-synchronised females. Although fawns were born at the pysiological time for roe deer births, i.e. in May and June, the hypothesis of an artificially shortened diapause could not be verified.

   Blood samples taken from all members of the three roe deer families (each family consisting of the putative parents, the biological parents and the offspring) for the

genetic allocation of parentage proofed that in all three cases putative parents and biological parents were identical. In conclusion, all fawns were the result of the natural matings in summer with the physiological duration of pregnancy and not the result of the embryo transfer program in autumn. No statement can be made about the possibility to influence embryonic diapause or about the dependence of eD on external or internal factors. It is still not clear who gives the signal for the resumption of embryonic development, mother or embryo.

In spite of induction of a new cycle via luteolysis going along with development of new corpora lutea and temporary decrease of progesterone concentrations, none of the pregnancies was terminated and embryos were not resorbed. All females carried their fawns to term, which leads to the conclusion that in contrast to other animal species in roe deer, during the diapause, maintenance of pregnancy must be largely independent from the presence of progesterone.

This finding is considered to be another reproductive particularity of European roe deer.