10 Summary

Improvement of the whole embryo culture (WEC) of gestational day 9.5 rat embryos by testing different buffer solutions and using a continuous gassing regime Prolonging the culture time up to 72 (96) hours

In the first part of this thesis, it was attempted to improve the culture conditions by optimizing the buffer system used. As culture medium a mixture of self produced bovine serum (43 %), purchasable fetal calf serum (43 %) and buffer (14 %) was used. The following different buffers were investigated for their potential to improve the culture conditions of the embryos: HEPES (hydroxy-ethyl-piperazine-ethane-sulfonic acid), Bufferall®, HBSS (Hank´s balanced salt solution) and Tyrode solution. The embryonic development was significantly improved by the use of HBSS and Tyrode solution in comparison to the usage of HEPES or Bufferall. However, the Tyrode solution revealed the disadvantage that its higher concentration of phosphates could precipitate under culture conditions. Therefore, HBSS was found to be the most preferable buffer for the culture medium of the WEC.

In the second part of this thesis the rotator culture system (continuous gassing of the culture medium) of the WEC was investigated in comparison to the standard roller culture system (discontinous gassing) regarding the development of the cultured embryos. The gas concentrations and the pH values in the culture medium following continuous gassing in the rotator stabilized the culture conditions significantly. However, the development of the embryos cultured for 48 hours (gestational day (GD) 9.5 to 11.5) in the rotator was not significantly different from the one in the roller. In both culture systems, the embryos reached a stage of development that was almost comparable to *in vivo* embryos of GD 11.2. Therefore, when using a culture time of 48 hours in routine WEC experiments it seems reasonable to use the common standard culture system (roller) because it is less labour-intensive and simpler to handle.

In the third part it was aimed to identify culture conditions which are essential for the prolongation of the culture for more than 48 hours. To reach this aim, different protocols to manipulate the yolk or amniotic sac of embryos, which were established before for the culturing of older embryos, were used and evaluated in comparison. Embryos cultured with any manipulation of the yolk sac or amniotic sac after 42 hours of culture led to significant lower development of the cultured embryos than without. Thus, the most suitable protocol for a prolongation to 74 hours was a culture for 42 hours, followed by a further culture for 32 hours with no opening of the yolk and amniotic sac, combined with a replacement of culture medium

after 42 hours. Thereby, the differentiation of embryos was almost comparable to *in vivo*, if the culture vessels were continuously gassed with increasing oxygen concentrations (10 % up to 60 %).

These embryos showed a degree of differentiation and growth which was above the one of *in vivo* embryos on GD 11.5 but significant lower that on GD 12.5. The histological examination revealed that the structures of the different organ anlagen, were developed without significant anomalies. Necrosis occurred only occasionally.

The prolongation of the culture time up to 96 hours did not been achieved - neither with the roller- nor with the rotator-system. Thereby, the number of necroses in the embryonic tissue increased significantly. The embryos did not show any progress in growth and differentiation beyond the developmental stage observed after 72 hours of culture.

Comparison of the culture conditions in the standard roller culture and the new rotator culture with the prolonged culture time of 72 (or 96) hours.

Culture-conditions day 9.5 – day 13.5	**Rotator** (continuous gassing)	**Roller** (discontinuous gassing)
Culturemedium	43 % bovine serum; 43 % fetal calf serum; 14 % HBSS (buffer); no antibiotics; heat-inactivated serum, filtrated (0.2 µm)	
Incubation - temperature	38.0 °C ± 0.5°C	
Flow rate of gassing:	250 ml / min for 2 min	4 l/min for 2 min
Embryos (E) / ml	GD 9.5 – 11.2: 2 E / 5 ml GD 11.2 – 13.5: 1 E / 5 ml	GD 9.5 – 11.2: 4 E / 7 ml GD 11.2 - 13.5: 2 E / 7 ml
Exchange_of culture medium	after 42 hours	
Gassing	t = 0-24 h: 5 % O_2 t = 24 – 42 h 30 % O_2 t = 42 – 72 h 60 % O_2 t = 72 – 96 h 85 % O_2	t = 0-36 h: 5 % O_2 t = 36 – 42 h 50 % O_2 t = 42 – 72 h 60 % O_2 t = 72 – 96 h 85 % O_2

In the last part of this work the direct effects of ethanol on rat embryos exposed to 1, 3 and

6 mg/ml were investigated in the WEC. The 48 hours exposure of 9.5 GD rat embryos

to 1 mg/ml caused no adverse effects on embryonic development. But when embryos were exposed to 1 mg/ml ethanol during a culture period of 72 hours a significant retardation in growth and differentiation could be observed in comparison to the development of the control embryos. Higher concentrations of ethanol (more than 3 mg) induced growth retardation and embryotoxic effects after an exposure of 48 hours as well as 72 hours.

But the prolonged culture period produced a different pattern of specific malformations; particularly differences in rotation became manifested in abnormal shape of embryos.