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THE INFLUENCE OF DONORS AND RECIPIENTS ON EMBRYOTRANSFER RESULTS IN RABBITS

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microinjection in zygote pronuclei.
In this work different influences of donor and recipient animals on embryotransfer results of microinjcted rabbit zygotes were examined.

Material and Methods

Three different rabbit races were used for the experiments:
- cross-breeding rabbits
- ZIKA-rabbit-hybrids
- Californian rabbits

The animals were kept in a 14 hours day and 10 hours night rhythm. The stable temperature varied between 16° C and 18° C. The donor rabbits were superovulated by a single injection of 40 I.U. PMSG per kg body weight (ROTTMANN and STRANZINGER, 1977; TSUTSUMI et al., 1980). After 72 to 76 hours the natural cover respectively artificial insemination with diluted fresh-sperm was performed. The ovulation was caused by application of 120 or 180 I.U. HCG (GABLER, 1970; MICHELMANN und PAUFLER, 1974).

One group of the recipient animals was presynchronized: 22 days before embryotransfer they were ovulated by 120 I.U. HCG or 0.0008 mg Buserelin. In this way a pseudopregnancy for 16 - 20 days was caused. On day 21 - one day before the embryotransfer - the recipients and donors were synchronized by application of 120 I.U. HCG.

The other group of the recipients was synchronized with the donor animals by a single injection of 120 I.U. HCG one day before embryotransfer.

19 - 20 hours after natural cover or artificial insemination the embryo collection was performed after slaughtering the donor animals (ROTTMANN, 1978; ADAMS, 1982).

The embryos were flushed out of the oviducts by BSM-II-medium (Gibco). Fertilized oocytes with visible pronuclei were microinjected. After microinjection of 1 - 2 pl DNA-solution (1000 copies/pl) the zygotes were cultivated in BSM-II-medium and classified after one hour.

The transfer of microinjected, vital zygotes was performed by laparotomy in the linea alba by use of Rompun-Ketamin-anaesthesia (CHANG and PICKWORTH, 1969). After eventration of the ovaries and oviducts the corpora rubra, cysts and corpora lutea were counted. After that, the embryos were deposited into the left and right oviduct by use of a curved transferpettor-cap which was connected with a tube and a syringe.

Results

Of 214 superovulated donors 23,2 oocytes in average were obtained. 18,4 oocytes in average were fertilized. Per animal 22,3 fertilized oocytes were obtained from cross-breeding animals, 17,9 from ZIKA-hybrids and 13,9 from Californian rabbits.

Table 1: Influence of the race on the superovulation results

race	no. donor animals	no.oocytes per animal	no. fertilized oocytes per animal	maximum no. embryos
cross-breeding animals	48	28,0	22,3	71
ZIKA-rabbit-hybrids	142	22,8	17,9	122
Californian rabbits	24	16,4	13,9	47
Sum	214	23,2	18,4	

In experimental period I (1985/86) 12 (23%) of 52 non presynchronized recipients and 9 (43%) of 21 presynchronized recipients got pregnant.
In experimental period II (1987/88) 1 (12%) of 12 non presynchronized and 49 (46%) of 106 presynchronized recipients got pregnant.

Table 2: Influence of the presynchronization on the pregnancy rate

	without presynchronization	with presynchronization
no. recipients I	52	21
no. pregnancies I	12 (23%)	9 (43%)
no. recipients II	12	106
no. pregnancies II	1 (8%)	49 (46%)
total recipients	64	127
pregnancies	13 (20%)	58 (46%)

In experimental period I 15 (41 %) of 37 recipients got pregnant in winter (November, December, January), in spring (February, March, April) no one of 12 recipients, in summer (May, June,

July) 5 (45 %) of 11 recipients and in autumn one (8 %) of 13 recipients got pregnant.

In experimental period II 22 (52 %) of 42 recipients got pregnant in winter, 13 (28 %) of 47 in spring, 14 (52 %) of 27 in summer and 1 of 2 in autumn.

Table 3: Influence of the season on the pregnancy rate

season	winter	spring	summer	autumn
no. recipients I	37	12	11	13
no. pregnancies I	15 (41%)	0 (0%)	5 (45%)	1 (8%)
no.recipients II	42	47	27	2
no. pregnancies II	22 (52%)	13 (28%)	14 (52%)	1
total				
no. recipients	79	59	38	15
no. pregnancies	37 (47%)	13 (22%)	19 (50%)	2 (13%)

The total pregnancy rate in period I was 29 %, whereas it was 42 % in period II.

If less than 20 embryos were transferred to one recipient (group I) 10,7 % of the embryos survived, whereas 6,3 % survived if 20 to 30 embryos were transferred (group II) and 4,5 % survived if more than 30 embryos were transferred to one recipient (group III). The survival rate in total was 5,7%.

Table 4: Embryo survival rate in relation to the no. of transferred embryos (X)

group	I X < 20	II 20 ≤ X ≤ 30	III X > 30	total
no. transferred embryos	326	1222	2011	3559
no. born animals or implanted fetusses	35	77	91	203
survival rate of embryos	10,7%	6,3%	4,5%	

In group I 8 (44 %) of 18 recipients got pregnant whereas in group II 22 (45 %) of 49 and in group III 20 (39 %) of 51 recipients got pregnant.

Table 5: Influence of the no. of transferred embryos (X) on the pregnancy rate

group	I X < 20	II 20 ≤ X ≤ 30	III X < 30	total
no. recipients	18	49	51	118
no. pregnant recipients	8 (44%)	22 (45%)	20 (39%)	50
no. embryos transferred to pregnant recipients	134	536	788	1458
no. born animals or implanted fetuses	35	77	91	203
survival rate of embryos in pregnant recipients	26%	14%	12%	

Discussion

The big differences in the number of the obtained oocytes per superovulated donor show a dependence of the race respectively a heterosis effect in the cross-breeding animals.
Obviously the pregnancy rates are dependent on the seasons. The decrease of the pregnancy rates in spring repeated for three years and there is no satisfactory explanation for this phenomenon.
The presynchronization of the recipients shows an increasing effect on the pregnancy rates because a possible pseudopregnancy at the time of ovulation can be excluded.
In the group of rabbits in which less than 20 embryos were transferred to one recipient the survival rate of embryos is much higher than in the group in which more than 20 embryos were transferred. Maybe a large number of transferred embryos brings along a negative effect on the developmental capacity in the uterus.

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