Acta vet. scand. 1980, *21*, 26—33.

From the Institute for Animal Reproduction, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

EMBRYO TRANSPLANTATION IN CATTLE

NON-SURGICAL RECOVERY OF EMBRYOS FROM REPEAT BREEDERS*

By

Torben Greve

GREVE, T.: *Embryo tranpslantation in cattle. Non-surgical recovery of embryos from repeat breeders.* Acta vet. scand. 1980, *21*, 26—33. — Fourteen true repeat breeders with entirely normal oestrous cyclicity more than 1 year after calving and 14 control donor cows were superovulated with PMSG (2000 i.u.) and flushed non-surgically 6—8 days after the superovulatory heat. The superovulatory response was identical for the 2 groups such as assessed by the number of corpora lutea (9.4 ± 1.8 C.L. per repeat breeder and 9.1 ± 1.5 per control cow), occurrence of ovarian overstimulation (polycysts), presence of a non-countable amount of corpora lutea, negative outcome of the flushings and the number of recovered embryos (5.8 ± 1.0 embryos per repeat breeder and 6.0 ± 1.8 embryos per control cow). The most pronounced difference between the 2 categories of animals was related to the fertilization rate of embryos. In the repeat breeder group only 2.4 embryos per cow or 41 % were fertilized, whereas the control animals attained a fertilization rate of 4.9 embryos or 82 %. Since most factors liable to interfere with the fertilization process were identical for both groups (age, breed, nutritional and management conditions, semen quality, dose, AI-technician e.g.), it is believed that intraovarian, follicular, or follicular-dynamic conditions were responsible for producing a high proportion of non-fertilizable oocytes.

non-surgical embryo transplantation; repeat breeders.

Experiments on the effect of V. fetus organisms in the female reproductive tract indicate that this organism does not interfere with the normal fertilization per se, but impairs or prevents the normal embryonic developmental process, thus resulting in early embryonic mortality and repeat breeding (*Adler* 1959). Newer investigations on the nature of viral infections in the uterus have

* This study was supported by a grant from the Danish Agricultural and Veterinary Research Council.

also established that the primary cause of sterility may be early embryonic degeneration (*Archbald et al.* 1979). Studies of *Ayalon* (1968, 1972) also indicated that in repeat breeder cows free from venereal diseases, with a history of normal calvings, and not suffering from endometritis or any other clinically detectable uterine, ovarian and oviductal disease, reduced fertility is due to early embryonic mortality (e.e.m.) prior to day 13 rather than to actual fertilization failure. In more comprehensive publications *Ayalon* (1964, 1968) reviewed possible causes for e.e.m., and although fertilization failure is more pronounced in repeat breeders than in animals with normal fertility, the most significant difference between these 2 groups is observed on day 6—7 where a high percentage of normal embryos from repeat breeders undergo degeneration (a decrease in normal embryos from 80 % on day 4—5 to 42 % on day 7—8). This degeneration coincides with the differentiation of the embryo (inner cell mass, endoderm and trophoblast), and if the uterine environment is detrimental to the embryos, it should be possible to rescue some of these by egg transplantation, i.e. recovery of 6—7 day old embryos and subsequent transfer to normal, synchronized recipients. Although several investigations (*Bowen et al.* 1978, *Elsden et al.* 1979) have indicated that superovulation and embryo transplantation from infertile cattle (including repeat breeders) are discouraging and probably unproductive from a commercial point of view, the present experiment was undertaken in an attempt to assess the superovulatory response and the quality of 6—8 day old embryos from repeat breeder cows and to compare these with embryos from control cows.

MATERIALS AND METHODS

Fourteen Jersey cows belonging to 2 herds comprised the repeat breeder group. The distance from calving was more than 365 days, and during that period of time the cows exhibited normal oestrus cyclicity and had no apparent signs of endometritis (vulvar discharge). All the cows had returned to heat after being inseminated at least 7 times, and prior to superovulation they were subjected to a detailed gynaecological examination at which no visible or palpatory abnormalities were observed.

The control group consisted of 14 Jersey cows deriving from 1 of the 2 herds, and all with a history of normal reproductive performance.

All the cows were superovulated, inseminated and flushed non-surgically as previously described (*Greve et al.* 1977), and the assessment of embryo quality was based on a stereomicroscopic evaluation (*Greve et al.* 1979).

RESULTS AND DISCUSSION

The main results of the superovulation are given in Table 1. Three repeat breeder cows and 4 control cows developed multiple anovulatory follicles (polycystic) resulting in a fluctuating consistency of the ovaries, a frequency that was also found in a large number of Jersey cows (*Greve* 1980). It is believed that the

Table 1. The superovulatory response in repeat breeders and control cows.

	Estimated number of corpora lutea* in			Number of animals with	
	left ovary	right ovary	left/right ovaries	non-countable corpora lutea	cystic corpora lutea
Repeat breeders (n=14)	3.8±1.0 (n=9)	5.6±1.0 (n=9)	9.4±1.8 (n=9)	4	3
Control cows (n=14)	4.1±0.6 (n=10)	5.0±1.0 (n=10)	9.1±1.5 (n=10)	4	4

* Values indicate mean ± s.e.m.
n = number of animals.

cystic condition developed after the superovulatory heat was a result of continuous stimulation of residual amounts of circulating PMSG (*Schams et al.* 1978). The anovulatory follicles are capable of producing oestradiol-benzoate (*Saumande* 1978) which ultimately may have an adverse effect on the embryo quality (degeneration, retardation). The number of corpora lutea could be assessed with a reasonable degree of certainty in 9 repeat breeders (9.4 ± 1.8 corpora lutea) and in 10 control cows (9.1 ± 1.5 corpora lutea), which is almost identical with the data presented by *Elden et al.* (1979) for repeat breeder cows. The number of corpora lutea was higher in the right than in the left ovary in both groups (5.6 and 5.0 versus 3.8 and 4.1), which was probably caused by a difference in the actual ovarian status at

the time of PMSG stimulation (follicle population, amount of receptors in the granulosa cells e.g.), since most extrinsic factors were constant (breed, time of PMSG treatment e.g.).

The proportion of negative flushings, i.e. recoveries where embryos were not isolated, was identical for both groups, and tubal occlusion was an unlikely cause of infertility. One of the control cows had a copious amount of pus in the recovered flushing medium, although the AI-technician and the stockmaster claimed that the cervical mucus was clear and stringy at the time of heat. This phenomenon has been observed in other instances, and it is possible that subclinical endometritis localized in the anterior part of the uterine horn may not give rise to vulvar discharge. Conversely, several cows with a history of pus-stained mucus at the time of heat have been found free from any cell debris in the flushing medium, and a local cervicitis may be the cause.

The main results of the flushings are given in Tables 2 and 3. There was no difference between the total amount of embryos in the 2 groups (av. 5.8 in repeat breeders versus 6.0 in control cows), which was contrary to the data presented by *Elsden et al.*, in which the fertile groups yielded a significantly higher amount of embryos (9.5/donor) than did the repeat breeder group (3.0/donor). More embryos were recovered from the right than from the left horn in the repeat breeders (3.6 versus 2.2). This observation was not consistent in the control group, but for 192 lactating dairy cows superovulated and flushed during the past 3

Table 2. Evaluation of embryos (day 6—8) from repeat breeders and control cows.

	Number of embryos*					Number of unfertilized eggs	
	total	viable	(%)	retarded and degenerated	(%)		(%)
Repeat breeders (n=11)	5.8±1.0	2.1±0.7	(36)	0.3±0.5	(5)	3.4±1.0	(59)
Control cows (n=13)	6.0±1.8	3.1±1.1	(52)	1.8±0.8	(30)	1.1±0.4	(18)

* Values indicate mean ± s.e.m.
n = number of animals.

Table 3. Embryo distribution between left and right uterine horn in repeat breeders and control cows.

	Number of embryos*							
	total		viable		retarded and degenerated		Unfertilized eggs	
	left	right	left	right	left	right	left	right
Repeat breeders (n=11)	2.2±0.6	3.6±1.0	0.6±0.3	1.5±0.6	0.1±0.1	0.2±0.1	1.5±0.7	2.0±0.8
Control cows (n=13)	3.3±1.0	2.6±0.9	1.7±0.5	1.4±0.7	1.1±0.4	0.5±0.2	0.6±0.3	0.8±0.2

* Values indicate mean ± s.e.m.
n = number of animals.

years more embryos have been recovered from the right (3.1/donor) than from the left (2.4/donor) uterine horn, and more ovulations occurred in the right (5.3/donor) than in the left (4.8/donor) ovary (*Greve et al.* 1979).

The number of viable embryos was lower in the repeat breeder group (2.1/donor) than in the control group (3.1/donor), but this difference was not nearly so pronounced as found by *Elsden et al.* where only 1.0 fertilized embryo was recovered from infertile cows in contrast to 6.8 fertilized embryos for fertile cows.

Although the criteria for embryo evaluation were identical for the 2 groups, it was still based on subjective, non-specific morphological assessments, and it is possible that apparently normally developed viable embryos (compacted morula, early expanding and expanded blastocysts) from the repeat breeders might possess defects detectable only by transmission-electron-microscopic studies.

The average fertilization rate of embryos was very low in the repeat breeders (41 %) in comparison with the rate in the control cows (82 %). The number of unfertilized eggs was 3.4/donor for repeat breeders and only 1.1/control donor, which indicated that fertilization failure per se rather than early embryonic mortality contributed to the repeat breeding in superovulated repeat breeders.

The reason for the difference in fertilization failure is more difficult to explain, since several extrinsic factors known to affect fertilization were constant, i.e. superovulatory treatment, age and lactational status of the animals, management, type, dose and quality of semen, AI-technician, and time of AI in relation to occurrence of heat.

Although the bacteriological status of the uterine flushings was not known, uterine infection is not generally believed to interfere with the normal fertilization procedure, whereas it is known to provoke embryonic degeneration (*Adler* 1959, *Ayalon* 1978).

The actual cause of fertilization failure in these repeat breeders can not be fully assessed, but certain morphological and biochemical defects in the production of oocytes may have resulted in a higher proportion of oocytes that could not be fertilized, and if fertilized, could produce embryos of inferior quality liable to stop their development or to degenerate at an early stage.

Two apparently normal 6½ day old embryos from 1 of the repeat breeders were transferred surgically to 2 synchronized recipient heifers of which 1 became pregnant and delivered a normal calf. This result indicated that it was possible to produce offspring from a repeat breeder by transferring a viable embryo from the uterine environment of a donor to a recipient with normal utero-ovarian functions. Several commercial embryo transfer units have been using this procedure in cows and have found it less profitable (*Elsden et al., Hasler* 1979, *Vincent* 1979, 1980). Since repeat breeding may have a hereditary basis, such an approach should probably be omitted in repeat breeder heifers.

This study has indicated that the superovulatory capacity of repeat breeders was identical with that of normal control cows. The main difference was found in the number of embryos which was significantly lower in the repeat breeders than in the control cows. Several influencing factors, such as infections, management, semen quality, insemination technique and time, known to influence the normal fertilization procedure and early embryonic development (*Ayalon* 1978), could be excluded as causes of this failure. Further studies on the ovarian morphology, mechanism of ovulation, gamete transport, uterine environment, and genetics will be required in order to find probable causes.

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SAMMENDRAG

Embryotransplantation på kvæg.
Ikke-kirurgisk opsamling af embryoner fra omløberkøer.

Fjorten regulære omløbere, d.v.s. køer som viste regelmæssig brunst efter kælvning, som ikke havde vist kliniske tegn på endometritis, og som var insemineret forgæves mere end 7 gange, blev

sammen med 14 kontrolkøer superovuleret med PMSG og skyllet ikkekirurgisk 6—8 dage efter den superovulerede brunst. Superovulationssvaret målt ud fra antallet af ovulationer (9,4 ± 1,8 C.L. for omløbere og 9,1 ± 1,5 for kontroldyrene), forekomst af polycystiske ovarier (3 for omløbere og 4 for kontrolkøer) og ovarier med et stort antal corpora lutea (4 i hver gruppe), samt ved antallet af identificerede embryoner (5,8 ± 1,0 embryon pr. omløber og 6,0 ± 1,8 embryon pr. kontrolko) var ikke forskelligt for de 2 kategorier af dyr. Derimod blev der observeret en meget betydelig forskel i befrugtningsprocenten af embryoner (41 % for omløbere og 82 % for kontrolkøer). De fleste af de faktorer, som kan påvirke den normale befrugtningsprocent, var identiske for de 2 grupper, og det er muligt, at dybtgående forstyrrelser i dyrenes endokrine og follikeldynamiske processer var medvirkende årsager til den lave fertilitetsprocent hos omløberne.

(Received April 25, 1979).

Reprints may be requested from: T. Greve, the Institute for Animal Reproduction, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Copenhagen V, Denmark.