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KARYOTYPES AND MORPHOLOGICAL AND HISTOLOGICAL ALTERATIONS OF THE GENITAL TRACTS OF REPEAT BREEDER HEIFERS WITH KNOWN BREEDING HISTORY

By

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GUSTAFSSON, H., K. LARSSON and I. GUSTAVSSON: *Karyotypes and morphological and histological alterations of the genital tracts of repeat breeder heifers with known breeding history.* Acta vet. scand. 1985, *26*, 1—12. — Forty-two repeat breeder heifers (RBH) and 27 virgin heifers (VH) were used in the study. The breeding history and the occurrence of chromosomal aberrations were investigated in the RBH. The genital tracts of the RBH and the VH were investigated at slaughter 3 to 35 days after artificial insemination and/or embryo transfer for the presence of morphological and histological changes. According to AI data the mean number of inseminations per RBH in the herd of origin was 4.9 and 110 interservice intervals out of 141 calculated were normal (17—24 days) or twice normal (34—48 days). Two RBH out of 28 investigated demonstrated the 1/29 translocation. The post mortem examination revealed corpus lutea and follicles normal for the stage of the cycle in all animals. In three RBH abnormalities of the uterus or cervix were found. A higher incidence of cystic glands and of focal accumulation of erythrocytes and lymphocytes in the endometrium was found in the RBH than in the VH ($P \leq 0.05$). The relation between the embryonic survival and the histological alterations of the uterus was not significant.

It is suggested that errors in heat detection, abnormal karyotypes and morphological defects of the genital tract play a minor role in the repeat breeding complex in heifers.

embryonic death; chromosomal aberrations; genital tract pathology; cattle; AI data.

A number of factors influence the conception rates in cattle (for reviews see *Laing* 1952, *Boyd* 1965, *Ayalon* 1981). Such factors will affect either ovum fertilization or embryonic survival. In repeat breeder animals fertilization failure and/or embryonic

death are elevated (*Tanabe & Almqvist* 1953, *Hawk et al.* 1955, *Ayalon* 1981, *Linares* 1981, *Gustafsson & Larsson* 1984).

Generally a repeat breeder is defined as a clinically normal female with a series of infertile services at normal intervals (*Tanabe & Casida* 1949). The importance of strict adherence to these criteria has been emphasized by *O'Farrel et al.* (1983) who found only 45 % normal interservice intervals when evaluating the breeding history in a group of cows culled as repeat breeders. A normal conception rate was found in these cows after an additional insemination. Similar findings have been reported from other experiments in which no comment is made on interservice intervals (*de Kruif* 1976, *Dawson* 1977, *Refsdal* 1979). This provides evidence that errors in oestrus detection may substantially contribute to repeat breeding in cattle.

The importance of chromosomal aberrations for the reproductive performance of domestic animals has been reviewed by *Gustavsson* (1980). Varying incidences of chromosome aberrations in repeat breeder animals have been reported (*Gustavsson* 1971, *Refsdal* 1979, *King & Linares* 1983, *Swartz & Vogt* 1983).

Functional disturbances such as ovulation failure and anatomical alterations of the genital tract which might prevent the union of the sperm and the ovum are causes of repeat breeding reported in clinical and abattoir surveys (*Tanabe & Casida* 1949, *Graden et al.* 1968, *de Kruif* 1976, *Dawson* 1977, *Refsdal* 1979).

The uterine environment is important for normal embryonic development. Under conditions when specific genital infections can be ruled out an improper uterine environment might be caused by the presence of low-grade non-specific infectious agents. Other possible alterations of the uterine environment involve abnormal endometrial secretions. Histopathological changes of the endometrium as indication of subclinical endometritis and other pathological alterations have been found in different proportions of repeat breeder animals (*Moss et al.* 1956, *Simon & McNutt* 1957, *Graden et al.* 1968, *Hartigan et al.* 1972, *Griffin et al.* 1974, *Dawson* 1977).

The objective of the present study was to evaluate the breeding history of a group of heifers culled as repeat breeders and to report the incidences of chromosomal aberrations and morphological and histological changes of the genital tract in this group of animals.

MATERIAL AND METHODS

Forty-two repeat breeder heifers (RBH) which had failed to conceive after 3 or more inseminations were bought from different herds around Uppsala from January 1981 to October 1983. During the same time 27 sexually mature but not previously inseminated virgin heifers (VH) were bought. The animals were all dairy heifers of the Swedish Red and White breed (RBH:36, VH:13), Swedish Friesian (RBH:4, VH:10), and crosses between these 2 breeds (RBH:2, VH:4). The heifers were housed in the veterinary clinic during an experimental period of approximately 5 months and were thereafter slaughtered. The mean age of the RBH animals at slaughter was 33 months (range 25—36) and of the VH 22 months (range 19—25).

The number of inseminations and the interservice intervals in the RBH in their herds of origin were calculated on available AI data.

Karyotype analysis was performed in 28 RBH. Cultures of peripheral blood lymphocytes were set up (*Moorhead et al.* 1960) and harvested in the conventional way. Giemsa-stained chromosomes were counted and morphologically studied in at least 50 cells of each animal. Three cells per animal were stained according to the RBA technique (*Dutrillaux et al.* 1971) as applied by *Gustavsson & Hageltorn* (1976) and analysed. When a possible deviating banding pattern was suspected the number of RBA banded karyotypes analysed was increased and sometimes complemented with analysis of GTG-banded (*Seabright* 1971) chromosomes until conclusive results could be obtained.

Endometrial tissues for histological examination were taken by biopsy from 24 RBH shortly after their arrival at the clinic. The specimens were taken during the luteal phase from the distal part of one uterine horn using Folmer Nielsen's instrument (*Rasbech* 1950).

During the course of the experiment the animals were subjected to artificial insemination (AI) and non-surgical embryo collection 7 days after AI on average three times. Finally most of the animals were used as recipients in a reciprocal embryo transfer experiment. These animals were inseminated and 7 days after AI an embryo was transferred to the uterine horn contralateral to the corpus luteum bearing ovary. The recipients were slaughtered either 16—17 days or 32—35 days after AI. Heifers not used as recipients for embryo transfer were inseminated and

slaughtered 3—17 days after AI. The design of the experiment has been described in detail by *Gustafsson* (1984) and *Gustafsson & Larsson* (1984).

Soon after stunning and bleeding the reproductive tract was removed from the animal and examined carefully for anatomical abnormalities. The ovaries were weighed and the diameter of the largest corpus luteum (CL) and follicle visible on the surface were measured. The oviducts were dissected free and embryos were collected by flushing the oviducts and the separated uterine horns over petri dishes (3—17 days after AI) or by careful dissection of each horn (32—35 days after AI). Tissues were taken from 41 RBH and 23 VH from the midportion of the uterine horns at slaughter. The endometrial specimens were dehydrated according to standard methods, embedded in paraffin, cut transversely in 5 μm sections and stained with hemotoxylin-eosin. The sections were evaluated microscopically for the presence of histological alterations. The evaluation was done so that the identity of the specimen was unknown to the investigator.

Conventional statistical methods were used for the evaluation of data.

RESULTS

Service intervals and oestrus

The number of services and interservice intervals in the RBH are given in Table 1. Accurate service dates were not available for 3 heifers. Eleven interservice intervals were excluded from the calculations due to prostaglandin treatments during the cycle. The number of inseminations per animal ranged between 3 and 7 with an average of 4.9 per animal. Two and 6 interservice inter-

Table 1. Number of inseminations and intervals between inseminations for repeat breeder heifers.

Number of inseminations	Number of heifers	Number of intervals	Insemination intervals (days)				
			< 17	17—24	25—33	34—48	> 48
3	4	8	0	4	0	4	0
4	10	30	1	19	0	6	4
5	14	53	0	26	2	11	14
6	8	34	0	26	2	3	3
7	3	16	1	9	2	2	2
Total	39	141	2	84	6	26	23

vals respectively were shorter than 17 days or between 25 and 33 days. Eighty-four intervals were normal, 26 were 34—48 days and the remaining 23 more than 48 days. Oestrus was observed in all animals except in one RBH within 24 days after their arrival at the clinic.

Karyotypes

Two cases demonstrated the 1/29 translocation, but the remaining RBH appeared o have a normal karyotype even when the blanding techniques were applied (Fig. 1). The chromosome counts deviated (most often hypodiploid) from the expected ones in approximately 5 % of the cells analysed, but this variation was assumed to be artefactual since it appeared to randomly concern the chromosomes of the karyotype.

Post mortem findings

The day of cycle at slaughter, the weights of the ovaries and the sizes of the CL and the largest follicle are shown in Table 2. The weights of the CL bearing ovary in the RBH slaughtered 3 days after insemination and of the ovary contralateral to the CL in the RBH slaughtered 32—35 days after insemination were significantly greater than of the corresponding ovaries in the VH ($P \leq 0.01$). The size of the CL as well as of the largest follicle varied considerably both within and between the selected stages of the oestrous cycle (Table 2). No differences was seen between RBH and VH in this respect.

Table 2. Gross morphology of ovaries from repeat breeder (RBH) and virgin heifers (VH) slaughtered 3—35 days after insemination.

Heifer category	Day of cycle	n	Weight of ovaries (g)		Size of the Cl (mm) $\bar{x}$, range	Size of the largest follicle (mm) $\bar{x}$, range
			Cl bearing ovary	Ovary contralateral to Cl		
RBH	3	10	6.8[a]	4.9	12 (5—20)	8 (5—12)
VH	3	4	5.3[b]	3.9	8 (5—10)	9 (5—11)
RBH	7	3	10.4	5.2	26 (22—30)	19 (5—20)
RBH	16—17	13	10.2	5.1	23 (18—25)	12 (4—15)
VH	16—17	9	9.8	4.5	22 (18—25)	12 (10—15)
RBH	32—35	10	9.9	5.5[a]	20 (12—25)	12 (10—15)
VH	32—25	7	8.4	4.0[b]	22 (20—25)	12 (6—15)

[a, b] Means with different superscripts differ significantly ($P \leq 0.01$).

Macroscopic abnormalities were found in the uterus or cervix in 3 out of 42 RBH, while no abnormalities were found in the 27 VH. The abnormalities found were uterus unicornis, duplication of cervix and the uterine body (uterus didelphys) and cervical diverticulum. In the latter case a fluctuating structure with a diameter of approximately 4 cm was palpable during oestrus but was not evident during dioestrus. Efforts to pass this structure with an insemination gun were unsuccessful. At necropsy a dilatation was found in the region between the two proximal cervical folds, filled with retained mucus. The cervical canal through the dilatation was not obstructed but extremely difficult to pass.

The results of the histological examination at necropsy are shown in Table 3. Cystic glands were detected in specimens from eight RBH and one VH. Diffuse cellular reactions were also ob-

Table 3. The incidence of uterine histopathological alterations in repeat breeder (RBH) and virgin heifers (VH).

Category of animals	No. of animals sampled	Cystic glands	Cystic glands + diffuse cellular reaction	Focal cellular reaction	Total alterations (%)
RBH	41	4	4	2	10 (24)[a]
VH	23	1	0	0	1 (4)[b]

[a, b] The difference is significant ($P \leq 0.05$).

served in the region of the cystic glands in four RBH. The cystic glands had a wide lumen and a low cuboidal or flattened epithelium. Layers of fibrous connective tissue were observed around some cystic glands in six specimens (Fig. 2). Only a limited number of cystic glands were observed in each of the sections. Focal accumulations of lymphocytes, eosinophiles and neutrophiles were found in specimens from two RBH. Two foci were found in each section, with an additional infiltration of cells in the peripheral parts of the endometrium. In total the incidence of histological alterations was significantly higher in RBH than in VH ($P \leq 0.05$).

The biopsies from the RBH taken in vivo were usually very small and consisted mostly of the most peripheral part of the endometrium. No histological abnormalities were found in the sections from these biopsies.

The histopathological findings in relation to the occurrence of embryos are shown in Table 4. Morphologically normal embryos were found in 35 uterine horns. In sections from 4 of these horns cystic glands were found. In sections from 6 out of 22 uterine horns with no or abnormal embryos histological alterations were found. Focal cellular reaction was found in 2 heifers with degenerated fetal membranes. From 1 heifer with cystic glands a retarded 17-day-old blastocyst was recovered. In the remaining 3 heifers, all with cystic glands, no embryos at all were found. The relationship between the embryo survival and the occurrence of histological alterations was not significant.

Table 4. The incidence of uterine histopathological alterations in relation to the embryonic survival to day 16—35.

Embryonic status	No. of uterine horns sampled	No. of uterine horns with alterations (%)
Normal embryo	35	4 (11)
Abnormal or no embryo	22	6 (27)

DISCUSSION

According to the breeding history (Table 1) the incidence of normal and twice normal interservice intervals, the latter most likely representing animals which came in heat at the normal interval but were not inseminated, is nearly 80 %. This indicates that errors in oestrus detection play a minor role as cause of repeat breeding in this group of RBH.

The proportion of animals with 1/29 translocation found in this experiment is comparable to that in another Swedish report (*King & Linares* 1983) but considerably lower than in an early investigation of repeat breeders within the Swedish Red and White cattle population (*Gustavsson* 1971). Cattle carrying the 1/29 translocation are known to have reduced fertility resulting from death of embryos which inherit abnormal karyotypes (*Gustavsson* 1969). Hence it is not surprising to find higher frequencies of the translocation in a group of repeat breeders than in the general population. The decrease in the incidence from 1971 to 1981—1983 apparently reflects the elimination of the 1/29 translocation from the population, achieved by selection according to cytogenetic means (*Gustavsson* 1984). Other chromosomal aberrations generally associated with complete sterility,

which have been reported from other countries in surprisingly high proportions in repeat breeders heifers (*Swartz & Vogt* 1983) could not be demonstrated in this experiment.

The size and the morphology of the CL and the largest follicle at slaughter (Table 2) are in agreement with descriptions by *Rajakoski* (1960), *Greve & Kendrick* (1973) and *Ireland et al.* (1980) and well correlated to the expected time of ovulation. This gives an indication of the accuracy of oestrus detection during the experiment. It also shows that delayed ovulation is unlikely to be the cause of the high incidence of apparently retarded embryos found in the RBH (*Gustavsson* 1984). The greater weights of the ovaries in the RBH than in the VH are most likely due to th higher ages of the RBH (*Hammond* 1927).

Anatomical defects of the genital tract are sometimes very difficult to detect clinically. Many are often detectable only at post mortem examination. The 3 heifers with anatomical defects found in the present experiment were selected from the herds as being clinically normal in the judgement of the AI technicians. However, the abnormalities were apparent at examination in the clinic. The present study indicates that the incidence of anatomical defects is higher in a group of heifers culled as repeat breeders than in virgin heifers. The incidence of anatomical defects in the RBH was also higher than in a slaughterhouse material of Swedish cows and heifers reported by *Alam* (1984). However, the incidence in the present investigation is lower than in other reports concerning repeat breeder heifers (*Tanabe & Almqvist* 1953, *Dawson* 1977, *Refsdal* 1979). Acquired defects, e.g. adhesions of the ovarian bursa and the oviduct, which in many reports constitute the majority of the alterations (*de Kruif* 1977), were not found in the present material. This might be explained by the low frequency of genital infections in Sweden.

The significantly higher incidence of histological alterations in the RBH than in the VH might indicate an improper uterine environment as a cause of repeat breeding. However, non-specific infections and/or inflammatory histological alterations have been found in the uterus both of repeat breeders (e.g. *Hawk* 1958, *Simon & McNutt* 1957, *Hartigan et al.* 1972, *Griffin et al.* 1974, *Dawson* 1977) and fertile cows (*Skjerven* 1956, *De Bois* 1961, *Griffin et* al. 1974). This suggests that moderate inflammatory lesions marginally predispose to the repeat breeding syndrome. Furthermore it is not possible to evaluate whether the inflamma-

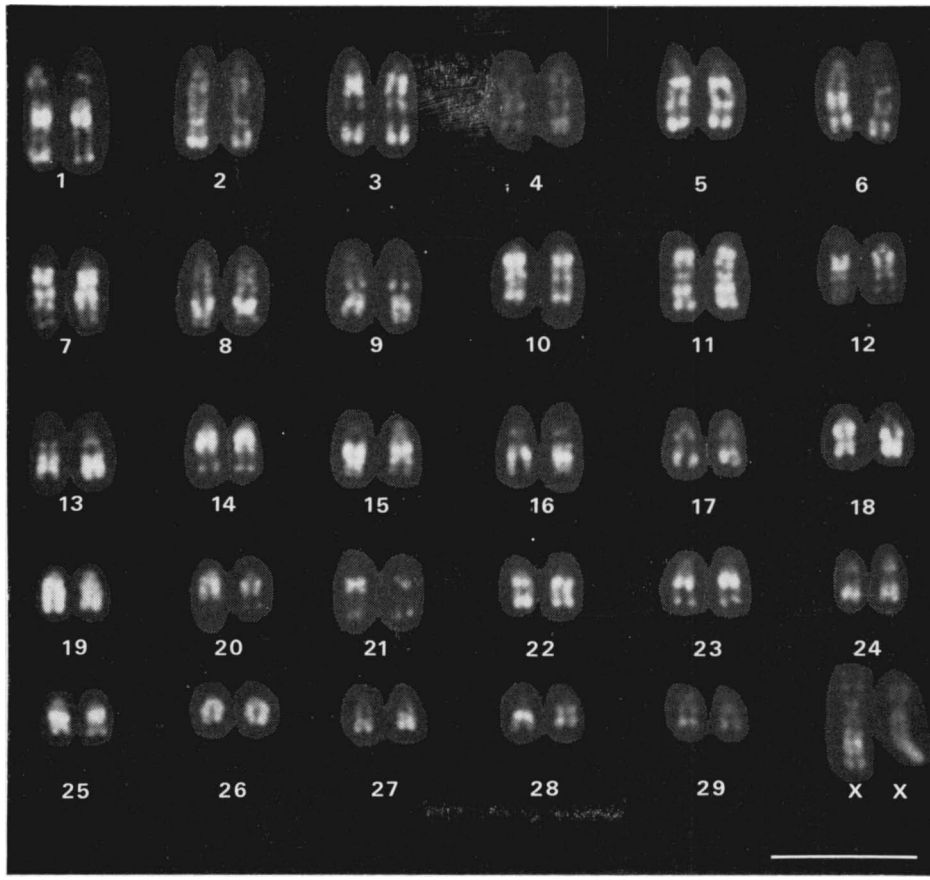

Figure 1. Representative RBA banded karyotype of a RBH classified as normal from the cytogenetical point of view. The cromosomes have been arranged as far as possible according to international agreements (*Ford et al.* 1980). Scale indicates 10 μm.

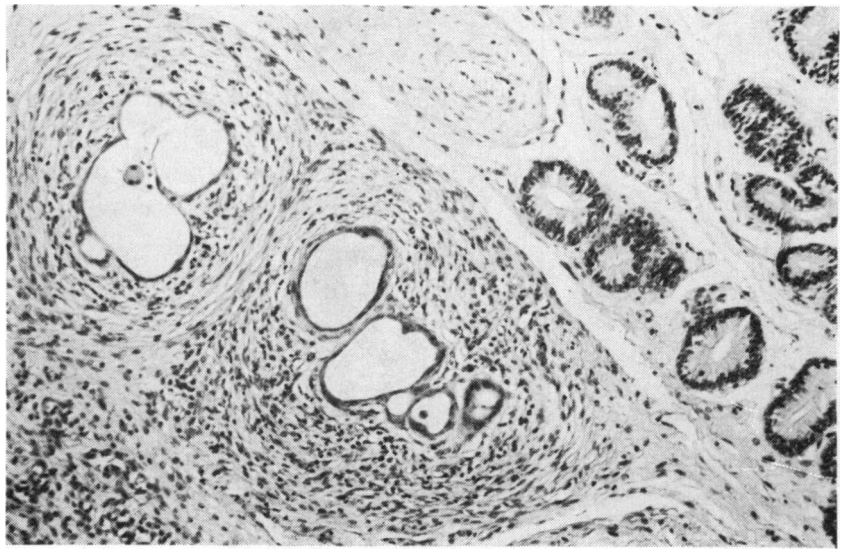

Figure 2. Section of a uterine specimen of a RBH showing normal uterine glands and cystic glands surrounded with connective tissue × 250.

tory reactions found are the cause of or a consequence of embryonic death. Cystic and atretic glands in the uterine mucosa of repeat breeders have been reported by *Moss et al.* (1956), *Simon & McNutt* (1957) and *Graden et al.* (1968) and in cows with cystic ovaries by *Garm* (1949). In the horse cystic distention of uterine glands is a characteristic of chronic degenerative endometritis (*Kenney & Ganjam* 1975, *Rossdale & Ricketts* 1980). It is tempting to speculate that the cystic uterine glands found in the RBH reflect the altered hormonal situation in these animals (*Gustafsson et al.* 1984). The relatively limited extension of the cystic glands in each section and the slight periglandular fibrosis indicate a minor importance of these alterations for fertility (cf. *Kenney* 1975). This is also suggested by the non-significant relation between the embryo survival and the occurrence of histological alterations (Table 4).

In conclusion the present study shows that the repeat breeding complex in heifers may be explained to a minor extent by morphological defects of the uterus and by abnormal karyotypes. Positive clues to the mechanism of the remaining embryonic loss in repeat breeder heifers could not be found.

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SAMMANFATTNING

Karyotyper och morfologiska och histologiska förändringar i könsorganen hos omlöparkvigor med känt antal inseminationer och inseminationsintervall.

Kvigor utslagna från besättningar på grund av symtomlös omlöpning (42 st) och förut ej inseminerade kvigor undersöktes enligt följande. Omlöparkvigornas inseminationer och inseminationsintervall i ursprungsbesättningarna beräknades från AI data. Omlöparnas karyotyper bestämdes. Könsorganen från båda kvigkategorierna undersöktes vid slakt, 3—35 dagar efter insemination och embryo transfer, avseende morfologiska och histologiska förändringar.

Omlöparna befanns vara inseminerade i genomsnitt 4.9 gånger med till största delen (ca 80 %) normala eller dubbelt normala intervall. Två av 28 undersökta omlöpare var heterozygota för 1/29 translokation. Alla kvigor hade gula kroppar och folliklar i ovarierna som väl överensstämde med cyclusstadiet vid slakt. Hos tre omlöpare sågs anatomiska förändringar i livmoder och livmoderhals. Cystiskt dilaterade körtlar och fokala ansamlingar av erytrocyter och lymfocyter i endometriet sågs hos en högre andel av omlöparna än hos de förut oinseminerade kvigorna ($P \leq 0.05$). Ingen signifikant relation sågs mellan förekomsten av normala embryon i livmodern och dessa förändringar.

Undersökningen ger vid handen att dålig brunstpassning, felaktiga kromosomuppsättningar och morfologiska förändringar i könsorganen sannolikt spelat en mindre roll för uppkomsten av symtomlös omlöpning i detta material.

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