Characterisation of the Repeat Breeding Syndrome in Swedish Dairy Cattle

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Introduction

Repeat breeding (RB) is a substantial problem in cattle breeding leading to large economic loss for the dairy producer due to more inseminations, increased calving interval and increased culling rates (Bartlett et al. 1986, Lafi et al. 1992). Repeat breeding has been defined as failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities (Zemjanis 1980).

The need for RB, ie a return to oestrus after a mating or artificial insemination (AI), could be caused by either fertilisation failure or embryonic death. Numerous studies have led to the conclusion that in female cattle with normal fertility the incidence of fertilisation failure is approximately 10% and early embryonic death within 3 weeks following fertilisation accounts for approx. 30% leading to a total early pregnancy loss of close to 40% during the first 21 days post AI (Roche 1981). This means that on average 40% females will return to oestrus after each AI or mating. Several environmental factors e.g. nutrition, climate, as well as intrinsic animal factors have been suggested to be the
cause behind this early embryonic loss in cattle (Ayalon 1978, Pope 1988). It has also been proposed that early embryonic loss should be regarded as "normal" due to an early elimination of unfit genotypes (Bishop 1964). During the last 50 years RB has been the object of several investigations (for reviews see eg. Laing 1952, Casida 1961, Ayalon 1984, Hyttel et al. 1996). There are different opinions among scientists about the cause of RB. A number of experiments have reported a higher proportion of embryos with deviated morphology collected from RB animals compared to control animals suggesting an increased embryonic death rate as the cause of RB (Ayalon 1978, Linares et al. 1980, Gustafsson 1985, Albihn 1991). Recently, higher progesterone levels during oestrus in RB heifers compared to control animals have been reported, indicating hormonal deviations as one possible cause of RB (Gustafsson et al. 1986, Båge et al. 1997).

These findings suggest physiological alterations linked to individual animals as a possible cause of repeat breeding. On the other hand, other investigators have reported normal pregnancy rates in repeat breeders when an additional insemination was performed under controlled conditions suggesting management and environmental imperfects as the most important factors behind the RB syndrome (de Kruif 1977, O’Farell et al. 1983). A third theory has been proposed by Hyttel et al. (1996) claiming the RB phenomenon as solely the result of a probability distribution since the same proportion of animals will be pregnant after each AI and there will always be a number of not pregnant individuals after a number of AIs. There are only a few studies analysing risk factors using field data (Hewett 1968, Lafi & Kaneene 1992, Bartlett et al. 1986, Brooks 1998). These studies have found factors such as season, herd size, age and nutrition influencing the incidence of RB. Some of the earlier studies, however, have the weaknesses that the material is restricted to a limited number of herds and animals and that the RB animals are not strictly selected according to the definition: "absence of detectable abnormalities".

The aims of the present study were to investigate factors associated with the RB syndrome both on herd and individual level, based on data on extracted animals strictly defined as repeat breeders from a large data set containing AI-, milk recording-, and disease records.

Materials and methods
Data for this study were from the official national Swedish production-, AI- and disease-recording schemes. Herds with more than 15 cows were eligible for inclusion in the study, and a 20% simple random sample of these herds was taken. Information on all individual cows calving during 1991 in these herds was retrieved and comprised complete identification (herd, breed, cow identification, etc), all dates (birth, calvings, breedings, etc), milk production, and diagnosed diseases, during the entire life-span of the cows. A cow was considered a repeat breeder (RB) if she had at least 3 AI and no subsequent calving or more than 3 AI irrespective of subsequent calving or not, with cows at risk being those with at least one AI. Additionally, cows treated at least once for the chronic reproductive diseases: cystic ovaries, anoestrous, suboestrus, endometritis and pyometra were not considered a RB, according to the traditional criterion of the ailment.

The characteristics of the RB syndrome were studied on 2 levels of aggregation, herd and individual cow. On herd level the outcome of interest was the frequency of RB cows and potential risk factors were herd size, level of milk production and somatic cell counts, age structure, seasonal calving pattern, breed, reproductive management, and disease frequency. Herd
size was number of cows calving in 1991, milk production was the average of daily fat-corrected milk yield recorded at second and third official test-month after calving, and somatic cell count was the average of cell counts in the period 1-150 days in milk (DIM). Cellcounts were adjusted for effects of breed, parity and milk yield as it is routinely done in the Swedish milk recording. The age structure of the herd was characterised by average lactation number and proportion primiparous cows, and seasonal calving pattern by average month of calving. Variables representing reproductive management were average days from calving to first AI, voluntary waiting period, and pre- and post-breeding oestrus detection efficiency. The voluntary waiting period (VWP) for a herd was defined as the number of days postcalving when 10% of the cows had received their first AI. From that date, 4 periods of 21 d (equivalent to one oestrus period) were defined. A fifth period covered the VWP +85 days up to 200 DIM. The prebreeding estrus detection efficiency (PREDE) for periods 1 through 4 was calculated as the number of first AI during the period divided by the number of cows available for AI:

\[
\text{PREDE}_w = \frac{n_w}{\sum_{i=1}^{s} n_i}
\]

where \( \text{PREDE}_w \) = estrus detection efficiency in period \( w \), and \( n_w \) = number of cows inseminated in period \( w \) (\( w=1 \) to 4)

The PREDE of the herd was calculated as the mean of \( \text{PREDE}_1 \) and \( \text{PREDE}_2 \). Postbreeding estrus detection efficiency was based on the interval between first and second AI, and calcu-

| Table 1. Descriptive statistics (median with inter-quartile range within parenthesis) for herds classified in thirds according to frequency of repeat breeder (RB)a. |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Variable                                      | RB-class 1 (n=489) | RB-class 2 (n=527) | RB-class 3 (n=525) |
| RB %                                          | 2.4             | 7.5             | 15.4             |
|                                               | (0-3.8)         | (5.9-9.1)       | (12.5-19.0)      |
| Herd size                                     | 31              | 30              | 28               |
|                                               | (26-40)         | (23-42)         | (21-37)          |
| Herd average FCM23b                          | 27.8            | 27.9            | 28.0             |
|                                               | (25.8-29.8)     | (26.2-29.9)     | (25.9-29.9)      |
| Herd average days from calving to 1st AI      | 82.6            | 79.3            | 77.1             |
|                                               | (75.4-90.4)     | (73.3-87.2)     | (71.1-83.9)      |
| Herd incidence reproductive disorders         | 0.04            | 0.04            | 0.04             |
|                                               | (0.00-0.07)     | (0.00-0.07)     | (0.00-0.08)      |
| Voluntary waiting period                      | 55              | 55              | 53               |
|                                               | (50-61)         | (50-59)         | (48-58)          |
| Prebreeding estrus detection efficiency       | 0.51            | 0.53            | 0.55             |
|                                               | (0.39-0.61)     | (0.43-0.63)     | (0.45-0.65)      |
| Postbreeding estrus detection efficiency      | 0.57            | 0.58            | 0.58             |
|                                               | (0.43-0.70)     | (0.45-0.70)     | (0.47-0.69)      |

\( ^a \) Thresholds used were 5.0% and 10.7%, respectively

\( ^b \) Average of daily fat-corrected milk yield recorded at 2nd and 3rd official test-month after calving

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lated as number of intervals of 18 to 24 days divided by number of intervals of 18 to 48 days. The herd incidence of diseases, grouped into mastitis, reproductive, digestive, and all other diseases, respectively, was calculated as the number of cows treated at least once by a veterinarian for the disease divided by number of cows in the herd.

Effects of risk factors on the herd frequency of RB cows were studied by logistic regression, using the SAS macro GLIMMIX (Littell et al. 1996) with a logit link. It was developed by backward stepwise elimination of non-significant (p>0.05) 2-factor interactions and main effects. A generalised linear mixed model with logit

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Table 2. Descriptive statistics (frequencies (%), or medians with inter-quartile ranges within parenthesis) for cows classified as repeat breeder (RB+) and not repeat breeder (RB-).a.

| Variable | RB+ (n=3,436) | RB- (n=34,051) |
|----------|---------------|----------------|
| Breed:  |               |                |
| Swedish Red and White Cattle | 1,973 | 18,976 |
| Swedish Fresian Cattle | 1,299 | 13,179 |
| Other or cross-bred | 164 | 1,896 |
| FCM23b | 28.4 (24.7-32.8) | 28.0 (24.1-32.2) |
| Lactation number: | | |
| 1 | 1,299 | 12,420 |
| 2 | 892 | 9,300 |
| 3+ | 1,245 | 12,331 |
| Parturition: | | |
| Normal or not known | 3,211 | 32,357 |
| Difficult or dystocia | 159 | 1,065 |
| Twins (normal parturition) | 66 | 629 |
| Stillbirth: | | |
| No | 3,261 | 32,677 |
| Yes | 175 | 1374 |
| Season at 1st service: | | |
| January | 439 | 3,293 |
| February-March | 666 | 6,263 |
| April-September | 701 | 9,282 |
| October-December | 1,630 | 15,213 |
| RB in previous lactation: | | |
| No | 2,916 | 28,802 |
| Yes | 194 | 1,255 |
| not known | 326 | 3,994 |
| Days in milk at 1st service | 68 (58-83) | 75 (63-93) |
| Veterinary treatedc cases of: | | |
| Clinical mastitis | 5.5 | 7.1 |
| Reproductive disorder | 4.5 | 3.8 |
| Digestive disorder | 6.7 | 6.6 |
| Other disorder | 0.9 | 0.8 |

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a The sum of number of cows is less than the total (57,616) since only cows with at least one AI were at risk for RB and some had missing information on explanatory variables.
b Average of daily fat-corrected milk yield recorded at 2nd and 3rd official test-month after calving.
c Only treatments occurring before 1st service.
link and accounting for herd-level variation by including a random effect of herd, was used to study the individual animal risk for RB. In addition to herd, the following potential explanatory variables were considered: breed, milk production, lactation number, calving performance, stillbirth, season at first service, RB in previous parity, DIM at first service, and veterinary treated cases of mastitis, reproductive, digestive, and all other diseases that occurred before the first service.

For this model, we also used the SAS macro GLIMMIX (Littell et al. 1996), and it was developed by backward stepwise elimination of non-significant (p>0.01) 2-factor interactions and main effects.

**Results**

The original dataset consisted of 68,117 animals, and after initial editing, the total material consisted of 57,616 cows in 1,541 herds. In 19,781 (34%) animals no further calving was recorded. Of these animals 9,697 were inseminated and 2615 (13%) were recorded pregnant. A total of 153 animals (0.3%) were inseminated 3 times or more, recorded pregnant and culled

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**Table 3. Final logistic regression model for herd frequency of repeat breeder cows**

| Variable                                      | β     | SE(β) | Prob.  |
|-----------------------------------------------|-------|-------|--------|
| Intercept                                     | -0.468| 0.347 | n.a. a |
| Herd size                                     | -0.023| 0.008 | n.a.  |
| Herd average log_{10}SCCb                     | -0.155| 0.276 | n.a.  |
| Herd average days from calving to 1st AI      | -0.019| 0.002 | >0.001 |
| Herd incidence clinical mastitis              | 0.594 | 0.209 | >0.004 |
| Herd incidence reproductive disorders         | -0.501| 0.239 | >0.036 |
| Herd incidence other vet. treated disorders   | 1.313 | 0.655 | >0.045 |
| Interaction herd size*log_{10}SCC             | 0.016 | 0.008 | >0.038 |

a not applicable

b Somatic cell count (SCC) was the average of log_{10} cell counts recorded, and adjusted for effects of breed, parity and milk yield, at official test-month in the period 1-150 days in milk

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**Figure 1**. Herd frequency of repeat breeder (RB) cows in relation to herd size and herd average log_{10}SCC as estimated from a logistic regression analysis (values given correspond to 25, 50 and 75 percentiles, respectively).
due to fertility reasons. Some descriptive statistics for herds and individual cows classified RB and not RB are given in Tables 1 and 2 respectively. The overall frequency of RB animals was 10.1%. The median proportion RB animals in the 1,541 herds studied was 7.5%. The median for the worst third of the herds was 15.4%.
The associations between risk factors and the herd frequency of RB animals in herds are shown in Table 3 and graphically in Figs. 1 and 2. The incidence of RB animals in herds increased with decreased herd size, decreased herd average somatic cell count, decreased herd average days from calving to first AI and decreased herd incidence of reproductive disorders but with increased herd incidence of clinical mastitis and increased herd incidence of other veterinary treated disorders. The association between herd size, SCC and frequency of RB is graphically exemplified in Fig. 1, showing that the frequency of RB is increasing by increased SCC and the increase is greater in larger herds than in smaller. As shown in Fig. 2 the frequency of RB increases by a decreasing CFI.
The risk of becoming a RB animal was positively correlated to lactation number, dystocia,
Table 4. Parameter estimates for the final generalised linear mixed model for the individual animal risk of repeat breeder

| Variable | β     | OR (95% confidence intervals) |
|----------|-------|-----------------------------|
| Intercept| -3.034| n.a. b                      |
| FCM23<sup>c</sup> | 0.030 | 1.28 (1.20-1.36)          |
| Lactation number: | | |
| 1      | 1.375 | n.a.                        |
| 2      | 0.629 | n.a.                        |
| 3+     | 0     | n.a.                        |
| Parturition | | |
| Normal or not known | -0.119 | 0.89 (0.69-1.14) |
| Difficult or dystocia | 0.301 | 1.35 (1.00-1.82) |
| Twins (normal parturition) | 0     | 1                           |
| Season at 1st service: | | |
| January | 0.177 | 1.19 (1.07-1.33) |
| February-March | -0.035 | 0.97 (0.88-1.06) |
| April-September | -0.259 | 0.77 (0.70-0.85) |
| October-December | 0     | 1                           |
| RB in previous lactation: | | |
| No | 1.084 | n.a.                        |
| Yes | 1.414 | n.a.                        |
| not known | 0     | n.a.                       |
| Days in milk at 1st serviced | -0.016 | 0.62 (0.59-0.66) |
| Veterinary treated e case of reproductive disorder | | |
| Yes | 0.254 | 1.29 (1.09-1.52) |
| No | 0     | 1                           |
| Interaction lactation number*RB in previous lactation | | |
| 1 No | -1.145 | 3.72 (2.65-5.23) |
| 1 Yes | -1.270 | 4.57 (2.84-7.34) |
| 1 not known | 0     | 3.96 (2.76-5.67) |
| 2 No | -0.631 | 2.95 (2.10-4.14) |
| 2 Yes | -0.699 | 3.84 (2.54-5.80) |
| 2 not known | 0     | 1.88 (1.22-2.88) |
| 3+ No | 0     | 2.96 (2.11-4.14) |
| 3+ Yes | 0     | 4.11 (2.76-6.14) |
| 3+ not known | 0     | 1                           |

<sup>a</sup> Odds ratio
<sup>b</sup> not applicable
<sup>c</sup> Average of daily fat-corrected milk yield recorded at 2<sup>nd</sup> and 3<sup>rd</sup> official test-month after calving; OR evaluated at an interquartile range of 8 kg
<sup>d</sup> OR evaluated at an interquartile range of 30 days
<sup>e</sup> Only treatments occurring before 1st service

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RB in previous lactation and reproductive disorder before the first AI and negatively correlated to days in milk at first service. The risk of becoming a RB was higher for cows first serviced during winter compared to other seasons (Table 4). The estimated incidence of RB was higher for cows in all parities being a RB in the previous service period (Fig. 3).

Discussion
A true calculation of the incidence of RB in a population based on field data is not possible due to a number of record errors, e.g. the fact that not all animals are given the chance of getting 3 inseminations. Animals subjected for the present study were selected among animals calved during 1991 which means that heifers inseminated but not calved are not present in the material. Hence, heifers culled as RB will not contribute to the material which may lead to the fact that estimated frequencies and effects rather may be under- than overestimated. Furthermore, we are not able to characterise RB heifers in which reproductive physiological factors may be more important than environment and management factors.

A further problem is that based on the definition per se a RB animal should not have any other disorders that can explain the pregnancy failure. Reproductive disorders such as cystic ovaries, anoestrus, and chronic endometritis increase the risk of pregnancy failure. To avoid these errors, animals treated for these disorders after the start of the service period were not included in the present study of individual risk factors.

The overall incidence of RB animals of 10.1% obtained in the present study corresponds to the mean of 10% reported by Hewett in an Swedish survey 30 years earlier (Hewett 1968). The fact that 15.4% of the cows were a RB in the worst third of the Swedish farms and the incidence varied between 14.5% to 36.8% among 22 US herds studied (Bartlett et al. 1986) underline the statement that the RB syndrome is a serious and costly syndrome. A number of risk factors for RB associated to the herd were identified. The finding that the number of RB animals increased by decreased size of the herd is opposite of what was found by Hewett (1968) who explained the negative association with herd size by the assumption that cows received more individual attention from the owner in smaller farms. The shift 30 years later may be due to owner of smaller herds being progressively only part time employed with the animal husbandry while the larger herds have become more aware of fertility management as an important economic factor. The similar oestrus detection efficiency in farms with a high compared to a low proportion of RB animals found in the present study indicates indirectly that individual care does not differ relative to herd size.

When looking at the combined effects of herd size and somatic cell counts there was a positive correlation to RB. This finding is also supported by other studies showing that the same management factors influence both udder health and reproduction (Ekman 1998). Herd incidence of RB increased by increased incidence of clinical mastitis and also by other disorders that needed treatment by a veterinarian. A negative effect of mastitis on conception rates mediated through endotoxins, causing prostaglandin release, has been hypothesised (Cullor 1991). This is supported by a recent study by Scott et al. (1999), in which clinical mastitis occurring after first AI caused a 50% reduction in pregnancy.

The risk of RB increased with a decreased interval from calving to first AI both on herd and individual level. It is well known that the conception rate will increase with the interval from calving up to about 60-80 days postpartum and then remain relatively constant (Britt et al. 2002).
This is probably due to a successive progesterone priming by subsequent oestrus which has been shown to increase conception rates (Bullman & Lamming 1978). In spite of the fact that the median CFI for the RB animals was 68 days compared to 75 days for the non-RB animals and thus not being any extreme values, this factor was one of the most significant individual risk factors.

On the individual basis an increasing milk production was found to be one risk factor for being a RB. An increase of daily milk yield of approximately 15 kg FCM increased the risk 1.5 times. This finding is in agreement with other studies (Hewett 1968, Bartlett et al. 1986). As pointed out by Bartlett et al. (1986) the association may in part be due to the dairymen’s willingness to invest more inseminations on high-producing cows before making a decision to cull them. It is, however, reasonable to suggest that a great part of the association between milk yield and RB depends on the vulnerability of the high producing cow to metabolic and endocrine disturbances which in turn will affect conception rates.

In general conception rates are reduced in those animals which have calved for the first time and in cows over 7 years of age (de Kruif 1978). In RB animals a positive correlation between RB and age has been found (Hewett 1968, Bartlett et al. 1986). On the contrary Brooks (1998) found no statistically significant difference in the incidence of repeat breeders occurring in first lactation heifers compared to older cows. In the present investigation there was a higher risk for a first calver of becoming a RB animal than for a multiparous cow. This again may reflect the difficulties of first parity cows to cope with the metabolic demands necessary for the lactation.

According to Swedish AI statistics there is a clear seasonal variation in 56 days non return rates with the lowest conception rates in January and the highest in August. This is also reflected in the present study showing that animals getting their first insemination during January have higher risk of becoming RB animals. In agreement with our findings, Hewett (1968) found the highest incidence of RB in cows calving during autumn and winter (September to February). Bartlett et al. (1986), however, found no significant seasonal distribution of RB in 22 Michigan dairy herds. It is plausible that the decreasing daylight during autumn culminating in December and January in Scandinavia negatively influences the hormonal secretion responsible for the reproductive functions.

There was a positive association between RB and calving difficulties. Dystocia was also the most significant risk factor directly associated with RB in the study by Lafi & Kaneene (1992). It has been well known since long time that problems during parturition lead to a delayed involution of the uterus and a delayed resumption of ovarian functions which in turn causes lower conception rates and longer calving intervals (Morrow et al. 1962).

Cows being RB animals in the previous lactation had a higher risk of becoming RB animals also in the present lactation. This indicates also intrinsic factors coupled to the individual cows as a cause of RB supporting the finding of hormonal disturbances in heifers culled as RB (Gustafsson et al. 1985, Båge et al. 1997). Our finding is, however, contradictory to Brooks (1998) who studied the pregnancy rate in 40 cows from 3 different farms which needed 4 or more services in a lactation to obtain pregnancy. Seventy-three per cent of these held to 3 or less services in the next lactation. Brooks suggested that there is no inherent infertility in the RB cow, also suggested by de Kruif (1977), O’Farrel et al. (1983) and Hyttel (1966).

In conclusion our study shows that RB in cows is a multifactorial problem involving both management factors and environmental factors as
well as factors coupled to the individual cow.

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Sammanfattning

Studier av symtomlös omlöpning hos svenska mjölkkor.

Symtomlös omlöpning, d.v.s. omlöpning hos kor eller kvigor med normala brunstintervall efter minst tre inseminationer med avsaknad av kliniska symtom som förklarar omlöpningarna, är ett kostsamt problem för mjölkbonden. För att undersöka omfattningen av symtomlös omlöpning i svenska mjölkko-besättningar och för att identifiera riskfaktorer analyserades produktions-, AI- och djursjukdata från 57,616 mjölkkor i 1,541 besättningar. Untersökningen gjordes både på besättnings- och djurnivå. Effekten av riskfaktorer på besättningsnivå studerades med hjälp av vanlig logistisk regression, medan riskfaktorer på individnivå studerades med en modell där besättningens slumpmässiga effekt togs hänsyn till.

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