

# Pregnancy Rates in Repeat-breeder Heifers Following Multiple Artificial Inseminations during Spontaneous Oestrus

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**Singh B, Saravia F, Båge R, Rodríguez-Martínez H: Pregnancy rates in repeat-breeder heifers following multiple artificial inseminations during spontaneous oestrus. Acta vet. scand. 2005, 46, 1-12** – Hormonal asynchronies during oestrus, related to the presence of suprabasal plasma-progesterone (P<sub>4</sub>) concentrations and a delayed ovulation, interfere with the fertility of repeat-breeder heifers (RBH). Since tubal dysfunction can occur in connection with hormonal asynchronies and constrained availability of fertile spermatozoa at the time of ovulation, the present study tested the hypothesis that frequent sperm deposition from onset of oestrus to ovulation may improve pregnancy rates in RBH. Five RBH and five virgin heifers (VH; controls) were repeatedly artificially inseminated (AI) at 6 h intervals from onset of oestrus to spontaneous ovulation. Hormone analyses revealed suprabasal P<sub>4</sub> concentrations and a delay in the occurrence of the luteinising hormone (LH) surge, but a normal cortisol profile in RBH. Compared with controls, RBH presented longer interval from onset of oestrus to ovulation, and therefore, received more AIs. Pregnancy rates in RBH reached control levels (60%; NS), indicating that the hypothesis might be correct. Pregnancy rates in VH were below the expected range, presumably attributed to a deleterious influence of the frequent handling. The study suggests that pregnancy rates can be improved in RBH by frequent AI in relation to spontaneous ovulation. However, this practice of repeated manipulations, while seeming not to show adverse effects, lacks practicality for routine use.

*Pregnancy rates; Repeat-breeder; Heifer; AI; Oestrus.*

## Introduction

Successful fertilisation is the result of well-timed sperm-oocyte interactions. Time of insemination and proper sperm transport to the site of fertilisation, within the functional life span of the oocyte, are major prerequisites (Hunter 1994). In cattle, repeat breeding is characterized by low fertilisation rates (Graden *et al.* 1968, O'Farrell *et al.* 1983) and/or early embryonic mortality (Linares 1981a, Gustafsson & Larsson 1985). Such events have been studied in repeat-breeder heifers (RBH), animals presenting hormonal and physiological

asynchronies during oestrus. These include suprabasal plasma-progesterone (P<sub>4</sub>) concentrations, a delayed surge of luteinising hormone (LH), and hence delayed ovulation (Linares 1981b, Gustafsson 1985a, Albiñ 1991a, Båge 2002) during spontaneous oestrus. A higher susceptibility to stress by RBH during oestrus is also suspected to contribute to these hormonal asynchronies, as evidenced by observations that the administration of exogenous adrenocorticotrophic hormone (ACTH) or cortisol during or around oestrus in heifers inter-

feres with the surge of LH (Stoebel & Moberg 1982, Li & Wagner 1983). Hyperactivity of the hypothalamo-adrenal axis and its interaction with the hypothalamo-pituitary axis are well-documented responses to stress (Dobson & Smith 2000). However, despite the bulk of gathered information, conclusive explanations for the etiology of the multifactorial repeat-breeding syndrome have yet to be provided.

Compared with normal virgin heifers (VH), RBH have longer interval from onset of oestrus to ovulation (Gustafsson et al. 1986, Båge et al. 2002a). Therefore, single artificial insemination (AI) following a routine AM-PM schedule may lead to constrained conception due to the shortage of spermatozoa with maintained fertilising ability by the moment ovulation occurs. The concerted contractile activity of the myometrium and myosalpinx plays a role during sperm transport to the site of fertilisation (Hawk 1987) and conditions the environment where fertilisation occurs. An altered tubal function in RBH, because of hormonal imbalance, may impair conditions for sperm transport and/or fertilisation (Båge et al. 2002b).

In ensuring fertilisation, the importance of AI timing with respect to ovulation has repeatedly been emphasized (Wilcox & Pfau 1958, Macmillan & Watson 1975, O'Farrell et al. 1983, Rodríguez-Martínez 2001). Inseminations early in oestrus yield low pregnancy rates either due to fertilisation failure (Graden et al. 1968, Hunter 1994) or due to early embryonic mortality (Salisbury & Flerchinger 1967). Despite a well timed (in respect to onset of oestrus), but single AI in RBH, these animals presented a lower pregnancy rate compared with controls (Båge et al. 2003). When delayed ovulation occurs, the poor tubal milieu and altered contractility compromises sperm survival and impairs proper gamete transport. Under these premises, re-inseminations during oestrus have been found to only marginally improve

fertility in spontaneous repeat-breeder animals (van Rensburg & de Vos 1962, Stevenson et al. 1990). Therefore, the problem in RBH might be alleviated if high numbers of fertilisable spermatozoa are available at the time of ovulation. However, in cases of experimentally induced repeat breeding in heifers, re-insemination 24 h after first insemination did not reach a pregnancy rate similar to that seen in controls, although the pregnancy rate was higher than that following a single AI (Duchens et al. 1995). This suggests a concrete problem either with sperm availability for fertilisation or with their capability to fertilise. Using semen from a highly fertile sire, the issue of sperm availability could be explored.

The present study aimed, therefore, to determine whether the application of multiple AIs with frozen-thawed semen of good fertility during spontaneous oestrus would improve pregnancy rates in RBH.

## Materials and methods

### Animals

Five RBH and five VH of the Swedish Red and White breed (SRB) were purchased from Swedish dairy farms. RBH were selected on the basis of their characteristic to return to oestrus following at least three consecutive AI performed at regular and normal inter-oestrous intervals. Further, RBH should be without any apparent pathological findings of the genital tract. The animals selected were free from bovine viral diarrhoea and bovine leukosis, and in good general health. The RBH were 3.4 to 4.5 yr of age (mean 3.11 yr) and weighed  $795 \pm 19.6$  kg (mean  $\pm$  SEM; range 746 to 850 kg), whereas the VH, here used as controls, were 3.8 to 4.0 yr of age (mean 3.9 yr) and weighed  $765 \pm 10.6$  kg (mean  $\pm$  SEM; range 653 to 792 kg). All the heifers were kept tethered in the same barn for 12 months before the initiation of the current experiment. They were accustomed to

daily handling and to oestrus detection, blood sampling and rectal palpation at regular basis. They were fed hay twice daily, and given continuous access to water. The Ethical Committee for Experimentation with Animals, Uppsala, Sweden, approved the experimental protocol before the commencement of the study.

#### *Oestrus detection and ovarian monitoring*

The animals were monitored twice daily for signs of oestrus, such as excitement, vocalization, licking, lordosis, vulvar oedema and redness, and presence and aspect of mucous discharge, as well as uterine tone assessed by palpation per-rectum. Oestrous signs were scored on a scale from 1 to 5. When the animals approached their second spontaneous oestrus (heifers were inseminated during this period) from the start of the trial, they were monitored for oestrous signs every 3 h. The ovaries were examined every 6 h by trans-rectal ultrasonography with an ultrasound scanner (485 Anser, Pie Medical, Maastricht, The Netherlands) equipped with an 8-MHz linear array endo-rectal transducer. Ultrasonographic examination of the ovaries provided additional clues in assigning the different stages of the oestrous cycle, especially the oestrus. Time of onset of standing oestrus was decided according to the combined criteria of primary and secondary oestrous signs, i.e. the animal exhibiting the primary oestrous sign "standing to be mounted", either spontaneously towards herd mates or when provoked by manually pressing the sacrum region, in combination with the secondary oestrous signs described above. Besides this, the decision was supported by information on uterine tone and ultrasonographic evaluation of the dominant follicle and the corpus luteum, as well as presence of increasing amounts of uterine fluid. The day of standing oestrus was assigned as Day 0 of the cycle.

#### *Blood sampling*

Blood samples were collected to analyse plasma (P) concentrations of P<sub>4</sub>, LH and cortisol in the peripheral circulation. Samples were collected by jugular venipuncture into sterile, evacuated blood-collection tubes (Venoject; Terumo Europe N.V.3001 Leuven, Belgium) with sodium heparin (100 IU) as anticoagulant. The plasma was immediately separated by centrifugation at 1,000 × g for 15 min and stored in plastic tubes at -20 °C until assayed at the laboratory. Samples were collected on the day of the first spontaneous oestrus, and from Day 16 of the first oestrous cycle up to the second spontaneous oestrus once daily. During this second spontaneous oestrus, blood samples were collected every 6 h, alternately with ultrasonographic examinations, and on the following Days 3 and 6.

#### *Hormonal analyses*

##### *Progesterone:*

Plasma from the blood samples collected from Day 18 of the first oestrous cycle to Day 6 of the next oestrous cycle were analysed for P<sub>4</sub> content using a solid-phase <sup>125</sup>I radioimmunoassay (Coat-A-Count® Progesterone; DPC®, Los Angeles, CA, USA). The assay had a working range of 0.3 to 127 nmol/l and the analytical sensitivity was 0.10 nmol/l. The intra-assay coefficient of variation (CV) was 12% for low (2.99 nmol/l), 7% for medium (19.37 nmol/l), and 3% for high (43.64 nmol/l) P<sub>4</sub> concentrations. All samples were analysed in single assay.

##### *Luteinising hormone:*

Plasma from the blood samples taken during the second spontaneous oestrus was analysed for plasma-LH by the method described by Forsberg et al. (1993). The intra-assay CVs were 9% for low (0.96 ng/ml), 3% for medium (1.23 ng/ml), and 11% for high (2.42 ng/ml)

LH concentrations. All samples were analysed in a single assay.

#### Cortisol:

Plasma from blood samples from 2 days before, and on Days 3 and 6 after the second spontaneous oestrus were analysed for cortisol concentrations. A single sample per heifer taken during the first spontaneous oestrus was also analysed. Cortisol concentrations were analysed by solid-phase  $^{125}\text{I}$  radioimmunoassay (Coat-A-Count<sup>®</sup> Cortisol; DPC<sup>®</sup>, Los Angeles, CA, USA). The assay had an analytic sensitivity of 4 nmol/l. The inter-assay CV was 12% for the standard cortisol concentration (14 nmol/l), and decreased  $\approx$  10% when standard cortisol concentrations increased from 28 nmol/l to 1,380 nmol/l. The intra-assay CVs were 19% for low (33.83 nmol/l), 16% for medium (82.88 nmol/l), and 12% for high (593.92 nmol/l) cortisol concentration for the first assay, and 23% for low (34.46 nmol/l), 12% for medium (75.98 nmol/l), and 11% for high (590.33 nmol/l) cortisol concentrations for the second assay.

#### Artificial insemination

Heifers of both groups (RBH and VH) were artificially inseminated every 6 h (alternately with blood samplings) during their second spontaneous oestrus, starting from the onset of oestrus

until ovulation. Each AI was preceded by a brief ultrasonographic examination of the ovaries to attempt to confirm that ovulation had occurred. Frozen-thawed semen from a highly fertile Swedish Holstein bull (56-d NRR = 74.6%) was used for AI in every heifer. Each straw (0.25-ml plastic straw) contained a minimum of 7.5 million live spermatozoa post-thaw.

#### Pregnancy diagnosis

After inseminations, the heifers were observed daily for signs of oestrus, especially 18 to 25 d post-AI. Eventual pregnancy status of those heifers not repeating oestrus past this period was determined by trans-rectal ultrasonography, which was performed 30 d post-AI.

#### Statistical analysis

The data were analysed statistically with the Statistical Analysis Systems package (SAS Institute Inc., Cary, NC, USA; V8, updated 2002) for variation in length of oestrous cycle, interval from onset of standing oestrus to ovulation, P-P<sub>4</sub> and -cortisol concentrations, and pregnancy rates in all heifers. To calculate the interval from onset of oestrus to ovulation, onset of oestrus and time of ovulation were set retrospectively to the mean time point between first detection and the preceding examination. A Fisher's exact test was used to compare differ-

Table 1. Mean  $\pm$  SEM of oestrous cycle length, interval from onset of standing oestrus to ovulation, P-P<sub>4</sub> concentration from onset of oestrus to ovulation, and numbers of artificial inseminations (AIs) in RBH (n = 5) and VH (n = 5).

Variable	RBH	VH	P Value
Oestrous cycle length (d)	20.8 $\pm$ 1.02	21.0 $\pm$ 0.84	0.84
Interval from onset of oestrus to ovulation (h)	50.8 $\pm$ 5.32	27.6 $\pm$ 4.49	< 0.05
P-P <sub>4</sub> concentrations from onset of oestrus to ovulation (nmol/l)	0.5 $\pm$ 0.02	0.2 $\pm$ 0.04	< 0.001
Total numbers of AIs	8.4 $\pm$ 0.93	4.6 $\pm$ 0.75	< 0.05

P values < 0.05 are considered statistically significant.

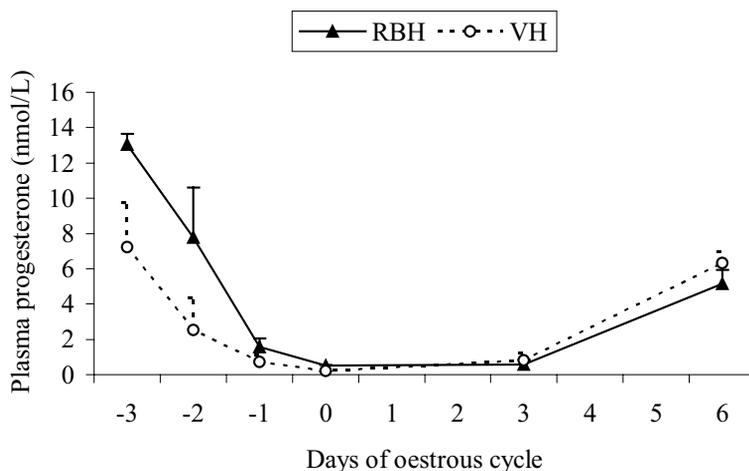


Figure 1. Plasma-progesterone concentrations (mean  $\pm$  SEM) in RBH (n = 5; solid line) and VH (n = 5; dotted line) from 3 days before onset of oestrus (day 0), during the interval from onset of oestrus to ovulation, and on the following Days 3 and 6 of the cycle.

ences in the interval from onset of oestrus to ovulation as well as to compare  $P_4$  concentrations between RBH and VH. A chi-square test was used to compare pregnancy rates between categories. Individual values of LH of both groups of heifers (RBH and VH) were presented against time. Data were normally distributed and were presented as means  $\pm$  standard error of the mean (SEM). Differences were considered statistically significant when  $P < 0.05$ .

## Results

### *Oestrous cycle length, oestrus and interval from onset of oestrus to ovulation*

Both RBH and VH had normal oestrous cycle lengths between 20 and 21 d (Table 1) with no significant difference between heifer groups ( $P = 0.84$ ). The average interval from onset of oestrus to ovulation was significantly longer ( $P < 0.05$ ) in RBH than in VH (Table 1). RBH generally expressed, in the present study, stronger oestrous signs than VH.

### *Progesterone concentrations*

Mean  $P_4$  concentrations from onset of standing oestrus to ovulation were significantly higher ( $P < 0.001$ ) in RBH than in VH (Table 1). Compared with VH, RBH revealed higher  $P_4$  concentrations during oestrus and early metoestrus, but  $P_4$  concentrations were lower in RBH than in VH during late metoestrus and onwards (Figure 1).

### *LH concentrations*

Plasma-LH concentrations of individual RBH and VH are presented in Figures 2A and B, respectively. Compared with VH, RBH had a delayed rise in P-LH. Although the LH surge was not clearly definable using this frequency of blood sampling, approximate onset and decline of the LH surge could be identified. In all heifers, ovulation occurred after the decline of the hereby-defined LH surge.

### *Cortisol concentrations*

All recorded values for P-cortisol concentra-

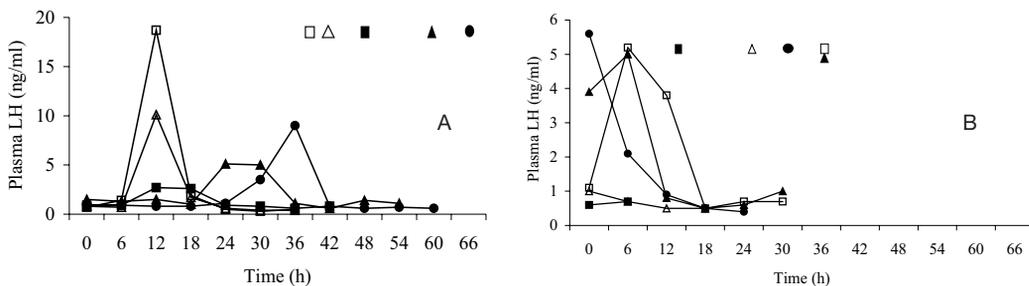


Figure 2. Individual P-LH concentrations in (A) RBH ( $n = 5$ ) and (B) VH ( $n = 5$ ) during spontaneous oestrus. Symbols representing data points indicate the time of ovulation for each heifer. Solid data-point symbols indicate those heifers that subsequently became pregnant.

tions below the analytical sensitivity (4 nmol/l) of the assay were adjusted to 4 nmol/l. The average P-cortisol concentration from a single sample per heifer during the first spontaneous oestrus was  $12.2 \pm 2.08$  nmol/l in RBH and  $15.6 \pm 4.74$  nmol/l in VH ( $P = 0.53$ ). The maximum average P-cortisol concentration during the sec-

ond spontaneous oestrus was  $15.0 \pm 6.7$  nmol/l in RBH (3 h after onset of oestrus) while  $17.5 \pm 10.5$  nmol/l in VH (15 h after onset of oestrus) and thus not statistically different from the average concentrations during the first spontaneous oestrus in RBH ( $P = 0.89$ ) or VH ( $P = 0.85$ ). Nor was there any significant difference

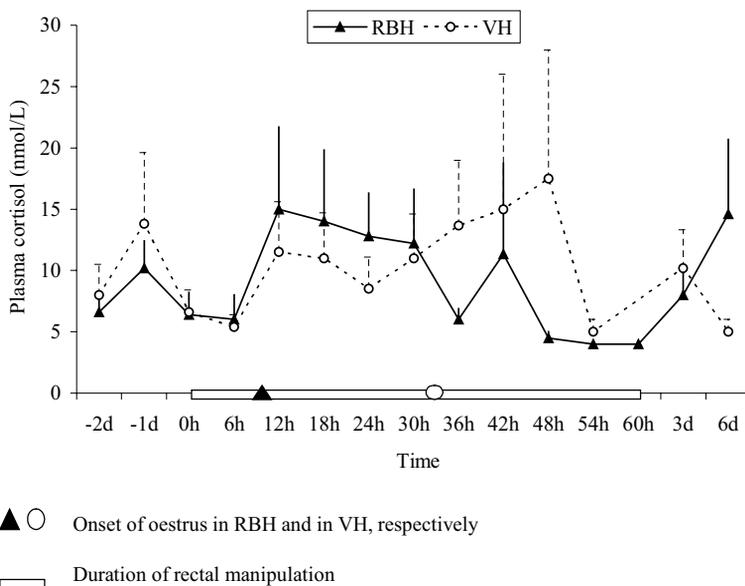


Figure 3. Plasma-cortisol concentrations (mean  $\pm$  SEM, nmol/l) in RBH ( $n = 5$ ; solid line) and VH ( $n = 5$ ; dotted line) on Days 19 and 20 of the first oestrous cycle, during repeated manipulations per-rectum (including AI) in the second spontaneous oestrus, and on Days 3 and 6 of the following oestrous cycle.

between the groups for maximum hormone concentrations ( $P = 0.58$ ). In either group, the rise in cortisol appeared to coincide with the onset of oestrus. There was, however, a large individual variation among heifers, as evident from the SEM (Figure 3).

#### *Numbers of inseminations*

The total number of AIs was significantly higher in RBH compared with VH ( $P < 0.05$ ; Table 1), with a mean value in the pregnant RBH of  $9.7 \pm 0.88$  and of  $6.5 \pm 0.50$  in the non-pregnant RBH ( $P = 0.07$ ). In VH, however, the values were  $4.3 \pm 1.21$  and  $5.0 \pm 1.00$ , respectively ( $P = 0.73$ ). Although, numerically, pregnant RBH were inseminated more times than non-pregnant RBH, the mean values did not differ statistically. On the other hand, pregnant VH received numerically fewer AIs than non-pregnant VH.

#### *Animal behaviour*

The behaviour of the heifers when manipulated rectally and vaginally during oestrus was very much individual. Some of the heifers in each category reacted to some extent by the manipulations, while others were not disturbed. One of the five VH showed discomfort during the palpation per-rectum, this being the one, out of all the heifers, that reflected maximum P-cortisol concentrations. Another VH and one RBH reacted nervously to noise in the barn but were otherwise cooperative during handling. Apparent signs of nervousness or discomfort were not evident in the remaining heifers.

#### *Pregnancy rates*

Two out of five (40%) VH returned to oestrus after 19 and 22 d post-AI, respectively, while none of the RBH returned to oestrus until the end of the observation period of 30 d post-AI. On the ultrasound examination 30 d post-AI, three out of five (60%) heifers in both groups

had live conceptuses. Thus, the pregnancy rates were equal in RBH and VH. The remaining RBH (two out of five, 40%) returned to oestrus after Days 40 and 47, respectively. The mean interval from onset of oestrus to ovulation in the pregnant RBH was  $58.0 \pm 5.29$  h, compared with  $40.0 \pm 2.00$  h in the non-pregnant RBH ( $P = 0.08$ ). This interval was  $26.0 \pm 7.22$  h in the pregnant VH and  $30.0 \pm 6.00$  h in the non-pregnant VH ( $P = 0.73$ ).

#### **Discussion**

The present study, including a small but well-defined group of animals, tested the hypothesis that pregnancy rates in RBH could be improved by multiple AIs during oestrus, performed until spontaneous ovulation occurred. The results showed that repeated inseminations, every 6 h from onset of behavioural oestrus to spontaneous ovulation, yielded a pregnancy rate in RBH comparable to that of controls (VH).

The RBH and VH had oestrous cycle lengths of normal duration. However, compared with VH, RBH displayed significantly longer interval from onset of oestrus to ovulation, periovulatory suprabaasal P-P<sub>4</sub> concentrations, and a delayed rise of P-P<sub>4</sub> during the beginning of the luteal phase. These findings have already been described in other studies in RBH (*Linares 1981b, Gustafsson 1985a, Albiñ 1991a, Båge 2002*), suggesting that the animals included here were to be considered strict repeat breeders.

Average P-cortisol concentrations during the period of frequent rectal and genital manipulation, in both groups, were within the normal physiological range, as could be expected during oestrus in normal dairy heifers (*Thun et al., 1985*). Between the groups, there was no significant difference ( $P = 0.58$ ) in maximum P-cortisol during the period of manipulation. Patterns of P-cortisol did not reveal any evident stressful experience by these heifers. On the

other hand, RBH expressed a delayed onset of P-LH surge, while onset of LH surge appeared to coincide with the onset of oestrus in VH. A similar observation was reported by *Båge et al.* (2002a), who found a significant ( $P = 0.02$ ) delay in onset of LH surge in RBH. Consequently, ovulation can be delayed, resulting in preovulatory ageing of the oocyte. Since administration of exogenous ACTH or cortisol to heifers can delay the LH surge (Stoebel and Moberg, 1982), it is suspected that a higher stress responsiveness (resulting in cortisol release during oestrus in RBH) might be responsible for the delay in LH surge. The present study, which demonstrated the presence of physiological P-cortisol concentrations in RBH, questions whether their delay in LH surge could be solely caused by cortisol release. Echterkamp (1984) has indicated that the two- to three-fold increase observed normally during oestrus is not enough to interfere with the LH surge, requiring a 10- to 20-fold increase to elicit this interference. Most likely, it is the suprabasal P4 that is responsible for the observed delay in LH surge in RBH.

More AIs were done in RBH than in VH, as an obvious consequence of their longer oestrus duration. Pregnant RBH exhibited a numerically longer interval from onset of oestrus to ovulation compared with those non-pregnant, and thus there were more AIs and a higher degree of manipulation done. However, pregnant VH expressed a numerically shorter interval from onset of oestrus to ovulation, thus receiving fewer AIs than that of non-pregnant VH, indicating their better efficiency to reproduce. Irrespective of the repeated manipulation, the behaviour of the heifers, subjectively addressed, appeared to be normal during the experimental period. There were no apparent signs of stress (which would have been reflected in P-cortisol concentrations) expressed by these animals. Maximum P-cortisol concentration was observed in the

VH that showed discomfort during rectal palpation and AI, but subsequently became pregnant. Overall, the experiment revealed no obvious negative effects of repeated manipulation on the reproductive performance in either group. *Alam & Dobson* (1986) found no adverse effect on reproductive parameters and LH surge on dairy cattle following rectal palpation and venipuncture.

Early embryonic mortality, a well-known reason for repeat breeding, is a consequence of either one of several possibilities (*Linares* 1981a, *Gustafsson & Larsson* 1985). Under normal physiological situations, low P- P<sub>4</sub> concentrations during oestrus relate to increased tubal spontaneous contractility, whereas a decrease in tubal spontaneous contractility is related to increasing P- P<sub>4</sub> concentrations during the luteal phase (*Bennett et al.* 1988). *Binelli et al.* (1999) have indicated that tubal function (in terms of gamete transport) during oestrus could be altered by manipulating steroid hormone concentrations. It is possible that the suprabasal P- P<sub>4</sub> concentrations present in RBH during oestrus reduced tubal contractility, resulting in an impaired or delayed sperm transport from the sperm reservoir to the site of fertilisation. A delay in sperm transport may contribute to substantial sperm death due to membrane and acrosome disruption, thus impairing fertilisation (*Salisbury & Flerchinger* 1967). This delay could also contribute to polyspermy by the ageing of the oocytes before they encounter the spermatozoa (*Hunter* 1994). In either case, the resulting zygotes would have poor developing capacity and would eventually undergo early embryonic mortality. The heifer would subsequently return to oestrus. Moreover, in vitro exposure of bovine spermatozoa to uterine lavage from repeat-breeding cows adversely affects sperm motility by lowering their ability to consume oxygen (*Peterson* 1965). Altered protein synthesis and secretions in the uterine tube of

the cow due to persistent dominant follicles (a phenomenon that also has been recorded in RBH; *Båge* 2002a) have been experimentally demonstrated by *Binelli et al.* (1999). Therefore, it appears that the tubal environment in RBH is not favourable for maintaining the optimal fertilising ability of spermatozoa. However, there is a need for detailed studies on the consequences of uterine milieu on the deposited spermatozoa and their transport from the site of semen deposition to the fertilisation place in RBH.

A pregnancy rate of 60% was achieved in both heifer groups, which is comparable to the pregnancy rate usually registered in normal healthy bovines following a routine single AI (*Diskin & Sreenan* 1980). A single second AI, in repeat-breeding animals, has led to an improvement in pregnancy rates. *Stevenson et al.* (1990) found a marginal increase in pregnancy rate from 32.1% to 33.5% when a single second AI was performed 12 to 16 h after first AI. *Bostedt* (1976) has reported an increase in pregnancy rate from 9.5% to 52.9%, in cows that ovulated 24 h after first AI, when a single second AI was performed 24 h (re-insemination close to ovulation) after first AI. *Albihn* (1991b) has reported pregnancy rates of 44% in strictly defined RBH when insemination was performed every 24 h post-first insemination, until spontaneous ovulation (re-insemination closer to ovulation). Improvement of pregnancy rate following re-inseminations in repeat-breeding animals should therefore not be disregarded. From the above-mentioned studies, it may be speculated that AI closer to ovulation may provide more spermatozoa with high fertilising capacity at the site of fertilisation and thus lead to improved conception rates. A possible reason for this improvement could be the shorter exposure to an adverse maternal milieu before they attempt fertilisation. In the present study, AI performed every 6 h would have replenished the

sperm reservoir, increasing the availability of potentially fertile spermatozoa at the time of ovulation.

Two of the five RBH, which were not pregnant at 30 d after AI, did not display signs of oestrus until the end of the observation period. Probably fertilisation had occurred in these two heifers but was followed by embryonic death. Embryos of RBH have compromised developing ability, as evident from a series of previous studies (*Linares* 1981a, *Gustafsson* 1985b, *Albihn et al.* 1989). The probability that polyspermy could occur is not to be disregarded, owing to the presence of large numbers of spermatozoa resulting from frequent inseminations. Polyspermy is a lethal condition in mammals (*Hunter* 1994). Another possibility may be that the uterine environment in these RBH was not competent to support embryonic growth and thus would lead to early embryonic death, with consequent prolongation of the oestrous cycle length (*Albihn et al.* 1991).

Virgin heifers, the control group in the present study, were treated in the same way as RBH and revealed a pregnancy rate of 60%. Fertilisation rates in normal healthy animals are reported to be near 100% (*Sreenan et al.* 2001), but pregnancy rates between 60% and 70% are achieved in highly fertile herds under conventional management conditions (*Roche et al.* 1981). In the present study, pregnancy rates in VH were in the same range as could be achieved in normal animals, but the treatment received by these heifers was different. Compared with routine single AI, these heifers were inseminated every 6 h, without concomitant increased pregnancy rates. Not only is there a probability of polyspermy in these non-pregnant VH (see above reasoning), but also the frequent AI and blood collection should be regarded as stressful to the animals. Animals respond individually to various stress conditions, resulting in variant endocrine changes that could alter their ability to

conceive. Frequent manipulation of the cervix during AI may initiate a local, acute inflammatory reaction along with an altered contractility of the genital tract that would interfere with the transport of gametes and embryos. A pilot study conducted to follow prostaglandin  $F_{2\alpha}$  release, as reflected by its circulatory main metabolite, in two extreme cases of the present heifer population (a pregnant RBH and a pregnant VH with longest and least interval from onset of oestrus to ovulation, respectively) revealed very low concentrations of the metabolite (Kindahl, personal communication). However, besides studying more animals for variations in prostaglandin  $F_{2\alpha}$  concentrations, the involvement of other inflammatory mediators should be evaluated. Contrary to the response in normal heifers, frequent manipulation of the genital tract during AI in RBH, which are supposed to have a slower tubal contractility due to suprabaasal  $P_4$  during oestrus, would possibly improve sperm and/or oocyte transport, thus explaining the improvement hereby seen in pregnancy rates.

### Conclusions

The results from the present study revealed an improvement in pregnancy rates in RBH following frequent inseminations during oestrus until spontaneous ovulation. The response to frequent AI differed between RBH and VH, probably owing to different physiological and endocrine response to the treatment. However, adverse effects of stress during oestrus, and of repeated rectal and vaginal manipulations on pregnancy rates were not evident in either RBH or VH.

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## Sammanfattning

*Dräktighetsresultat hos symptomlösa omlöparkvigor efter att de inseminerats upprepade gånger under spontan brunst.*

Hormonstörningar under brunsten, t ex suprabasala progesteronnivåer, och påverkat brunstförlopp med fördröjd ovulation har setts i samband med symptomlös omlöpning hos SRB-kvigor. Det är känt att hormonstörningar i sin tur kan störa äggledarfunktion-

nen och påverka tillgången på befruktningssdugliga spermier vid ovulationstidpunkten. I det aktuella försöket inseminerades omlöparkvigor och normala kvigor med 6 h intervall från högbrunstens start till ovulation för att se om dräktighetsresultatet kunde påverkas positivt. Hormonanalyser visade att omlöparkvigor hade suprabasala progesteronnivåer under brunsten och fördröjd preovulatorisk LH-frisättning, medan kortisolnivåerna var normala. Högbrunsten varade längre hos omlöparkvigor, vilka

därför inseminerades fler gånger än de normala kvigor. Dräktighetsprocenten hos omlöparkvigor steg till normala nivåer (60%), medan de normala kvigor hade lägre dräktighetsprocent än väntat, vilket kan bero på negativ effekt av de frekventa inseminationerna. Resultaten från försöket visar att dräktighetsresultaten kan förbättras hos symptomlösa omlöparkvigor om de insemineras upprepade gånger tills spontan ovulation sker.

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