Fixed time deep intracornual insemination of heifers at synchronized estrus

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Abstract

The aim of the study was to determine the efficiency of single fixed time deep intracornual insemination using $2 \times 10^6$ spermatozoa compared with single standard dose deep intracornual insemination and single and dual standard dose ($40 \times 10^6$) uterine body (conventional) insemination in heifers at synchronized estrus. Estrus was synchronized in 275 virgin heifers by administration of two doses of PGF$_2\alpha$ 14 days apart. Deep intracornual inseminations with low (ICI-LD1, $n = 102$) and standard (ICI-SD1, $n = 56$) dose of semen and the single standard dose conventional inseminations (AI-SD1, $n = 66$) were performed 80–82 h after the second PGF$_2\alpha$ treatment. Ultrasonography was used to identify the first dominant (presumed ovulatory) follicle, and semen was deposited either close to the utero-tubal junction ($n = 69$ in ICI-LD1 and $n = 23$ in ICI-SD1) or in the middle part of the uterine horn ($n = 28$ in ICI-LD1 and $n = 28$ in ICI-SD1) ipsilateral to the ovary bearing the first dominant follicle. The dual standard dose conventional inseminations were performed 72 and 96 h after the second PGF$_2\alpha$ treatment (AI-SD2, $n = 51$). The pregnancy rate in the ICI-LD1 group (68.0%) did not differ significantly ($P > 0.05$) from the ICI-SD1 group (56.9%) or the AI-SD2 group (65.9%) and was significantly higher ($P < 0.05$) than in the AI-SD1 group (54.2%). The site of intacornual deposition of semen, near the utero-tubal junction or in the middle of the horn, had no effect on the pregnancy rate. The pregnancy rate in all the groups was not affected by the intensity of expression of estrous signs.

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1. Introduction

Deep intracornual deposition of semen in cattle was proposed in the late 1950s (see the review by Lopez-Gatius [1]) to increase the efficiency of artificial insemination and to
reduce the required number of spermatozoa per insemination dose and thus enhance the usage of semen from genetically superior bulls.

The precise relationship between site of insemination and the optimal number of spermatozoa in the insemination dose has not been defined. Studies on the optimal number of spermatozoa for insemination have not produced consistent results. With the use of the conventional insemination technique, reduction of the dose from $20 – 25 \times 10^6$ to $12 – 15 \times 10^6$ did not reduce the non-return rate (NRR) [2,3], whereas in another study it was lower after use of $8 \times 10^6$ and $12 \times 10^6$ than after $16 \times 10^6$ spermatozoa [4]. In a further study, the pregnancy rate was similar (70.5 and 70.1%) after insemination with $4.6 \times 10^6$ or $21.6 \times 10^6$ motile spermatozoa [5].

The efficiency of deep deposition of frozen–thawed semen has been evaluated and found not to differ from [6–9] or to be 11–20% higher [10–12] than that of conventional uterine body insemination. It has been suggested that the semen should be deposited near the utero-tubal junction ipsilateral to the ovary bearing an ovulatory follicle [10,13], but the importance of the actual site of intracornual insemination has also been questioned [14]. Intracornual insemination requires greater care on the part of the technician because of the risk of perforating the uterine wall due to the tonicity of the uterus at estrus and the danger of rupturing the ovulatory follicle whilst palpating the ovaries per rectum to determine the side of ovulation. Insemination close to the utero-tubal junction may result in more spermatozoa reaching the site of fertilization and increased chance of polyspermy [13]. Dalton et al. [15] found more spermatozoa in the zona pellucida after insemination close to the utero-tubal junction than after conventional insemination. On the other hand, Hawk and Tanabe [6] found no increase in the number of accessory spermatozoa after deep deposition of semen.

Achievement of acceptable pregnancy rates with low numbers of spermatozoa would greatly enhance the potential for the use of sexed spermatozoa [16,17]. With current technology, the number of sexed spermatozoa available for insemination is severely limited by the rate of sorting and the dilution requirements for sorting [18]. Seidel et al. [19,20] reported 54% pregnancy rate after deep intracornual insemination with as few as $3 \times 10^5$ non-frozen sexed spermatozoa. It is clear that further studies are needed to ascertain the efficacy of deep intracornual insemination with small numbers of spermatozoa especially after the synchronization of estrus.

The main aim of the present study was to compare a single fixed time deep unilateral intracornual insemination of $2 \times 10^6$ spermatozoa with deep insemination of $40 \times 10^6$ spermatozoa and single and double conventional insemination of $40 \times 10^6$ spermatozoa in heifers at synchronized estrus. Other aims were to determine the effect of intensity of expression of estrous signs and site of deep deposition of semen on pregnancy rate.

2. Materials and methods

2.1. Animals and experimental procedures

The experimental animals consisted of 275 virgin heifers of the Estonian Holstein breed (EHF) aged 13–15 months with body weight of 330–350 kg and housed in tie-stalls on two
farms. All heifers were treated twice intramuscularly at 14-day interval with 25 mg of PGF$_2\alpha$ (Pharmacia & Upjohn Co.) to synchronize estrus. At the time of each treatment, the ovaries of all heifers were visualized with a real-time B-mode diagnostic ultrasound scanner equipped with a 5.0 MHz linear array intrarectal transducer (Honda, HS-120, Japan). Measurements of the visualized ovarian structures were recorded. The insemination methods used were: single low dose deep intracornual (ICI-LD1); single standard dose deep intracornual (ICI-SD1); single standard dose conventional (AI-SD1); and dual standard dose conventional (AI-SD2). Heifers were randomly allotted to groups and inseminated irrespective of the presence of estrous signs. All the single dose inseminations were performed 80–82 h after the second PGF$_2\alpha$ treatment [21]. The dual inseminations were performed 72 and 96 h after the second PGF$_2\alpha$ treatment. Ultrasonography was used for deep intracornual insemination to identify the first dominant (presumed ovulatory) follicle, and semen was deposited either close to the utero-tubal junction (n = 69 in ICI-LD1 and n = 23 in ICI-SD1) or in the middle part of the uterine horn (n = 28 in ICI-LD1 and n = 28 in ICI-SD1) ipsilateral to the ovary bearing the first dominant follicle using a 0.25 ml straw embryo transfer catheter covered with stainless steel tipped side-opening sheaths (IMV, France). For conventional insemination the semen was deposited in the uterine body using a 0.25 ml stainless steel insemination catheter (IMV, France). One AI technician performed all conventional inseminations and two experienced embryo transfer technicians performed the deep intracornual inseminations. At the time of insemination the heifers were examined for expression of estrous signs (vaginal mucus discharge, vulvar edema, reddening of mucosa, relaxation of the cervix), and the intensity of estrus was evaluated as strong or weak according to the criteria of Callesen et al. [22]. Pregnancy status was diagnosed either by ultrasonography 35–40 days after insemination or by palpation of the uterus per rectum 45–60 days after insemination. The side of pregnancy was recorded to confirm its accordance with the ultrasonographic prediction of side of ovulation.

2.2. Semen processing

The semen of three EHF bulls with similar field fertility was used (average NRR in 2000/2001 ranged from 57.4 to 58.0%). The total number of spermatozoa in standard and low insemination doses was $40 \times 10^6$ and $2 \times 10^6$ with at least $20 \times 10^6$ and $1 \times 10^6$ of progressively motile spermatozoa, respectively. The semen was diluted with Triladyl® (Minitüb GmbH & Co., Germany) and an egg yolk extender. First, the semen was diluted at +20 °C to achieve $40 \times 10^6$ spermatozoa in a standard dose. After chilling the initially diluted semen at +4 °C for 2 h, the semen was additionally diluted in a 1:1 ratio. The low dose straws were then manually filled by depositing a 1 cm column with $2 \times 10^6$ spermatozoa in the middle part and two columns of pure extender at both ends of the straws, separated from the semen by air bubbles, as previously described by Seidel et al. [23]. After equilibration for 2 h at +4 °C, the straws were frozen. Sperm motility in each frozen semen batch was evaluated before the experiments using Sperm Motility Analyser (Minitüb GmbH & Co., Germany). For insemination the straws with frozen low and standard doses were thawed in water at +35 °C for 15 s and insemination was performed immediately after thawing.
2.3. Analysis of data

Effect of the insemination method, bull, farm, technician, the presence of CL in the ovaries on the day of the second PGF<sub>2α</sub> treatment, expression of estrous signs, the size of the presumed ovulatory follicle, and the site of semen deposition in the uterine horn on the pregnancy rate were analyzed using the general linear-models procedure [24]. As there were no statistically significant effects of bulls, farms, and technicians on pregnancy rates, the data for these variables were pooled to compare the methods of insemination. Differences between the pregnancy rates were subjected to the t-test and considered to be significant at P values of <0.05.

3. Results

On the day of the second PGF<sub>2α</sub> treatment, CL were present in 89.4–95.1% of heifers (Table 1). Although 3 of the 21 heifers without detectable CL were pregnant, they were considered as non-synchronized and were eliminated from subsequent analysis. The insemination results were not affected by bulls (P = 0.29), farms (P = 0.53), or technicians who performed the inseminations (P = 0.52). In synchronized heifers (the presence of CL at the time of the second PGF<sub>2α</sub> treatment) the pregnancy rate in the ICI-LD1 group did not differ significantly (P > 0.05) from that in the ICI-SD1 group or the AI-SD2 group and was significantly higher (P < 0.05) than in the AI-SD1 group.

Intensity of estrous signs at the time of insemination was evaluated as strong in 51.1–66.7% and weak in 33.3–49.2% of synchronized heifers (Table 2). However, the pregnancy rates in the ICI-LD1, ICI-SD1, AI-SD1, and AI-SD2 insemination groups were not significantly affected by the intensity of the estrous signs (P > 0.05).

The pregnancy rates did not differ significantly (P > 0.05) after the deposition of low and standard doses of semen either near the tip or in the middle part of the horn (Table 3).

Table 1
The effect of presence of a CL at the second PGF<sub>2α</sub> treatment on pregnancy rates following single fixed time low and standard dose deep intracornual and single and dual standard dose conventional inseminations at synchronized estrus

<table>
<thead>
<tr>
<th>Insemination method</th>
<th>Total number of heifers</th>
<th>Presence of CL at the second PGF&lt;sub&gt;2α&lt;/sub&gt; treatment</th>
<th>Absence of CL at the second PGF&lt;sub&gt;2α&lt;/sub&gt; treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pregnancy rate</td>
<td>Pregnancy rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n  %</td>
<td>n  %</td>
</tr>
<tr>
<td>ICI-LD1</td>
<td>102</td>
<td>97  95.1</td>
<td>66  68.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ICI-SD1</td>
<td>56</td>
<td>51  91.1</td>
<td>29  56.9&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AI-SD1</td>
<td>66</td>
<td>59  89.4</td>
<td>32  54.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AI-SD2</td>
<td>51</td>
<td>47  92.2</td>
<td>31  65.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ICI-LD1: single low dose deep intrauterine insemination; ICI-SD1: single standard dose deep intrauterine insemination; AI-SD1: single standard dose conventional insemination; AI-SD2: dual standard dose conventional insemination. Values in columns with different superscripts (a, b) differ significantly (P < 0.05).
In ICI-LD1 and ICI-SD1 insemination groups, the pregnancy side coincided with the side of presumed ovulation in 83.2% (79/95) of the pregnant heifers. In 16 heifers pregnancies were found in the horn contralateral to the semen deposition. In eight heifers ovulations occurred in the right ovary instead of the left as predicted and in eight heifers it was vice versa. The mean size ($C_6S.D.') of the presumed ovulatory follicle was 15.3 ± 3.2 mm in pregnant and 13.9 ± 4.4 mm in non-pregnant animals ($P < 0.05$).

### Table 2

<table>
<thead>
<tr>
<th>Insemination method</th>
<th>Total number of heifers</th>
<th>Strong estrous signs</th>
<th>Weak estrous signs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$n$</td>
<td>$%$</td>
</tr>
<tr>
<td>ICI-LD1</td>
<td>97</td>
<td>56</td>
<td>57.7</td>
</tr>
<tr>
<td>ICI-SD1</td>
<td>51</td>
<td>34</td>
<td>66.7</td>
</tr>
<tr>
<td>AI-SD1</td>
<td>59</td>
<td>30</td>
<td>50.8</td>
</tr>
<tr>
<td>AI-SD2</td>
<td>47</td>
<td>24</td>
<td>51.1</td>
</tr>
</tbody>
</table>

ICI-LD1: single low dose deep intrauterine insemination; ICI-SD1: single standard dose deep intrauterine insemination; AI-SD1: single standard dose conventional insemination; AI-SD2: dual standard dose conventional insemination. Values with similar superscripts (a) in rows do not differ significantly ($P > 0.05$).

### Table 3

<table>
<thead>
<tr>
<th>Insemination method</th>
<th>Total number of heifers</th>
<th>Near the utero-tubal junction</th>
<th>Medial part of the horn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inseminated</td>
<td>Pregnant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$n$</td>
<td>$%$</td>
</tr>
<tr>
<td>ICI-LD1</td>
<td>97</td>
<td>69</td>
<td>47</td>
</tr>
<tr>
<td>ICI-SD1</td>
<td>51</td>
<td>23</td>
<td>14</td>
</tr>
</tbody>
</table>

ICI-LD1: single low dose deep intrauterine insemination, ICI-SD1: single standard dose deep intrauterine insemination. Values with similar superscripts (a) in rows do not differ significantly ($P > 0.05$).

In ICI-LD1 and ICI-SD1 insemination groups, the pregnancy side coincided with the side of presumed ovulation in 83.2% (79/95) of the pregnant heifers. In 16 heifers pregnancies were found in the horn contralateral to the semen deposition. In eight heifers ovulations occurred in the right ovary instead of the left as predicted and in eight heifers it was vice versa. The mean size (±S.D.) of the presumed ovulatory follicle was 15.3 ± 3.2 mm in pregnant and 13.9 ± 4.4 mm in non-pregnant animals ($P < 0.05$).

### 4. Discussion

In synchronized heifers the pregnancy rate after a single low dose deep intracornual insemination did not differ significantly from that after a single standard dose deep intracornual or dual standard dose conventional insemination. However, it was significantly higher than the pregnancy rate obtained after a single conventional insemination with a standard dose of semen. Our results are similar to those reported from other studies where deep intracornual insemination also yielded a significantly higher pregnancy rate compared to the conventional uterine body insemination [10,12]. It is evident that the lower pregnancy rate after the single standard dose conventional insemination was caused by an inappropriate time interval between the onset of the estrus and insemination. It is likely that
in some heifers the single fixed time conventional insemination with standard dose of semen was performed too late. The results of our study indicate that when a small number of spermatozoa is deposited close to the site of fertilization by deep intracornual insemination, an insemination time of 80–82 h after the second PGF$_2\alpha$ treatment is appropriate. According to our results the main advantage of a single low dose deep intracornual insemination in synchronized heifers, compared to the dual standard dose conventional insemination, is effective use of valuable semen. When compared with the single standard dose conventional insemination, there is an advantage of higher pregnancy rate.

Regardless of the method of insemination, there was a consistent trend towards lower pregnancy rates in heifers with weak estrous signs, but the difference was not statistically significant. This compares with the report of Hyttel and Greve [25], in which weak intensity of estrus was associated with a lower pregnancy rate in cows synchronized with two injections of PGF$_2\alpha$. With intracornual insemination, using low or standard dose of spermatozoa, pregnancy rates were not significantly affected by the site of insemination, near the utero-tubal junction or in the middle of the uterine horn. Likewise, Hawk and Tanabe [6] found no difference in pregnancy rates between the deposition of semen in the curvature or the uterine body. On the other hand, there are reports of 11–20% increase in pregnancy rates from deposition of semen in the curvature [11] or in the cranial half [12] of the horn compared to deposition in the uterine body. The results reported here with an insemination dose of $2 \times 10^6$ spermatozoa indicate that there is no need to deposit semen close to the utero-tubal junction because similar efficiency can be achieved by deposition of semen in the middle part of the uterine horn. With the use of lower numbers of spermatozoa, there may be an effect of site of deposition within the uterine horn.

The use of ultrasonography to determine the ovulation side did not cause the rupture of any follicles and the mean size of the presumed ovulatory follicle agrees with the reported data [26,27]. The side of diagnosed pregnancy did not accord with the ultrasonographically presumed ovulatory side in 16 heifers. Obviously, in these heifers the first dominant follicle was not ovulatory (functionally non-dominant) and later was replaced by the second one [28,29]. The disagreement found between the presumed and diagnosed pregnancy sides indicates the ability of spermatozoa to migrate in the uterus toward the ovulated oocyte even when deposited deep contralaterally. After a deep intracornual insemination pregnancy can be achieved even if a mistake is made in the prediction of the ovulation side. This assumption is confirmed in a study by Seidel et al. [19], where no difference was found between the pregnancy rates after the deep intrauterine deposition of liquid semen ipsilaterally or contralaterally with regard to the ovulation side.

The difference in the mean diameter of the ovulatory follicle between the pregnant and non-pregnant animals was significant ($P < 0.05$), which is in agreement with findings of another study [30]. It supports the suggestion that ovulation of smaller follicles after a synchronized estrus may produce a lower pregnancy rate, probably due to the development of smaller CL and decreased concentrations of circulating progesterone [30]. We cannot exclude the possibility that the lower pregnancy rate in heifers with the smaller dominant follicles at insemination was due to slower follicular development resulting in a prolonged interval from insemination to ovulation. This would reflect the fact that two injections of PGF$_2\alpha$ result in better synchronization of estrus than of ovulation [31].
In conclusion, the results of this study show that with deep intracornual insemination of synchronized heifers 80–82 h after the second PGF$_2\alpha$ treatment a dose of $2 \times 10^6$ spermatozoa is as efficient as a dose of $40 \times 10^6$ and is similar in efficiency to dual uterine body insemination with $40 \times 10^6$ spermatozoa at 72 and 96 h after the second PGF$_2\alpha$ treatment. With low dose deep intracornual insemination the pregnancy rate was not affected by the intensity of the signs of estrus or the exact site of semen deposition, near the utero-tubal junction or in the middle part of the horn.

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References


