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Original article

Incidence of clinical form of anestrus after unsuccessful service in cows in eight dairy herds in north-east of Poland

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Abstract

There is no information available about the incidence of anestrus and its clinical forms after service in dairy cows in Poland. Thus, the aim of this study was to investigate the incidence of clinical forms of anoestrus after unsuccessful artificial insemination in dairy cows based on ultrasound examination. The study was carried out on 1543 Polish Holstein-Friesian cows in 8 dairy herds in north-east Poland over a three-year period. Cows were examined for pregnancy on day 35 after AI using a Honda 1500 portable ultrasound scanner equipped with a 5 MHz linear-array transducer. Cows diagnosed as pregnant were re-examined on day 45. Of the 1543 inseminated cows, 408 (26.4%) showed no estrus signs and were diagnosed not-pregnant by ultrasonography, 328 (21.3%) returned to estrus within 35 days, and 807 (52.3%) were pregnant via artificial insemination. The incidence of anestrus after service in non-pregnant cows varied among herds from 10.3% to 32.9% of cows ($p < 0.05$). Based on ultrasound examination silent heat was diagnosed in 324 (79.4%), *corpus luteum pseudograviditatis* in 36 (8.8%), ovarian cysts in 26 (6.4%), and ovarian afuction in 22 (5.4%) of 408 anestrus, non-pregnant cows. The results of this study showed that the incidence of anestrus after service in dairy herds in North-East Poland was high. The most prevalent clinical form of post-service anestrus was silent heat.

Key words: anestrus after service, dairy cows, silent heat

Introduction

Increased milk production demands have led to a decline in the reproductive performance of dairy cows due to a prolonged intercalving period (Lucy 2001, Walsh et al. 2011). One of the major factors contributing to an extended interval from calving to conception is anestrus after service. The cows being not-pregnant after the first service should have been observed in estrus for a second service. The majority of non-preg-

nant cows exhibit heat before pregnancy examination which is usually performed between 35 to 45 days after initial insemination. Non-pregnant cows that do not return to estrus within the expected time are termed “phantom cows” because they appear to be pregnant, but in reality are not (Nation et al. 2001, Lucy et al. 2004).

In the literature the incidence rate of anestrus after service ranges from 20% to 50% (Martinez and Thibier 1984, Bartlett et al. 1987, Nation et al. 2001). There are

four clinical forms of anestrus: (1) silent heat; (2) cystic ovarian disease; (3) ovarian afunction; (4) *corpus luteum pseudograviditatis* (Opsomer 1999, Mwaanga and Janowski 2000). However, studies about clinical conditions in cows with post-service anestrus are very limited and in part have been performed on a population of cows with lower milk yield (Bartlett et al. 1987, Nation et al. 2001).

There is no information available about the incidence of anestrus and its clinical forms after service in dairy cows in Poland. Thus, the aim of this study was to investigate the incidence of clinical forms of anestrus after unsuccessful artificial insemination in dairy cows based on ultrasound examination.

Materials and Methods

All treatments, housing and animal care reported below were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments. The study was carried out on 1543 Polish Holstein-Friesian cows in 8 dairy herds in north-east of Poland over a three-year period. The average number of cows in the herds ranged from 60 to 200. The cows were housed in free stall barns with a total mixed ration (TMR) feeding system. The TMR was based on maize and grass silage, concentrate, and vitamin and mineral supplements. The animals had free access to water. Cows were milked twice daily and the average milk yield was about 7000 kg per cow, per year. Cows included into the study were 2 to 7 year old. Estrus was checked three times a day for twenty - thirty minutes by the owner or herdsmen during the daytime and in the night-time by the care takers working on the night shifts. Cows detected in estrus were artificially inseminated by AI technicians at the first estrus occurring after 60 days post-partum. The cows were examined for pregnancy on day 35 after AI using a Honda 1500 portable ultrasound scanner equipped with a 5 MHz linear-array transducer. Positive diagnosis of pregnancy was based on the presence of a viable embryo and a normal appearance of chorioallantoic fluid. Cows without an visualized embryo, but with the presence of amniotic fluid and the corpus luteum, and cows with a non-viable embryo or with signs of embryo degeneration were re-examined by transrectal ultrasonography 7 days later. Non-pregnant cows were examined by transrectal ultrasonography at least twice in a 7-10 day interval to diagnose the clinical causes of anestrus. The ovarian findings were interpreted as follows:

1. Small ovaries with follicles of < 8 mm diameter and without corpus luteum on both examinations were interpreted as ovarian afunction;

2. The presence of follicles with diameter > 8 mm and corpus luteum during one of examination was interpreted as a silent heat;

3. The presence of follicles with diameter ≥ 2 cm with absence of corpus luteum on both examination was interpreted as ovarian cysts;

4. In the case of presence of corpus luteum at both examination the third examination was performed. The presence of corpus luteum at three examinations was interpreted as *corpus luteum pseudograviditatis*. The absence of corpus luteum at third examination was interpreted as a silent heat.

Cows diagnosed as pregnant were re-examined after day 45.

Differences in the incidence of anestrus after service among herds were statistically analyzed by the Mann-Whitney test (Yates corrected Chi-square test) using GraphPad Prism version 6.00 (GraphPad Software, San Diego, CA, USA). The level of significance was considered as $p < 0.05$.

Results

Of the 1543 inseminated cows, 408 (26.4%) showed no estrus signs and were diagnosed not pregnant using ultrasonography, 328 (21.3%) returned to estrus within 35 days, and 807 (52.3%) were pregnant by artificial insemination (Fig. 1).

The incidence of anestrus after unsuccessful service in cows varied among herds from 10.3% to 32.9% of cows (Fig. 2). The differences in incidence of post-service anestrus among herds were statistically significant ($p < 0.05$).

Based on ultrasound examination silent heat was diagnosed in 324 (79.4%), *corpus luteum pseudograviditatis* in 36 (8.8%), cystic ovarian follicles in 26 (6.4%) and ovarian afunction in 22 (5.4%) of 408 anestrus, non-pregnant cows (Table 1).

Discussion

In this study 26.4% of inseminated cows showed no estrus signs within 35 days and were diagnosed not pregnant. This result is in accordance with the study of Martinez and Thibier (1984), in which 30% of cows in two dairy herds in France were diagnosed as having anestrus after service. Bartlett et al. (1987) reported a higher incidence of post-service anestrus of 47% in twenty-two Michigan dairy herds. Nation et al. (2001) found lower incidence rate of anestrus after service of 19% in four Australian dairy herds.

The incidence of anestrus after service varied significantly among herds. In the present study nutrition

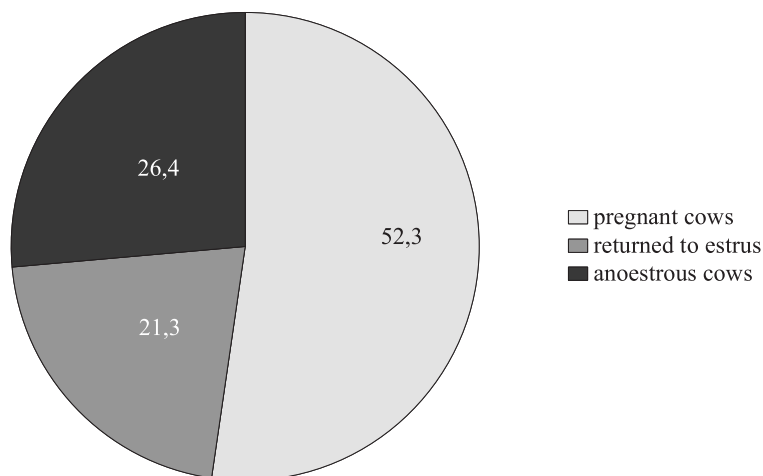


Fig. 1. Incidence of pregnant cows, cows returned to estrus and anestrus, and non-pregnant cows in the study population (n=1543).

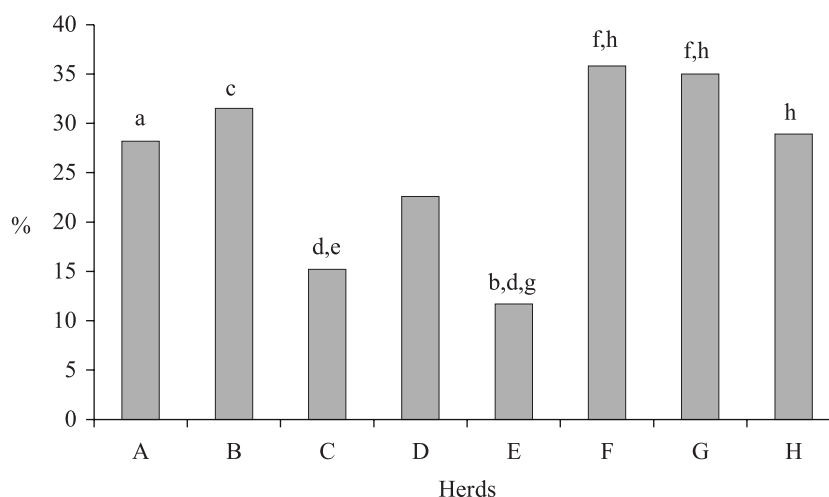


Fig. 2. Prevalence of anestrus in non-pregnant cows in 8 herds in North-East Poland. a,b; c,d; e,f; g,h – pairs of small letters present significant differences ($p < 0.05$).

Table 1. Incidence of clinical form of post-service anestrus in eight dairy herds.

Clinical form of anestrus	Anestrus cows	
	n	%
Silent heat	324	79.4
<i>Corpus luteum pseudogaviditatis</i>	36	8.8
Ovarian cysts	26	6.4
Ovarian afuction	22	5.4

and housing conditions in the herds were similar, thus, the variations among herds were probably attributed to differences in the managerial factors, mainly efficiency of estrus detection.

The main clinical form of anestrus after service was silent heat, affecting 79.4% of cows that failed to show estrus within 35 days after insemination. Silent heat or subestrus is defined as a condition in which the behavioral signs of heat are not observed at the expected

time although the cows are cycling regularly (Opsomer 1999, Mwaanga and Janowski 2000). Bartlett et al. (1987) classified 80% of cows with post-service anestrus as having functional anestrus. Those cows showed evidence of cyclic activity and no organic reason could be found. Nation et al. (2001) reported that 35% of anestrus cows following unsuccessful insemination (phantom cows) did not return to service within 24 days. They presume that the major cause of the phantom cow syn-

drome is the failure to detect a return to estrus. Poor rates of detected estrus may be due to insufficient observation by herd personnel (Mwaanga and Janowski 2001, Rorie et al. 2002, Roelofs et al. 2005) or limited expression of estrus. High-producing dairy cows display estrus signs for a shorter time and less intensely (Lucy 2001, Lopez et al. 2004). With increasing milk yield the percentage of cows in estrus that stand-to-be-mounted has declined from 80% to 50% and duration of detected estrus was reduced from 15 h to 5 h over the past 50 years (Dobson et al. 2008).

Corpus luteum pseudograviditatis (prolonged luteal phase) was diagnosed in 36 (8.8%) of 408 anestrous cows after service. *Corpus luteum pseudograviditatis* is a failure of the corpus luteum to regress despite the absence of pregnancy. Prolonged luteal activity is usually associated with late embryo mortality or abnormal uterine content such as pus or a mummified fetus (Olson et al. 1986, Opsomer 1999, Mwaanga and Janowski 2000, Humblot 2002). Embryonic losses are classified as late embryonic mortality when they occur between day 25 and 45 of gestation (Humblot 2002). The late embryonic mortality rate varies between 3% and 12% (Humblot 2002, Silke et al. 2002, Starbuck et al. 2004, Barański et al. 2012). In most cases pyometra develops as a consequence of post-partum endometritis (Sheldon et al. 2006). Pyometra can also result from contamination during insemination. In this study prolonged luteal activity was mainly related to late embryonic mortality, and pyometra was found only in 1 cow. Bartlett et al. (1987) reported that 0.6% of cows that failed to show estrus within 35 days after insemination had pyometra. Nation et al. (2001) found prolonged luteal activity in 39% of cows that were not confirmed pregnant after the first insemination and did not return for a second service within 24 days.

In 6.4% of cows with anestrus after service cystic ovarian follicles were diagnosed. Ovarian cysts were defined as follicular structures with a diameter over 25 mm that persist on the ovary with the absence of a corpus luteum (Mwaanga and Janowski 2000, Vanholder et al. 2006). Cystic ovarian follicles are a common pathological condition during the post-partum period in dairy cattle and are mainly associated with anestrus (Opsomer 1999, Mwaanga and Janowski 2000, Zduńczyk et al. 2002). There are only few studies about ovarian cysts as a cause of anestrus after service. Bartlett et al. (1987) found ovarian cysts in 16.5% of cows with post-service anestrus. Watson and Cliff (1997) detected ovarian cysts in 12% of cows with a negative pregnancy diagnosis. Nation et al. (2001) reported that ovarian cysts, pyometra and uterine adhesions were diagnosed in < 5% of Australian dairy cows that did not return to estrus within 24 days of an unsuccessful insemination.

In this study ovarian afuction (anestrus type I) was found in 5.4% of anestrous cows that failed to conceive after insemination. Ovarian afuction is defined as small ovaries with follicle growth up to deviation phase (follicle diameter < 9 mm) and the absence of a corpus luteum (Wiltbank et al. 2002). Bartlett et al. (1987) demonstrated that 3.7% of cows with anestrus after service had inactive ovaries. Nation et al. (2001) diagnosed inactive ovaries in 19% of non-return, non-pregnant cows; these cows apparently had a cessation of cyclicity after estrus or were inseminated at a falsely detected estrus. The incidence of inactive ovaries in cows with anestrus after service was especially high (28%) in cows treated with a CIDR device for cycle induction (Rhodes et al. 1999, Nation et al. 2001).

In conclusion, this study has demonstrated high incidence of anestrus after service in dairy herds in North-East Poland. The most prevalent clinical form of post-service anestrus was silent heat, followed by prolonged luteal phase associated with late embryo mortality.

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