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Hormone use for reproductive diseases and heat induction in relation to herd-level reproductive performance in Dutch dairy farms

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A R T I C L E I N F O

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A B S T R A C T

This ecological study aimed to associate hormone use for reproductive diseases and heat induction with reproductive performance at herd level. Hormone use, herd characteristics, and test-day recording data were obtained from 754 representative Dutch dairy farms belonging to five large veterinary practices from 2017 to 2019 (1679 observations in total). Hormone use was classified into prostaglandin, gonadotropin-releasing hormone (GnRH), and progesterone, and was expressed at herd level as the annual number of hormone doses per 100 adult dairy cows. Hormone use was categorized into four levels (no usage, low, medium, and high use), following the 33rd and 66th percentiles of herds that applied them. Three herd-level reproductive performance indicators (calving interval, calving-to-1st insemination interval, number of inseminations per cow) were analyzed using multivariable General Estimating Equations models. The median annual total hormone use was 36.1 (mean=43.1; min=0.0; max=248.2) doses per 100 adult dairy cows in all herds while the median was 39.2 (mean=46.8; min=0.4; max=248.2) doses per 100 adult dairy cows among the user-herds. The median annual group-specific hormone use was 21.3 (mean=26.1; min 0.0; max=180.0), 11.0 (mean=15.3; min=0.0; max=127.0) and 0.0 (mean=1.8; min=0.0; max=40.3) doses per 100 adult dairy cows for prostaglandin, GnRH, and progesterone, respectively. The final statistical models identified that herds with a high hormone use had a calving interval and a calving-to-1st insemination interval that was 9.3 ± 2.6 and 16.4 ± 2.1 days shorter than that of non-user herds (424.0 ± 2.7 and 114.0 ± 2.1 days), respectively. Furthermore, high-user herds needed on average 0.3 ± 0.04 inseminations more to get their cows pregnant compared to non-user herds (1.83 ± 0.04 no. of inseminations per cow). Medium-user herds had a 6.5 ± 2.6 days shorter calving interval and a 12.0 ± 2.1 days shorter calving-to-1st insemination interval with 0.2 ± 0.04 additional inseminations per cow compared to non-user herds. Low-user herds had a 6.2 ± 2.7 days shorter calving interval and a 7.9 ± 2.2 days shorter calving-to-1st insemination interval compared to non-user herds. The model produced the same trend for prostaglandin and GnRH use, with the higher use being associated with a shorter calving interval, a shorter calving-to-1st insemination interval, and a higher insemination per cow number. For progesterone use the opposite effect was observed. In conclusion, using a large representative herd-level dataset, hormone use was associated with a better reproductive performance in terms of calving interval and calving-to-1st insemination interval but gave extra average number of inseminations per cow. It should be monitored how reproduction performance changes when striving for a more prudent hormone use.

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1. Introduction

Reproductive hormones have been advocated for several decades to maintain a good reproductive performance and are regularly applied as part of dairy cows' reproductive management (Higgins et al., 2013; Moore and Hasler, 2017; Stevenson and Britt, 2017). Reproductive hormones are used to mitigate reproductive diseases. For instance, prostaglandins are used to treat cows with corpus luteum persistence (Gundling et al., 2015; Lüttgenau et al., 2016), gonadotropin-releasing hormone (GnRH) for cystic ovarian disease (Lüttgenau et al., 2016), and progesterone is proposed after insemination to reduce pregnancy loss (Friedman et al., 2014; Bisinotto et al., 2015). Besides curative treatments, hormones are also regularly applied to induce heat in order to improve reproductive performance. Routine application of hormones may conceal fertility management problems which may ignore the need to overcome the primary cause. Hormone use may thus be over-prescribed and potentially misused, and cost-effectiveness may be falsely assumed if outcomes are not monitored (Higgins et al., 2013).

Although hormones are intended to improve reproductive performance but results on the effectiveness are inconclusive. In earlier research, treatment of a prolonged luteal phase with prostaglandin decreased the calving-to-conception interval and the number of insemination-per-conception and increased the first-insemination conception rate (Lüttgenau et al., 2016). On the other hand, the study on the use of progesterone showed no effect on the probability of conception-to-first-insemination and the probability of pregnancy in anoestrus cows (Rabiee et al., 2004). Also, there were no differences in reproductive performance between follicular and luteal cysts in cows treated with progesterone-releasing intravaginal devices compared to non-treated animals (Rudowska et al., 2019). Furthermore, negative effects of treating endometritis with prostaglandin on reproductive performance (Haimerl et al., 2018) were found. A Dutch study, conducted more than 20 years ago, investigated the effect of GnRH treatment on cystic ovarian cows and showed no effect on the insemination-to-conception interval (Hooijer et al., 2001).

The effectiveness of reproductive hormones is commonly studied at the cow level in randomized clinical trials (e.g., Hooijer et al., 2001; Lüttgenau et al., 2016; Rudowska et al., 2019). The herd level effect of hormone use on reproductive performance under field circumstances seems not been studied. Quantifying the effects of reproductive hormones on reproductive performance at the herd level is important in order to evaluate the actual advantage of hormone use in dairy practice. Moreover, the impact of hormone use may be different at herd level compared to cow level because the treatment effect of problematic cows might be alleviated by the reproductive performance of non-problematic cows.

The aim of this study was to investigate the association of total hormone use for reproductive diseases and heat induction with reproductive performance at the herd level in Dutch dairy herds. Additionally, the herd level association between the use of different groups of hormones (prostaglandin, GnRH, and progesterone) and reproductive performance was determined.

2. Materials and methods

2.1. Data collection

Data on the sales of hormones and herd size were obtained from five large veterinary practices (Kernpraktijken Rundvee, Harmelen, the Netherlands) located in different regions of the Netherlands. The sales figures from the veterinary practices represented the total hormone use in each farm considering that hormones are only provided through the veterinary practices which follows the legislation in the Netherlands. The anonymised data consisted of information from 754 farms during the years 2017–2019 with each farm having three observations. The original data thus contained 2262 observations. Three common groups of reproductive hormones, which are frequently applied in dairy farms, namely prostaglandin, GnRH, and progesterone, were evaluated. The hormone groups were categorized based on its product name in the sales invoices. Further details on this data collection are described elsewhere (van der Laan et al., 2021).

Additional data from the regular test-day recording was provided by the Dutch Cattle Improvement Cooperative - CRV (CRV Holding BV, Arnhem, the Netherlands). It included herd level data from 3 years and consisted of yearly averages on reproductive performance and monthly milk production records. The yearly averages on reproductive performance included herd calving interval, herd calving-to-1st insemination interval, and herd number of inseminations per cow. The herd calving interval represented the observed herd average interval between two subsequent calvings in adult dairy cows that survived to the next lactation. The herd calving-to-1st insemination interval represented the observed herd average interval from calving to the first insemination in all adult dairy cows that received at least one insemination in a herd, including those that were culled in the current lactation. The number of inseminations per cow represented the observed herd average number of inseminations to all adult dairy cows in a herd. Artificial insemination (AI) records were provided at cow level and contained information on the breeding strategy: bred by insemination or bred by natural breeding (for those farms which has bull to breed) and who performed the insemination: AI company's person did the insemination or farmer did the insemination. Also, information about the milking system, automatic milking system (AMS) or conventional milking system, was provided. A description of the provided datasets is shown in Table 1.

2.2. Data preparation

Hormone data for each herd consisted of the sum of ml or number of devices sold by the veterinary practice per quarter year for that herd. Subsequently, the sum of ml or number of devices were converted in the number of doses per quarter by dividing the sum of ml or number of devices by the prescribed dose of the hormone's product following the package leaflet. Then, the hormone use was summed into yearly data and standardized as the number of hormone doses per 100 adult dairy cows, and consisted of 2262 observations (Fig. 1). A detailed description of the calculation of the hormone use can be found in van der Laan et al. (2021).

Data on 305-day milk yield and whether farms had an AMS were available at a monthly basis and were aggregated to get the annual average 305-day milk yield. The AMS herds were defined to be the farms that used an AMS for the whole year, or the herds which transitioned from a conventional milking system to an AMS somewhere during the concerning year. Farms that used a conventional milking system throughout the whole year were defined non-AMS herds. Data on the breeding strategy and the person executing AI were recorded at cow level. For the breeding strategy, the data was adjusted to annual herd level data and herds were categorized into two groups, being (1) herds

Table 1
Description of the analyzed datasets (2017 – 2019).

Datasets	Contents	Source
Hormone use	Records of reproductive hormone sale on prostaglandin, GnRH, and progesterone per quarter at the herd level	5 Veterinary practices
Herd size	Records of average number of adult cows per dairy herd per quarter	5 Veterinary practices
Reproductive performance	Yearly herd-level records of calving interval, interval calving to first artificial insemination (AI), and number of AI	Test day recording
Milk production	Monthly herd-level records of 305-day milk yield and automatic milking system use	Test day recording
Insemination records	Cow-level records on the breeding strategy (AI or natural breeding) and who performed AI (AI company or farmer)	Test day recording

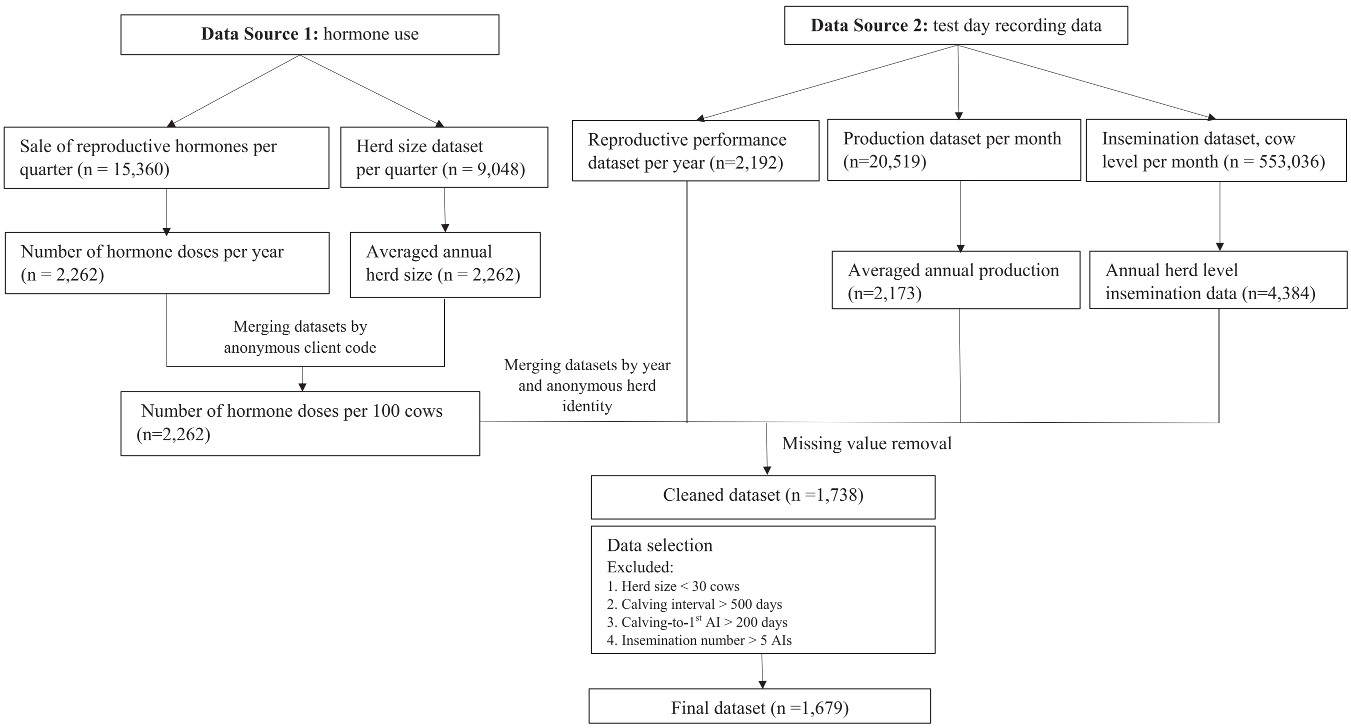

Fig. 1. Data flow chart on hormone use, reproductive performance, production, and insemination records into the final herd level analytical dataset.

where all cows during a year were bred by AI; and (2) herds that applied both AI and natural breeding. The adjustment process to the annual herd level was similar for the "who performed AI" variable and was categorized into three groups namely (1) herds where all cows during a year were inseminated by an AI company technician; (2) herds where all cows during a year were inseminated by the farmer, and (3) herds where cows were inseminated by either an AI company technician or the farmer.

All datasets were merged. During this process, records were excluded because of missing values in one of the datasets, which was mostly a result of herds not participating in the test-day recording (88.4% of farms participated in the test-day recording in 2019; CRV, 2020). This resulted in a dataset containing 1738 observations. Subsequently, only herds with more than 30 producing dairy cows were selected, which were assumed to represent commercial dairy farms in the Netherlands (Kulkarni et al., 2021). In addition, variables containing unrealistic records, i.e., those records having uncommon values and that are likely wrongly recorded, were excluded based on clear cut-off values (an average calving interval of more than 500 days; an average calving-to-1st insemination interval of more than 200 days; and an average number of AI of more than 5 inseminations per cow). Based on descriptive analysis, those values were categorized as the outliers. The final analytical dataset consisted of 1679 observations from 560 farms.

Hormone use had a non-normal distribution due to an excess of non-user herds (i.e., those herds that did not apply any hormones in a specific year; van der Laan et al., 2021). Since no transformation resulted in a linear relationship with the dependent variables, hormone use was categorized into four levels (no usage, low, medium, and high use) following the 33rd and 66th percentiles of the herds applying hormones. The same process was applied for prostaglandin and GnRH use, while progesterone use was categorized into two levels only (non-user and user) due to its low usage. Similarly, the 305-day milk production was also categorized into three levels of low, medium and high milk production following the 33rd and 66th percentiles.

2.3. Statistical analysis

As dependent variables, herd calving interval, herd calving-to-1st insemination interval, and herd number of inseminations per cow were selected. The continuous independent variables on hormone use, herd size, and 305-day milk production were checked for their linear relationship with the dependent variables. The evaluated independent variables are presented in Table 2.

All three reproductive performance indicators were analyzed using multivariable General Estimating Equations (GEE) models. To correct for repeated herd level observations over time, the autoregressive correlation structure was determined with the best model fit among competing correlation structures based on the quasi-likelihood under the independence model criterion (Cui, 2007). The general structure of the multivariable GEE model was defined as follows:

$$Y_i = \beta_0 + \beta_1 HU + \beta_2 year + \beta_3 herdsize + \beta_4 practice + \beta_5 milk + \beta_6 AMS + \beta_7 insem + \beta_8 breeding + \varepsilon_i$$

where Y_i represents one of the three reproductive performance indicators in year *i*, β_0 the intercept, $\beta_1 - \beta_8$ the regression coefficients, *HU* the categorical variable describing the total hormone use (non-user, low user (>0–21.6 doses per 100 cows), medium user (>21.6–50.6 doses per 100 cows), high user (>50.6 doses per 100 cows)), *year* the categorical variable indicating the year of data observation (2017–2019), *herdsize* the mean annual herd size, *practice* the categorical veterinary practice identification number (1−5), *milk* the mean annual herd level 305-day milk production (low (4900–8735 kg), medium (8736–9616 kg), high (9617–12,900 kg)), *AMS* the binary variable indicating whether an AMS was used, *insem* the categorical variable indicating whether cows were inseminated by a technician from a AI company only, or additionally also by the farmer; *breeding* the categorical breeding strategy variable (AI vs AI and natural breeding), and ε the residual error term.

Since the main interest of this study was in the association between hormone usage and reproductive performance at the herd level, the hormone use variable was forced in all regression models. Bi-variable models were created by pairing the total hormone use variable (as the

Table 2
Description and distribution of the independent variables used in the final statistical models.

Variables	Levels	Explanation on transformed data[a]	Number of observations (%)
Total hormone use	non-user	0 doses per 100 cows	134 (8.0)
	low-user	> 0 – 21.6 doses per 100 cows	420 (25.0)
	medium-user	> 21.6 – 50.6 doses per 100 cows	554 (33.0)
	high-user	> 50.6 doses per 100 cows	571 (34.0)
Prostaglandin use	non-user	0 doses per 100 cows	216 (12.9)
	low-user	> 0 – 12.1 doses per 100 cows	338 (20.1)
	medium-user	> 12.1 – 31.0 doses per 100 cows	554 (33.0)
	high-user	> 31.0 doses per 100 cows	571 (34.0)
GnRH use	non-user	0 doses per 100 cows	273 (16.3)
	low-user	> 0 – 5.9 doses per 100 cows	281 (16.7)
	medium-user	> 5.9 – 16.3 doses per 100 cows	555 (33.0)
	high-user	> 16.3 doses per 100 cows	570 (34.0)
Progesterone use	non-user	0 doses per 100 cows	952 (56.7)
	user	≥ 1 doses per 100 cows	727 (43.3)
Year	2017		556 (33.1)
	2018		566 (33.7)
	2019		557 (33.2)
Herd size	Continuous		120 ± 2.0[b]
Veterinary practice	Practice 1		363 (21.6)
	Practice 2		273 (16.3)
	Practice 3		208 (12.4)
	Practice 4		235 (14.0)
	Practice 5		600 (35.7)
305-days milk production	low	4900 – 8735 kg	554 (33.0)
	medium	8736 – 9616 kg	554 (33.0)
	high	9617 – 12,900 kg	571 (34.0)
Automatic milking system	yes		408 (24.3)
	no		1271 (75.7)
Who performed AI	AI company		764 (45.5)
	farmer		354 (21.1)
	AI company and farmer		561 (33.4)
Breeding strategy	AI		1414 (84.2)
	AI and natural breeding		265 (15.8)

[a] Hormone user herds and 305-day milk production were categorized following the 33rd and 66th percentiles
[b] mean±SD (continuous variable)

main predictor of interest) with each other independent variable in association with each of the 3 reproductive performance indicators. Candidate predictor variables were chosen when the *P*-value was below 0.15 based on the Type 3 test. A correlation check was carried out among selected independent variables. For the non-linear associations, an eta correlation test was performed (Garson, 2012) while the Cramer's V correlation test was used when paired variables were both categorical. By using a correlation coefficient cut-off below 0.5, no correlations were identified. Lastly, the multivariable regression modeling process consisted of a backward selection procedure until all predictor variables were significantly associated with the outcome variable. During the backward selection procedure, the presence of confounding was determined, which was assumed when one or more estimates changed more than 25% among nested models. A confounding variable would be maintained in the model but none were identified during this process. Two-way interaction terms between hormone use and all other predictors significantly contributing to each final model were also examined.

The effect of the usage of the three individual hormone groups on reproductive performance was evaluated separately. For this purpose, the variable describing the total hormone use (*HU*) was replaced by the hormone group-specific variables of prostaglandin, GnRH, and progesterone use in the final models. No additional variable removal processes were conducted. All data preparation and regression analyses were performed using R-studio for Windows version 1.4.1103 (R Core Team, 2020) using the 'tidyr', 'tidyverse', 'dplyr', 'lubridate', 'foreign' and 'geepack' packages (Halekoh et al., 2006). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Descriptive statistics

The distribution of the independent variables is described in Table 2. The average proportion of total hormone user-herds was 92.0% while 8.0% of the observations concerned herds with no use. For the average proportion of hormone use per hormone group, 87.1% of the observations concerned herds that used prostaglandin, 83.7% GnRH, and 43.3% progesterone. Most of the farmers (75.7%) were not using an AMS and milked their cows with a conventional milking system. The AI technician did most of the inseminations (on 45.5% of the farms only the AI technician performed them), 33.4% of the farmers inseminated their cows themselves, and on the remaining farms the farmers applied the inseminations together with the AI company (Table 2). Regarding the breeding strategy, the majority of farms were only inseminating their cows (84.2%) while the remaining farms also bred naturally (15.8%; Table 2). The average herd size was 120 ± 2.0 adult dairy cows with the 305-day milk production being on average 9124 ± 26.4 kg (Table 3).

The total hormone use on 560 Dutch dairy farms increased over the three years (Table 3). Across the 3 study years, the median was 36.1 (mean = 43.1; min = 0.0; max = 248.2) doses per 100 adult dairy cows in all herds while the median was 39.2 (mean = 46.8; min = 0.4; max = 248.2) doses per 100 adult dairy cows among the user-herds. The median prostaglandin use was 21.3 (mean = 26.1; min = 0.0; max = 180.0) doses per 100 adult dairy cows, the median GnRH use was 11.0 (mean = 15.3; min = 0.0; max = 127.0) doses per 100 adult dairy cows, and the median progesterone use was 0.0 (mean = 1.8; min = 0.0; max = 40.3) doses per 100 adult dairy cows.

Regarding the reproductive performance, the herd calving interval increased over the three years, with a 3-year average of 411.0 ± 0.6

Table 3
Mean (and SD) hormone use, farm characteristics and reproductive performance in 2017, 2018, and 2019 in 560 Dutch dairy farms.

Variable		Year			
		2017	2018	2019	2017–2019
Hormone use (doses per 100 cows)					
total use in all herds	mean	40.0 ± 1.5	43.8 ± 1.5	45.4 ± 1.6	43.1 ± 0.9
	median	32.3	37.6	38.9	36.1
	(min;max)	(0.0;243.2)	(0.0;200.4)	(0.0;248.2)	(0.0;248.2)
total use in user-herds	mean	43.5 ± 1.5	47.9 ± 1.6	49.2 ± 1.6	46.8 ± 0.9
	median	35.8	41.0	42.9	39.2
	(min;max)	(0.5;243.2)	(0.4;200.4)	(1.1;248.2)	(0.4;248.2)
prostaglandin use	mean	25.7 ± 1.0	25.8 ± 1.0	26.8 ± 1.0	26.1 ± 0.6
	median	20.7	20.6	21.9	21.3
	(min;max)	(0.0;180.0)	(0.0;163.1)	(0.0;145.2)	(0.0;180.0)
GnRH use	mean	12.8 ± 0.6	16.2 ± 0.7	16.7 ± 0.7	15.3 ± 0.4
	median	9.4	11.6	12.0	11.0
	(min;max)	(0.0;125.4)	(0.0;127.0)	(0.0;106.5)	(0.0;127.0)
progesterone use	mean	1.5 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	1.8 ± 0.1
	median	0.0	0.0	0.0	0.0
	(min;max)	(0.0;40.3)	(0.0;24.7)	(0.0;31.6)	(0.0;40.3)
Herd size (cows)		122 ± 3.0	118 ± 3.0	120 ± 4.0	120 ± 2.0
305-day milk production (kg)		8954 ± 44.5	9178 ± 45.0	9240 ± 46.8	9124 ± 26.4
Reproductive performance					
herd calving interval (days)		408.5 ± 0.9	411.4 ± 1.0	413.0 ± 1.0	411.0 ± 0.6
herd calving-to-1st insemination (days)		92.3 ± 0.8	93.5 ± 0.8	93.9 ± 0.9	93.3 ± 0.5
herd inseminations (number)		2.1 ± 0.0	2.2 ± 0.0	2.2 ± 0.0	2.2 ± 0.0

Table 4
Results of the three final generalized estimating equations (GEE) models on the association between total hormone use and herd calving interval, herd calving-to-first insemination interval and herd number of inseminations in 560 Dutch dairy farms.

Variable	Herd calving interval			Herd calving-to-1st insemination			Herd number of inseminations		
	Coefficient	SE	P-value	Coefficient	SE	P-value	Coefficient	SE	P-value
Intercept	423.99	2.72	< 0.0001	113.99	2.13	< 0.0001	1.83	0.04	< 0.0001
Hormone use									
non-user	Reference			Reference			Reference		
low-user	-6.17	2.71	0.02	-7.87	2.20	0.0003	0.03	0.04	0.43
medium-user	-6.51	2.61	0.01	-12.00	2.11	< 0.0001	0.15	0.04	< 0.0001
high-user	-9.25	2.61	0.0004	-16.35	2.09	< 0.0001	0.31	0.04	< 0.0001
Year									
2017	Reference						Reference		
2018	2.92	1.32	0.03	NS[a]			0.04	0.02	0.11
2019	4.82	1.33	0.0003				0.09	0.03	0.0003
Herd size	-0.03	0.01	< 0.0001	-0.05	0.01	< 0.0001	0.001	0.0002	< 0.0001
Veterinary practice									
practice 1	-7.17	1.52	< 0.0001	-5.32	1.27	< 0.0001	-0.04	0.03	0.13
practice 2	-7.75	1.72	< 0.0001	-5.25	1.27	< 0.0001	-0.06	0.03	0.04
practice 3	-6.18	1.69	0.0003	-6.48	1.39	< 0.0001	0.09	0.04	0.01
practice 4	-4.42	1.80	0.01	-3.72	1.46	0.01	0.07	0.03	0.02
practice 5	Reference			Reference			Reference		
305-day milk production									
low	NS			NS			Reference		
medium							0.08	0.03	0.001
high							0.12	0.03	< 0.0001
Who performed AI									
AI company	NS			Reference			Reference		
AI company and farmer				-2.65	1.09	0.01	0.03	0.03	0.38
Farmer				0.74	1.04	0.48	-0.14	0.03	< 0.0001
Breeding strategy									
AI	Reference			NS			Reference		
AI and natural breeding	-3.68	1.62	0.02				-0.21	0.03	< 0.0001

[a] Non-significant

days (Table 3). The herd calving-to-1st insemination interval also increased over the three years with a 3-year average of 93.3 ± 0.5 days, while the herd average number of inseminations per cow was 2.2 ± 0.0.

3.2. *Association of hormone use with reproductive performance indicators at the herd level*

Table 4 shows the results of the final statistical models on the association of hormone use with the three reproductive performance indicators at the herd level. The models identified that herds with a high hormone use had a herd calving interval and a herd calving-to-1st insemination interval that was 9.3 ± 2.6 and 16.4 ± 2.1 days shorter than non-user herds, respectively. Furthermore, herds with a high hormone use needed on average 0.3 ± 0.04 inseminations more to get their cows pregnant compared to non-user herds. Medium-user herds had a 6.5 ± 2.6 days shorter herd calving interval and a 12.0 ± 2.1 days shorter herd calving-to-1st insemination interval with 0.2 ± 0.04 additional inseminations per cow compared to non-user herds. Low-user

Table 5
Results of the three final generalized estimating equations (GEE) models on the association between the three groups of hormone use and herd calving interval, herd calving to first insemination interval and herd number of inseminations in 560 Dutch dairy farms.

Variable	Herd calving interval			Herd calving-to-1st insemination			Herd number of inseminations		
	Coefficient	SE	P-value	Coefficient	SE	P-value	Coefficient	SE	P-value
Intercept	424.07	2.36	< 0.0001	113.32	1.82	< 0.0001	1.85	0.04	< 0.0001
Prostaglandin use									
non-user	Reference			Reference			Reference		
low-user	-4.98	2.54	0.04	-5.17	2.01	0.01	-0.01	0.04	0.90
medium-user	-5.25	2.45	0.03	-7.17	1.93	0.0002	0.06	0.04	0.13
high-user	-4.99	2.59	0.05	-10.30	2.06	< 0.0001	0.23	0.05	< 0.0001
GnRH use									
non-user	Reference			Reference			Reference		
low-user	-1.33	2.33	0.57	-2.51	1.90	0.19	0.01	0.04	0.85
medium-user	-1.90	2.08	0.36	-4.22	1.70	0.01	0.06	0.04	0.09
high-user	-5.21	2.11	0.01	-6.93	1.75	< 0.0001	0.10	0.04	0.01
Progesterone use									
non-user	Reference			Reference			Reference		
user	0.85	1.21	0.48	0.35	0.96	0.72	-0.01	0.02	0.85
Year									
2017	Reference			NS[a]			Reference		
2018	3.03	1.32	0.02				0.04	0.02	0.06
2019	4.90	1.34	0.0002				0.09	0.02	0.0002
Herd size (mean and SD)	-0.03	0.01	< 0.0001	-0.05	0.01	< 0.0001	0.001	0.0002	< 0.0001
Veterinary practice									
practice 1	-7.66	1.54	< 0.0001	-5.52	1.28	< 0.0001	-0.05	0.03	0.07
practice 2	-8.05	1.75	< 0.0001	-5.32	1.28	< 0.0001	-0.07	0.03	0.02
practice 3	-6.03	1.68	0.0003	-6.21	1.38	< 0.0001	0.08	0.04	0.03
practice 4	-5.21	1.83	0.004	-4.21	1.49	0.005	0.07	0.03	0.03
practice 5	Reference			Reference			Reference		
305-day milk production									
low	NS			NS			Reference		
medium							0.09	0.03	0.0009
high							0.12	0.03	< 0.0001
Automatic milking system									
yes	NS			NS			NS		
no									
Who performed AI									
AI company	NS			Reference			Reference		
AI company and farmer				-2.63	1.10	0.02	0.02	0.03	0.45
farmer				0.95	1.04	0.36	-0.15	0.03	< 0.0001
Breeding strategy									
AI	Reference						Reference		
AI and natural breeding	-3.78	1.60	0.01	NS[a]			-0.21		< 0.0001
							0.03		

[a] Non-significant

herds had a 6.2 ± 2.7 days shorter herd calving interval and a 7.9 ± 2.2 days shorter herd calving-to-1st insemination interval than herds with no hormone use.

The hormone-group specific models of reproductive hormone use with reproductive performances are described in Table 5. The models produced the same trend for prostaglandin and GnRH use as for the total hormone use, with the higher use being associated with a shorter herd calving interval, a herd shorter calving-to-1st insemination interval, and a higher insemination number per cow. Farms with a high prostaglandin use had a herd calving interval and a herd calving-to-1st insemination interval that was 5.0 ± 2.6 and 10.3 ± 2.1 days shorter than non-user farms, respectively. Meanwhile, they needed on average 0.2 ± 0.05 inseminations more to get their cows pregnant compared to non-user herds. Medium prostaglandin user herds had a 5.3 ± 2.5 days shorter herd calving interval and a 7.2 ± 1.9 days shorter herd calving-to-1st insemination interval compared to non-user herds. Low prostaglandin user herds had a 5.0 ± 2.5 days shorter herd calving interval and a 5.2 ± 2.0 days shorter herd calving-to-1st insemination interval compared to non-user herds.

The high-user GnRH farms had a 5.2 ± 2.1 days shorter herd calving interval and a 6.9 ± 1.8 days shorter herd calving-to-1st insemination interval with 0.1 ± 0.04 additional inseminations per cow compared to non-user farms. Medium GnRH user herds had a 4.2 ± 1.7 days shorter herd calving-to-1st insemination interval compared to non-user herds. For progesterone use, the opposite effect was observed for all three reproductive performance indicators but none of the associations were significant.

4. Discussion

This observational study showed that the total hormone was associated with better reproductive performances, aside from the insemination number per cow. This finding could be explained by the fact that especially problem herds with reproductive diseases occurrence and poor reproductive performance are likely to apply hormones, and thus had a better reproductive performance than low or non-user herds. This study result would fit the farmers' expectation of the positive effect on the use of reproductive hormones. In addition, farmers having already a good reproductive performance may still want to maintain or improve their reproductive performances by using hormones. Furthermore, the shorter herd calving interval and herd calving-to-1st insemination interval and the higher number of inseminations per cow are likely a result of farmers aiming to achieve their reproductive goals within pre-set boundaries using hormones. Therefore, farmers appear to accept the extra use of hormones and inseminations to minimize the possibility of a delayed pregnancy or involuntary culling caused by poor reproductive performance. These observations correspond with earlier research where re-insemination of cows was found economically beneficial (van

Arendonk and Liinamo, 2003), with a maximum of three inseminations per cow (Inchaisri et al., 2011).

Compared to cow level studies, the positive effect of hormone use on reproductive performance was also revealed by a bio-economic simulation model (Ricci et al., 2020). Simulation results showed that a higher hormonal use resulted in an increased rate of pregnancy per insemination but the modeling was done in the context of intensive reproductive programs whereas synchronization was hardly applied in the herds investigated here (van der Laan et al., 2021). Also, a randomized controlled trial determined that use of GnRH is the best choice for early postpartum dairy cows to have fewer days open and a high conception per first insemination rate, resulting in a higher total milk production per lactation (El Tahawy and El Sharkawy, 2014).

The significant associations of prostaglandin and GnRH use with better reproductive performance agreed with some of the previous cow level studies. Prostaglandin use decreased the incidence of prolonged luteal phase and, subsequently, reduced the calving to conception interval (Lüttgenau et al., 2016). On the other hand, GnRH decreased the incidence of cystic ovarian follicle but did not improve the calving to conception interval (Lüttgenau et al., 2016) and insemination to conception interval (Hooijer et al., 2001). Prostaglandin treatment of subclinical endometritis also improves conception rate and days open (Kasimanickam et al., 2005) and first-insemination pregnancy per AI (Galvão et al., 2009). Contrary to the positive effect of prostaglandin, further studies described negligible effects for reproductive performance improvement after applying prostaglandin (Dubuc et al., 2011; Giuliodori et al., 2017; Borchardt et al., 2018; Haimerl et al., 2018). In terms of progesterone use, non-significant effects on reproductive performance were found (Rabiee et al., 2004; Marques et al., 2014; Rudowska et al., 2019). It has to be noted though that comparing the herd level associations from the current study with cow level results from previous studies has to be done with caution considering the aggregated individual factors in the current herd-level study (Bello et al., 2012). Furthermore, for most of the farms, herd level reproductive performance is mostly based on the untreated cows, which is the majority of the herd, and could thus result in bias due to ecological fallacy. Nonetheless, the current herd level study captures the situation in dairy practice and provides insights in the herd level effect of hormone use on herd reproductive performance.

For the association between total hormone use and herd calving interval, the estimates varied between 6.2 and 9.3 days for low and high users, respectively. For herd calving-to-1st insemination interval, the estimates varied between 7.9 and 16.4 days for low and high users, respectively (Table 4). In absolute numbers, the effect on calving interval is thus smaller while the calving-to-1st insemination interval is part of the calving interval. This discrepancy is due to the difference in cows being included in the interval calculations. For calculation of the herd calving interval, the culled dairy cows which did not survive to the next lactation were excluded while they were included for calculation of the herd calving-to-1st insemination interval.

The association of prostaglandin and GnRH use had the same direction with the total hormone use, but not for the progesterone. Among the three hormone groups, the use of prostaglandin was the highest and followed by GnRH, while progesterone was the lowest. The reason for this is that prostaglandin and GnRH are commonly used in the Netherlands, while the use of vaginal progesterone devices is low. Furthermore, the use of progesterone should always be combined with a prostaglandin and GnRH injection. This means that the effect of progesterone on herd reproductive performance as a single dose is difficult to evaluate, since it is a combination of three different doses of reproductive hormones.

In this study, using herd calving interval as one of the reproductive performance indicators has a disadvantage over other indicators because it excludes cows that received at least one insemination but did not survive until the next lactation, introducing potential bias. Therefore, the herd calving-to-1st insemination interval was additionally analyzed which includes cows that did not survive until the next lactation. Given their high correlation, those two reproductive performances gave the same direction of the association. On the other hand, conception indicators (e.g., calving to conception interval, insemination to conception interval, number of insemination-per-conception and conception rate) were frequently used in previous studies on measuring reproductive performance (Hooijer et al., 2001; Rabiee et al., 2004; Lüttgenau et al., 2016; Haimerl et al., 2018). The conception data based on veterinarian diagnosis by rectal palpation, ultrasonography, or blood sample hormonal testing was however not available in routine Dutch test-day recording data.

It was assumed that hormones were applied only to dairy cows, while in fact they could also have been applied on some farms to young stock (van der Laan et al., 2021). Hormone use in young stock is expecting to be lower since their reproductive performance is better than of lactating cows (Morton and Hons, 2004; Hare et al., 2006). Nevertheless, the total number of hormone use could be slightly overestimated which might lead to the underestimation of the effect of hormone use on reproductive performance of the lactating herd. Furthermore, the initial hormone sales dataset was provided without knowing the purpose of the application, so whether it was used for treatment of reproductive diseases or heat induction. Nonetheless, we assumed that there would be no other purpose, such as oestrus synchronization since that is hardly applied in the Netherlands (van der Laan et al., 2021). Finally, it should be noted that the herd-level hormone data represents the number of doses per 100 adult dairy cows and not the number of treated cows. The possibility of cows receiving numerous doses of hormones should be considered. The proportion of problematic cows within a herd is therefore unknown, but might affect the overall herd reproductive performance.

Hormone administration is an intervention to treat reproductive diseases or initiate oestrus when oestrus signs are not expressed or not observed. Using hormones might thus be a result of suboptimal reproductive management. Consequently, it is important to find the primary cause and prevent further reproduction problems. Poor reproductive performance is multifactorial and enhancing herd fertility often needs an improvement of multiple interfering managerial points (Opsomer et al., 2006). Good reproductive management practices include reproduction data recording, genetic selection, nutritional strategies, and biosecurity measures to prevent reproductive diseases. If hormones are applied, its use should be based on an accurate diagnosis (Refsdal, 2000) and followed by veterinary advice to improve reproduction management in the farm (Opsomer et al., 2006). Moreover, in order to reduce the use of hormones for heat induction due to missed oestrus observation, a better heat detection is needed through improving visual observations by farmers or using sensors for heat detection (Crowe et al., 2018). Responsible use of hormones needs to be considered as part of ethical concerns and to respond to the consumer's perspective on hormone use in dairy cows (Higgins et al., 2013; Pieper et al., 2016). As a start, in the Netherlands, prudent hormone use is promoted by a recently published guideline for practicing cattle veterinarians on optimizing hormone use and reproduction management (KNMvD, 2020). Whether such a promotion leads to a more prudent hormone use or a change in reproductive performance remains uncertain though.

In conclusion, using a large representative herd level dataset, hormone use was associated with a better reproductive performance in terms of herd calving interval and herd calving-to-1st insemination interval. However, the herd average number of inseminations per cow was slightly higher on farms with a high hormone use. Similarly, a higher prostaglandin and GnRH use was associated with a shorter herd calving interval and herd calving-to-1st insemination interval but with a higher number of inseminations per cow. The opposite was found for progesterone use, but with non-significant associations. It is uncertain how a more prudent hormone use will associate with a change in reproductive performance.

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Declaration of Competing Interest

The authors report no declarations of interest.

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