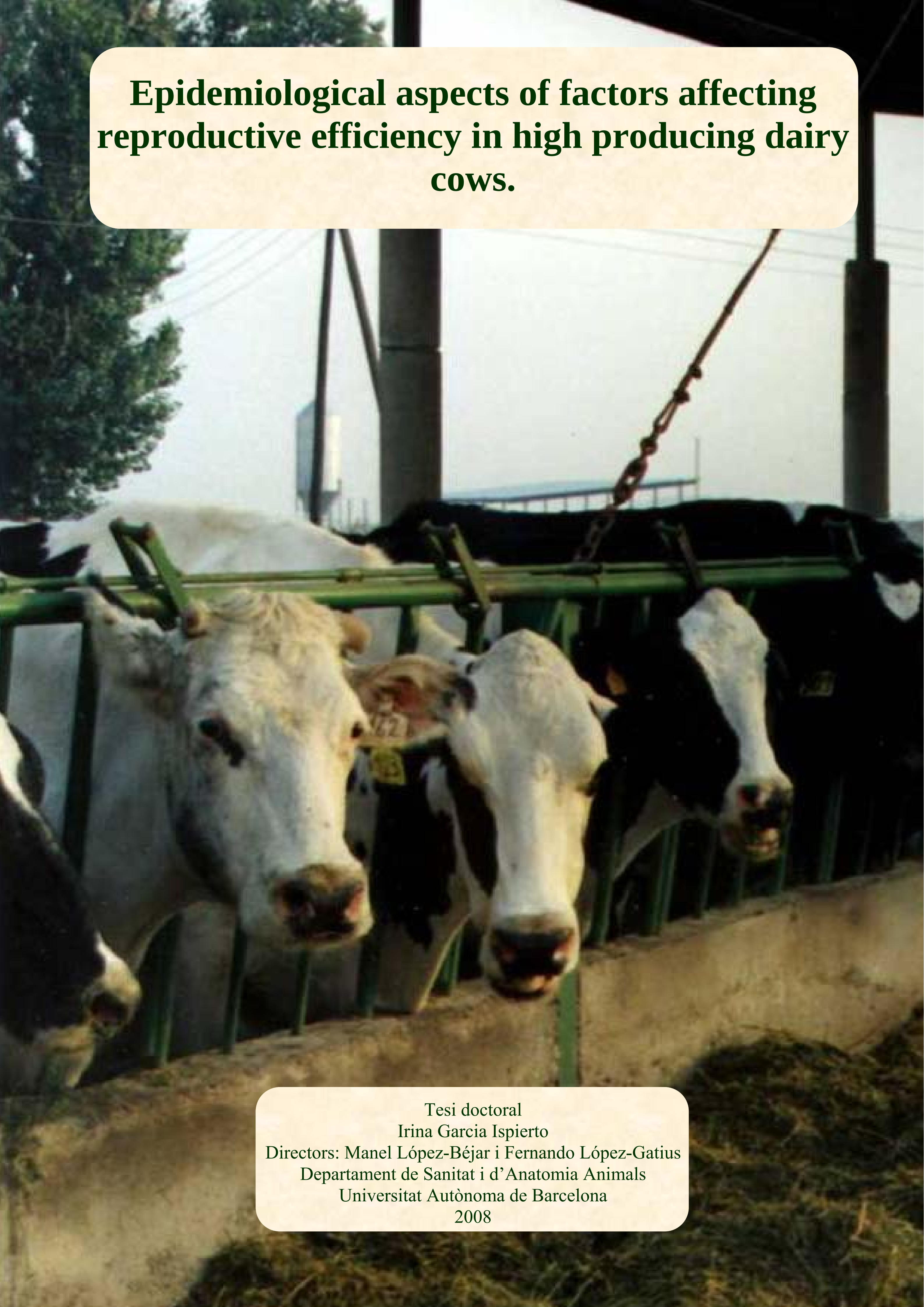
Epidemiological aspects of factors affecting reproductive efficiency in high producing dairy cows.



Tesi doctoral
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El genio comienza las grandes obras,
pero sólo el trabajo las acaba.

Joseph Joubert

Un amic és aquell que ho sap
tot de tu, i malgrat tot t’estima.

Elbert Hubbard

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Agraïments

Sempre he pensat que els agraïments d'una tesi són molt importants per dos motius bàsics: perquè és la única part que es llegeix tothom (i no em convencereu del contrari), però també perquè penso que els agraïments diuen molt de la persona que els escriu. Per això intentaré fer-los el millor que pugui.

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Abstract

The rapid worldwide progress in management and genetics of dairy herds has culminated in an increase of milk production and number of animals per herd. But despite this rapid progress, reproductive performance and reproductive disorders of high producing dairy has suffered a dramatic decrease and increase, respectively, since the mid 1980's. Management practices have also suffered significant changes during the last decades besides an increase in milk production. The most comfortable temperatures for dairy cows ranged from 5 to 25ºC. Therefore, heat stress in not confined in tropical regions of the world and has a great impact on farm economy. The main objectives of this thesis were to study environmental and management factors that affect fertility and pregnancy losses in high producing dairy cattle in northeastern Spain, a country with a seasonal warm weather. The studies were performed in high producing dairy herds in Lleida. Climatic data were obtained from a meteorological station located less than 6 km from the herds and management data were recorded in each herd.

In the first study results indicated that temperature humidity index during the periimplantational period and warm season were a risk factor for pregnancy losses.

In the second, cows that get pregnant before 90 days postpartum were those who produce more milk on 50 days postpartum. Management practices of these herds offset the negative effects of milk production.

In the third, management practices demonstrated to be an important factor for conception rate. Three times per day milking, inseminating bull and AI technician were a risk factors for infertility.

In the fourth, environment proved to be directly affecting conception rate especially around AI period.

In the fifth, monthly summary records provide risk markers for fertility: low milk production and ovarian cysts.

In conclusion, to increase reproductive performance of high producing dairy herds, it is necessary to monitor their status periodically. Moreover, management and environmental factors of dairy herds have a great importance on economic success of high producing dairy farms.

General Introduction

Introduction.

The principal objective of dairy herds is to produce economic milk. In our countries, the production is based in high industrialization of the process, where only herds with high production per cow are economically rentable (Line, 1986). The rapid worldwide progress in management and genetics of dairy herds has culminated in an increase of milk production and number of animals per herd. But despite this rapid progress, reproductive performance of high producing dairy herds not only have not increased, but also has suffered a dramatic decrease since the mid 1980's (Beam and Butler, 1999; Royal et al, 2000; Lucy, 2001; López-Gatius, 2003). The reason for the decrease in fertility has been solely attributed to the selection for milk production, but it is possible that the causes are multifactorial and not only related to this fact (Lucy, 2003). Many management practices and environmental factors could influence reproductive performance of dairy herds.

The impact of high environmental temperatures on reproductive performance of dairy cows has been extensively reviewed (Hansen and Arechiga, 1999; De Rensis and Scaramuzzi, 2003; López-Gatius, 2003;West, 2003; Collier et al, 2006). Heat stress could be defined as the sum of forces external to a homeothermic animal that acts to displace body temperature from the resting state (Yousef, 1984). This factor may increase in magnitude if global warming occurs (Hulme, 1997). The most comfortable temperatures for dairy cows ranged from 5 to 25ºC, which is the thermal comfort zone (Mc Dowell, 1972). Homeotherms have optimal temperature zones for production within which no additional energy above maintenance is expended to heat or cool the body. In this comfort temperature zone, minimal physiological cost and maximum productivity arises (Folk, 1974). In countries with tropical and subtropical climate or even in Spain, the 25ºC temperature is exceeded in many days in summer, but even in spring or autumn. Therefore, the problem of heat stress in not confined in tropical regions of the world and has a great impact on farm economy. Such stress can disrupt both the physiology and productive performance of dairy cows. The most important effect of heat stressed cows is the increase in infertility (DeRensis and Scaramuzzi, 2003; López-Gatius, 2003).

Effective environmental temperature can be affected by 4 factors (Bufimgton et al, 1981): air temperature, solar radiation, air movement and relative humidity. The temperature–humidity index (THI) incorporates the effects of both ambient temperature and relative humidity (RH) in an index (Thom, 1958). This index was created by Thom (1958) to analyze the comfort thermal sensation in humans, and later the Livestock Conservation Institute observed that this index was a good heat stress predictor also for dairy cows. This index has become recently one of the most used worldwide in hot areas to assess the impact of heat stress on dairy cows (Hahn, 1969; Fuquay 1961).

The decrease in conception rate can range between 20-30% in the hot season compared to the winter season (Cavestany et al 1985; Badinga et al, 1985). Heat stress not only contributes to the low fertility in summer months, but also increases early fetal losses. For example, in Spain, based on the odds ratio, cows that became pregnant during the warm period, bearing singletons or twins, were 3.7 and 5.4 times more likely to miscarry, respectively, compared to the cool period (López-Gatius et al, 2004). The profitability of dairy herds greatly depends on cows ability to get pregnant and to maintain the gestation. Although most pregnancy losses occur during the early embryonic period (Peters, 1996), the risk of non-infectious early fetal loss appears to increase under the conditions of intensive management systems (Forar et al, 1995; Hanzen et al, 1999). For that reason early fetal losses have a greater impact on dairy herd economy. There is a lack of studies analyzing the direct effects of temperature or THI on conception rate or pregnancy losses in high producing dairy cows. To try to solve heat stress problem, more studies are needed to specify the effects of heat stress on dairy farms and to know the most important factors affecting early fetal losses in dairy cattle.

Many factors can affect reproductive performance of high producing dairy cows apart from environment. As it has been said, milk production correlates with decrease in fertility (Pryce et al, 1998; Raw et al, 1998; Abdallah et al, 2000), but it is only evident under suboptimal conditions (Fahey et al, 2002; Calus et al, 2005). There is reasonable evidence that nutrition limits fertility and milk yield trough the mechanism of energy balance (Spicer *et al* 1990, Zurek *et al* 1995, Senatore *et al* 1998). Then, management factors such as nutrition and comfort of animals and cleanness as well, can play an important role in fertility of high producing dairy cows (Windig *et al* 2005). Better management practices may compensate for a slight decline in reproduction effects caused by high level of milk production of best dairy cows (Hansen 2000). Higher producing cows are often the healthiest cows because of better feeding and reproductive management, and may actually cycle earlier in the post-partum period (Staples et al, 1990; López-Gatius, 2003). The main question is whether there is phenotypic variation in relationship between milk yield and fertility and whether this variation can be related to management practices. Then, it is needed to gain further knowledge on high producing management routines to improve not only milk production, but also reproductive performance of high producing dairy cows.

The past five decades there has been an increase of reproductive disorders worldwide, accompanied with decreasing fertility and increasing milk production despite the programs developed by veterinarians to improve reproductive herd health, at least in countries with intensive dairy industry (Foote, 1996; López-Gatius, 2003). Moreover, they have been described as a risk factor for infertility (Jooster et al, 1988; Borsberry and Dobson 1989; Eiler, 1997; Fourichon et al, 2000). However, in our geographical area of study, when reproductive disorders are stratified by season, the cool season

(October to April) is related to a reduction in reproductive disorders and an increase in infertility compared with the warm season (May to September) (López-Gatius, 2003). An optimal study of records of monthly and annual reproductive parameters has become a useful tool to help in management of high producing dairy cows. Annual and monthly records provide commonly access to the reproductive production performance results of management on the dairy herd, either to the dairy owner as to the veterinarian. Computerized records have become a great tool and nearly a necessity to manage data efficiently for high producing dairy herds (Spahr, 1993). However, numerous studies have described associations between reproductive disorders and fertility from the individual cow but not by using summary records of farms of high producing dairy cows.

In conclusion, the aims of this thesis are to study the effects of management practices and environmental factors on fertility and pregnancy losses of high producing dairy cattle in Northeaster Spain.

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Aims of the study

Main objectives.

- Establish whether THI values from Days 1 to 40 of gestation could be associated with the pregnancy loss rate of high producing dairy cows during the early fetal period.

- Examine the impact of several climate variables such as temperature, rainfall, and THI on the conception rate of high producing dairy cows in northeastern Spain.

- Establish management risk factors for early pregnancy loss in high producing dairy cows.

- Evaluate the effects of a series of management indicators on the fertility of high producing dairy herds.

- Evaluate relationships between management, production and reproductive data, and high fertility (represented by cows becoming pregnant before 90 days in milk), in two high-producing dairy herds.

- Examine possible associations between production, the incidence of several reproductive disorders and the fertility of high producing dairy herds under warm conditions by using annual and monthly summary records.

Relationship between heat stress during the peri-implantation period and early fetal loss in dairy cattle

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Abstract

The aim of the present study was to establish whether temperature–humidity index values, as a measure of heat comfort, from Days 1 to 40 of gestation could be associated with the pregnancy loss rate in high producing dairy cows. Data from 1391 pregnancies were recorded. Pregnancy was diagnosed by transrectal ultrasonography between Days 34 and 45, and again 90 days after insemination. Pregnancy loss was assumed when the second pregnancy diagnosis on Day 90 proved negative and was registered in 7.8% (108/1391) of pregnancies. Mean and maximum temperature–humidity index values were established for each cow for Days 0 (day of insemination), 1, 2 and 3 after insemination, and averages established for Days 0–3, 0–10, 11–20, 21–30 and 31–40 after insemination. Cow and management variables previously found to be significantly correlated with the early fetal loss in the same geographical area were also recorded. The relative contribution of each factor to the probability of pregnancy loss was determined using logistic regression models. Based on the odds ratio, a strong association with pregnancy loss of the factors warm period of pregnancy (warm period–May to September versus cool–October to April), twin pregnancy (as negative factors: odds ratios 3.1 and 3.4, respectively) and an additional corpus luteum (as a positive factor: odds ratio 0.32) was confirmed. The likelihood of pregnancy loss increased by a factor of 1.05 for each additional unit of the mean maximum temperature–humidity index from Days 21 to 30 of gestation.

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Logistic regression analysis revealed no significant effects of temperature–humidity index values for the remaining gestation periods. Our results indicate that heat stress can compromise the success of gestation during the peri-implantation period, such that high temperature–humidity index values for the period 21–30 days of gestation are a risk factor for subsequent early fetal loss.

Keywords: Pregnancy loss; Early fetal period; Heat stress; Dairy cows

1. Introduction

Prenatal loss is probably one of the most important factors affecting the reproductive performance of high producing dairy cattle and has a substantial impact on the profitability of cow production [1,2]. Although most pregnancy losses occur during the early embryonic period [3], the risk of non-infectious early fetal loss appears to increase under the conditions of intensive management systems [4,5]. The embryonic period of gestation extends from conception to the end of the differentiation stage (about 42 days) and the fetal period spans from Day 42 to parturition [6]. An early fetal loss rate of 10% is a commonly accepted figure [2,7] and is often multifactorial in origin and difficult to diagnose [2]. In the geographical area of our study, there are only two distinguishable seasonal periods: warm (May–September) and cool (October–April). We previously demonstrated that reproductive variables are significantly impaired during the warm period [8,9] and also noted a significant correlation between season of insemination and early fetal loss [10–13]. For example, based on the odds ratio, cows that became pregnant during the warm period, bearing singletons or twins, were 3.7 and 5.4 times more likely to miscarry, respectively, compared to the cool period [13]. To our knowledge, however, the possible direct effects of heat stress on early fetal loss have not been previously explored. The temperature–humidity index (THI) is a measure of heat comfort used to characterize or quantify thermoneutral zones, and has been proposed as a tool for estimating the heat stress suffered by lactating dairy cows [14–16]. The THI includes factors related to both the heat environment and the stress caused by the environment [17,18]. The maximum THI, defining maximum temperature and minimum humidity, is considered among the factors that most effectively assesses the effect of heat stress on livestock [19–21]. Using this measure, a THI value above 72 units can be taken to indicate heat stress in animals, especially cows under intensive management conditions [22]. The aim of this study was to establish whether THI values from Days 1 to 40 of gestation could be associated with the pregnancy loss rate of high producing dairy cows during the early fetal period. The effect of cow and management variables previously found to be significantly correlated with early fetal loss in the same area were also analyzed.

2. Materials and methods

2.1. Cattle and herd management

The study was performed on three commercial dairy herds of approximately 210, 510 and 1080 heads of mature lactating Holstein–Friesian cows in Northeast Spain over a 33-month period (January 1, 2002–September 30, 2004). All animals were reared within the herds. The cows, kept in open stalls and milked three times daily, calved all year round. For the study period, mean annual milk production was 10890 kg per cow and the culling rate was 30% for all the herds. Only cows free from detectable reproductive disorders and with a body condition score between 2.5 and 3.5 were inseminated. Insemination was performed using frozen semen from 35 independent bulls of proven fertility. Artificial insemination (AI) was the only form of breeding used in these herds and the voluntary waiting period from calving to first AI was 50 days.

As part of a weekly reproductive health program, pregnancy was diagnosed by transrectal ultrasonography or by palpation per rectum between 34 and 45 days after insemination. A second diagnosis was made by palpation per rectum on Day 90. Only cows diagnosed pregnant by ultrasound in their first pregnancy diagnosis were included in the study. A cow was included once per lactation such that only the first diagnosed pregnancy within a lactation period was entered in the study. Only animals free from clinical disease from first pregnancy diagnosis to 90 days of gestation were used in the study. The final study series was comprised of 1391 pregnancies.

2.2. Pregnancy diagnosis

A portable B-mode ultrasound scanner (Scanner 100 Vet equipped with a 5.0 MHz transducer; Pie Medical, Maastricht, The Netherlands) was used for pregnancy diagnosis. Scanning was performed along the dorsal/lateral surface of each uterine horn to determine the presence of twins. Each ovary was also scanned to identify luteal structures. Pregnancies in which the number of corpora lutea exceeded the embryo number were recorded as pregnancies with an additional corpus luteum. Pregnancy loss was recorded when the Day 90 pregnancy diagnosis proved negative. All pregnancy diagnoses were performed by the same operator.

2.3. Climate variables

Climatic data such as daily mean and maximum temperatures, and mean and minimum relative humidity were obtained from a meteorological station located less than 6 km from the herds. Temperature–humidity indices (THI) were calculated. The mean and maximum THI indices were fitted to the law equations [19,20,23]:

$$\text{Mean THI} = \left(0.8 \times \text{mean}\ \ T + \frac{\text{mean}\ \ \text{RH}\ \ (\%)}{100} \times (\text{mean}\ \ T - 14.4) + 46.4\right);$$

$$\text{Maximum THI} = \left(0.8 \times \text{maximum}\, T + \frac{\text{minimum RH}\, (\%)}{100} \times (\text{maximum}\, T - 14.4) + 46.4\right)$$

where, T is temperature and RH is relative humidity.

2.4. Data collection and analysis

The following data were recorded for each animal: herd, lactation number, milk production at pregnancy diagnosis, semen-providing bull, interval from calving to pregnancy, number of services, pregnancy loss, presence of an additional corpus luteum, presence of twins, season of insemination (warm–May to September versus cool –October to April). Mean and maximum THI were calculated for each cow for the time-points Days 0 (day of AI), 1, 2 and 3 after insemination. Averages were furthermore established for the periods 0–3, 0–10, 11–20, 21–30 and 31–40 days.

The relative contribution of each factor to the probability of pregnancy loss was determined using logistic regression models. Pregnancy loss was considered as the dependent variable and presence of an additional corpus luteum, presence of twins, and season (warm-period) as dichotomous variables (where "1" denotes presence and "0" absence). Milk production, number of services, the interval from calving to pregnancy, lactation number and THI values (continuous variables), and the semen-providing bull and herd (class variables) were factors in the analysis.

Regression analysis (SAS software, Logistic procedure; [24]) was conducted according to the method of Hosmer and Lemeshow [25]. Basically, this method involves five steps as follows: preliminary screening of all variables for univariate associations; construction of a full model using all the variables found to be significant in the univariate analysis; stepwise removal of non-significant variables from the full model and comparison of the reduced model with the previous model for model fit and confounding; evaluation of interactions among variables and assessment of model fit using Hosmer–Lemeshow statistics. Variables with univariate associations showing P values <0.25 were included in the initial model. We continued modeling until all the main effects or interaction terms were significant according to the Wald statistic at $P < 0.05$.

3. Results

Mean monthly climate variables for the study period are provided in Table 1. There were significant differences in temperature and THI between the warm seasonal period (May–September) and the cool seasonal period (October–April).

The mean lactation number was 2.4 ± 1.5 (mean $\pm$ S.D.; range: 1–9 lactations). Mean daily milk production at AI was 40.8 ± 9.2 (range: 18–74 kg). The mean number of days in milk at AI was 141 ± 93 (range: 48–396). The mean service number at the time of pregnancy diagnosis was 2.8 ± 2.1 (range: 1–15). Six hundred and nineteen cows (44.5%) became pregnant during the warm and 772 (55.5%) during the cool period.

One thousand two hundred and sixty nine cows (91.2%) bore singletons and 122 (8.8%) carried twins. No triplets were recorded. One hundred and twenty three cows (8.9%) had and additional corpus luteum: 120 (9.5%) in single and 3 (2.5%) in twin pregnancies. Pregnancy loss was registered in 7.8% (108/1391) of pregnancies: 9.4% (29/310), 7.4% (65/876) and 6.8% (14/205) in herds 1, 2 and 3, respectively.

Logistic regressions analysis revealed no significant effects of herd, number of services, the interval from calving to pregnancy, milk production at AI, semen-providing bull, mean and maximum THI values for Days 0–3 after insemination, and for the averages for Days 0–3, 0–10, 11–20 and 31–40 after insemination, and mean THI values from 21 to 30 days of gestation. Table 2 shows the pregnancy loss rates and odds ratios of the variables finally included in the logistic model. No significant interactions were found. Based on the odds

Table 1
Mean monthly climate variables for the study period (33 months)

Month	T (°C)	Maximum T (°C)	RH (%)	Minimum RH (%)	THI	Maximum THI
January	5.6	10.8	91	74	42.9	52.2
February	6.2	11.6	86	67	44.1	52.2
March	10.3	17.2	82	56	51.1	61.4
April	12.7	19.4	80	53	55.0	64.1
May	16.5	23.5	76	51	61.0	69.3
June	23.2	31.0	72	47	71.2	78.9
July	23.7	31.4	79	52	72.8	80.3
August	23.8	31.5	81	54	73.0	80.7
September	19.5	26.8	89	64	66.7	75.8
October	14.3	20.2	91	72	57.7	66.4
November	10.1	15.1	92	80	50.4	58.7
December	6.8	11.1	94	85	44.6	52.4

T, temperature; RH, relative humidity; THI, temperature–humidity index.

Table 2
Odds ratios and pregnancy loss rates of variables included in the final logistic regression model for early fetal loss

Factor	Class	n	% Pregnancy loss	Odds ratio	95% Confidence interval	P
Lactation	Continuous	1391		1.24	1.09–1.40	0.001
Season	Cool	13/619	2.1			
	Warm	95/772	12.3	3.05	1.20–7.73	0.019
Additional corpus luteum	0	104/1268	8.2			
	1	4/123	3.3	0.32	0.11–0.99	0.032
Twins	0	81/1269	6.4			
	1	27/122	22.1	3.44	2.07–5.73	<0.0001
Maximum THI during gestation Days 21–30	Continuous	1391		1.05	1.01–1.09	0.025

Likelihood ratio test, 658; 5 d.f., $P = 0.0001$. Hosmer and Lemeshow Goodness-of-fit test, 4.41; 8 d.f., $P = 0.82$ (the model fits).

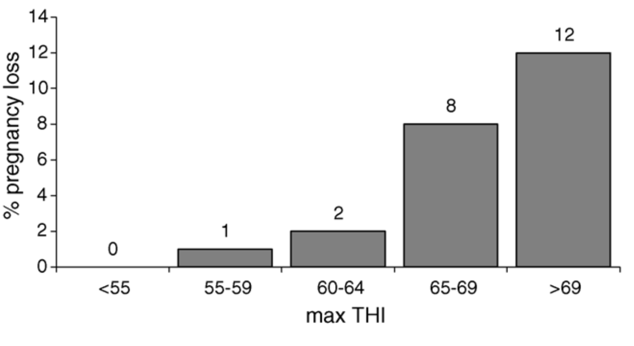

Fig. 1. Pregnancy loss rates for different maximum temperature–humidity indices during Days 21–30 of gestation.

ratio, a one-unit increase in lactation number yielded a 1.24-fold increase in the risk of pregnancy failure. Insemination during the warm period led to a 3.05-fold increase in pregnancy loss. The likelihood of pregnancy failure was 0.32 times lower for cows showing an additional corpus luteum. The presence of twins led to a 3.44-fold increase in pregnancy failure. The likelihood of pregnancy loss increased by a factor of 1.05 for each unit increase in mean maximum THI from Days 21 to 30 of gestation. Fig. 1 shows pregnancy loss rates for the different maximum THI values during this period.

4. Discussion

The most significant finding of our study was the clear relationship between mean maximum THI over the period Days 21–30 of gestation and subsequent fetal loss. The factors lactation number, season, twin pregnancy and additional corpus luteum were also found to significantly affect the rate of early fetal loss.

Heat stress is a greater problem, especially in high producing dairy cows [9,26], and even though the temperature–humidity index (THI) quantifies the effect of heat stress on livestock [19,20], we tested whether temperature alone or humidity alone could be a better predictor of pregnancy loss than THI. Two further logistic regression analyses including humidity alone or maximum temperature alone, respectively, were performed without THI. Both models (not presented) were practically the same from that presented in Table 2. Humidity did not affect pregnancy for any point time considered, and maximum temperature from Days 21 to 30 of gestation was a risk factor for pregnancy loss. However, including temperature alone, the model was worse fitted ($P = 0.50$) than that including THI ($P = 0.82$). Therefore, under our work conditions, we considered that THI was a better predictor of fetal loss than just temperature alone or humidity alone.

Although there was a logical high correlation between the THI values in any 10 day period and the THI values in any preceding or subsequent 10 day period, we were able to observe a clear relationship between the maximum THI during Days 21–30 of gestation and pregnancy loss. Thus, animals subjected to maximum THI values lower than 55 during this period underwent a subsequent pregnancy loss of 0% in contrast to that 12% of losses for THI values higher than 69. It is during Days 21–30 of gestation, that implantation of the embryo occurs in the cow [27].

The peri-implantation period has long been identified as a period of significant conceptus loss in cattle and other species [28–30] with rates ranging from 8 to 25% for dairy cattle [31]. Implantation requires intricate signaling interactions between the conceptus and mother [32–34]. Aside from changes in the endometrial stroma, the embryo undergoes dramatic morphological changes, including angiogenesis, as a prerequisite to formation of the placental cotyledons [35–37]. On day 19 of pregnancy, superficial implantation between the trophoblast and uterine epithelium begins. From Days 21 to 22 approximately, epithelial layers start to adhere by interdigitation of the microvilli [36,38] and this adhesion process continues for 1–2 weeks [27]. The peri-implantation period is therefore critical in the life of the embryo. Our results indicate that acute heat stress during this period predisposes pregnant cows to subsequent early fetal loss.

Although the effects of cow and management factors on early fetal loss were analyzed in previous studies [10–13], we also examined the effects of these factors here since they show a wide variation range. This analysis was able to confirm our previous findings of a strong association with pregnancy loss of the factors twin pregnancy, warm period of pregnancy (as negative factors: odds ratios 3.4 and 3.1, respectively) and an additional corpus luteum (as a positive factor: odds ratio 0.32).

Twin pregnancies exert a substantial negative influence in the reproductive cycle in dairy cows, they increase the risk of pregnancy failure throughout the gestational period [39]. In the present study, 9% of total cows carried twins, 22% of them suffered pregnancy loss and the risk of pregnancy loss during the early fetal period was 3.4 times greater for cows bearing twins than for cows with singletons, figures similar to those previous studies [10,13,40]. Such negative effects of twinning might be diminished if there was any way of reducing embryo number in the dairy cow. Embryo/fetal reduction methods are used in assisted reproduction in humans [41,42] and in the treatment of twin pregnancies in mares [43]. In a recent therapeutic approach [44] we showed that the procedure of progesterone administration after manual rupture of the amnion on Day 34 of gestation may provide a satisfactory way for twin reduction in dairy cattle.

A positive association was found between additional corpora lutea (pregnancies in which the number of corpora lutea exceeded the embryo number) and the maintenance of pregnancy. The likelihood of pregnancy loss was 0.32 lower for cows showing an additional corpus luteum. These results are consistent to those previous studies [10,13]. Sub-optimal progesterone concentrations during the early fetal period may probably compromise conceptus development. Indeed, progesterone supplementation has the potential to reduce the incidence of pregnancy loss during this period [11].

Data presented here show a total incidence of fetal loss in 7.8% of pregnancies. Ball [1] suggested that 5% of pregnancies ending in late embryo/early fetal death may be a basic level of loss, and losses exceeding these levels are likely due to specific causes. In our study region, warm season is a main cause of early fetal loss. Influences in the present study of season of the year add strength to existing published data from this geographical area [10–13]. The figure of 12.3% (95/772) of losses for cows that became pregnant during the warm period contrasts clearly with that 2.1% (13/619) losses for cows that became pregnant during the cool period. Cool environment appeared to preserve gestation, probably as a reflection of cow well-being. However, if heat stress during the implantation period is a risk factor for pregnancy loss, the manner in which season acts as a negative factor compromising the success of gestation would need to be established.

It is well documented that heat stress can cause a reduction in dry mater intake [45–47] and may influence the uterine environment by decreasing blood flow to the uterus with consecutive increase of uterine temperature [48,49]. The metabolic demands placed upon high-yielding dairy cows should also be added to the metabolic stress related to the warm period. Numerous management factors could therefore be potentially stressful for the conceptus from conception to early fetal development during the warm season. Our findings prompt a need to concentrate on improving management practices to reduce the effects of heat stress. For example, shades, showers, fan cooling or air-conditioned structures should in due course favor pregnancy maintenance during the hot periods. An appropriate feed supply, including high quality of food and adequate feeding time, and readily available cool drinking water should moreover compensate the dry matter intake reduction under heat stress conditions. Finally, calm areas where cows were not annoyed with a controlled hygiene and physical comfort should reduce the effect of environmental stressors. Probably, preventing heat stress rather than to try to solve their consequences is the best approach for reducing the impact of pregnancy losses on profitability of cow production.

As a general conclusion, the practical implication of our findings is that heat stress can compromise the success of gestation during the peri-implantation period to the extent that a high temperature–humidity index during Days 21–30 of gestation is a risk factor for early fetal loss. Warm period of pregnancy, twin pregnancy (as negative factors) and an additional corpus luteum (as a positive factor) were confirmed as main factors associated with pregnancy loss up until 90 days of gestation.

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Screening for high fertility in high-producing dairy cows

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Screening for high fertility in high-producing dairy cows

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Abstract

A retrospective study involving 2756 pregnancies from two commercial dairy herds in north-eastern Spain determined relationships between management, production and reproductive data, and high fertility (conception before 90 days in milk) in high-producing dairy cows. High fertility was registered in 989 (35.9%) cows. The following data were recorded for each animal: herd, repeated animal (cows included two or more times within the study in which data were obtained from different lactational periods), parity (primiparous versus multiparous), previous twinning, reproductive disorders following calving (retained placenta, primary metritis) and at postpartum gynecological examination (incomplete uterine involution, pyometra and ovarian cysts), days in milk at conception, previous estrous synchronization and season of calving and conception. In order to evaluate the possible effect of high production during the peak milk yield on subsequent fertility, daily milk production at Day 50 postpartum was also recorded and cows were classified as high ($\geq$50 kg) and low ($<$50 kg) producers. Logistic regression analysis indicated no significant effects of herd, repeated animal, previous twinning, reproductive disorders such as primary metritis, incomplete uterine involution, pyometra and ovarian cysts, previous estrous synchronization and season of calving and insemination. Based on the odds ratio, the likelihood of high fertility increased in high-producer cows by a factor of 6.8. High fertility was less likely for multiparous cows (by a factor of 0.35) and for cows suffering placenta retention (by a factor of 0.65). High fertile cows produced a mean of 49.5 kg milk at Day 50 postpartum, in contrast to that 43.2 kg milk of the remainder cows. These findings question

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the negative effect of high production on fertility. Our results indicated that high individual cow milk production can be positively related to high fertility.

Keywords: High fertility; Milk production; Dairy cattle

1. Introduction

In recent years, genomic information and molecular biology are rapidly expanding, but our understanding of fertility remains still scarce. This problem acquires special relevance for dairy cows. Fertility prediction can be more hazardous than estimating fertility because estimation of fertility may be applied under very numerous conditions and factors affecting fertility [1]. It is evident that fertility was declining besides rising milk yields during the past few decades [2–6]. However, it is difficult to determine which mechanisms are associated with the effect of milk production on fertility. Fertility declining is generally associated with genetic progress as well as improvements in nutrition and management practices, which have led to a continuous increase in the milk yield. Thus, the following general statements appear to hold true for high-producing dairy cows:

1) Genetic correlations between milk production and reproductive efficiency are unfavorable [7].
2) The negative energy balance during the early postpartum period affects subsequent fertility [5].
3) Herd environment and management practices influence fertility [8].
4) Expansion of the herds by using their own heifer replacement is only possible with a good herd fertility level [9].

Taken together, these constraints are restrictive enough to consider that expanding herds where all animals are reared within the herd can provide an interesting study population of high fertile cows. No investigations on risk factors for high fertility have been performed in high-producing dairy cows. Therefore, the purpose of this study was to examine, using logistic regression procedures, relationships between management, production and reproductive data, and high fertility (represented by cows becoming pregnant before 90 days in milk), in two expanding high-producing dairy herds.

2. Materials and methods

2.1. Cattle and herd management

In the present study, only healthy cows free of detectable reproductive disorders and free of clinical disease during the interval from 50 days in milk to pregnancy diagnosis were included. Exclusion criteria were the disorders: mastitis, lameness, digestive disorders, abnormal genital discharges and pathological abnormalities of the reproductive tract detectable on palpation per rectum. Efforts were made to reduce variation in the general status of the animals, so that failure to exhibit estrus or conceive could be attributed to factors other than the clinical condition of the cows during the study.

We analyzed data from 2756 pregnancies registered in two well-managed, high-producing Holstein–Friesian dairy herds over a 28-month period (1 January 2003 to 30 April 2005) in northeastern Spain. The two herds comprised a mean of 1518 mature animals (502 and 1016, respectively). Mean annual milk production of the herds for this period was 11,720 kg per cow. The cows, calved all year round, grouped according to their milk production, were milked three times daily and fed complete rations. Feeds consisted of cottonseed hulls, barley, corn, soybean and bran, and roughages, primarily corn, barley or alfalfa silages and alfalfa hay. Rations were in line with NRC recommendations [10]. Dry cows were kept in a separate group and transferred, depending on their body condition score and age, 7–25 days prior to parturition to a ‘‘parturition group’’. An early postpartum group was established ‘‘fresh cows group’’ for postpartum nutrition and controls, and 7–20 days postpartum primiparous and multiparous lactating cows were transferred to separate groups. All the animals were tested free of tuberculosis and brucellosis. The voluntary waiting period from calving to first AI established for these dairy herds was 50 days. All cows were artificially inseminated, and the study population only included pregnancies registered in parous cows with no abortions after the last parturition. The mean annual culling rates for the study period were 30%:7.6%, 1.1% and 21.3% excluded before 50, 50–200 and after 200 days in milk, respectively. Cows producing less than 25 kg per day were not inseminated.

The criteria used to select the herds were similar management practices and an annual herd expansion rate (by using their own heifer replacement) of 8% during two years prior to and during the study period. Management practices included the use of pedometers, housing in free stalls with cubicles and a concrete slatted floor, daily strict veterinary supervision for all cows, a rigorous postpartum revision protocol, application of the same reproductive management program, confirmation of estrus at AI, and the fact that most AI (more than 90%) were performed by veterinarians.

2.2. Reproductive health management, insemination and pregnancy diagnosis

Postpartum checks (daily control) involved the treatment of the following puerperal diseases until resolved or until culling: metabolic diseases such as hypocalcemia and ketosis (the latter diagnosed during the first or second week postpartum), a retained placenta (fetal membranes retained longer than 12 h after parturition), or primary metritis (diagnosed during the first or second week postpartum).

The herds were maintained on a weekly reproductive health program. This involved examining the reproductive tract of each animal by palpation per rectum within 30–36 days postpartum to check for normal uterine involution and ovarian structures. Reproductive disorders diagnosed at this time such as incomplete uterine involution, pyometra or ovarian cysts were treated until resolved. Involution of the uterus was defined as incomplete when the uterine horns and/or cervix were larger than those in non-pregnant cows. Detectable intrauterine fluid was interpreted as pyometra. An ovarian cyst was diagnosed when a structure 20 mm in diameter or larger in either or both ovaries persisted for at least 7 days in absence of a palpable corpus luteum.

Boluses containing oxytetracicline were always administered into the uterus for cows suffering retained placenta or primary metritis. Cloprostenol (500 μg i.m.; Estrumate, Schering Plough Animal Health, Madrid, Spain) was the luteolytic agent used to treat incomplete uterine involution, pyometra and ovarian cysts. In the latter case, treatment was applied following manual rupture of the cystic structure per rectum.

Cows 60 days in milk and not detected to be in estrus in the last 21 days were examined weekly until estrus synchronization or until AI was performed during a natural estrus. At the weekly visit, ovarian structures and uterine status or contents were recorded. If a cow had a corpus luteum estimated to be at least 15 mm (mean of the maximum and minimum diameter), the animal was treated with cloprostenol. Cows with anovulatory follicles (anestrous cows) were also synchronized for estrus. A cow was considered to suffer follicular anovulation when a follicular structure of at least 8–15 mm was detected in two consecutive examinations in the absence of a corpus luteum or cyst, and no estrous signs were noted during the 7-day period between the exams [11,12]. A follicular structure of this size was always detected in anestrous cows. These cows were fitted with a progesterone releasing intravaginal device (PRID, containing 1.55 g of progesterone, without the estradiol benzoate capsule; CEVA Salud Animal, Barcelona, Spain). The PRID was left in the animal for 9 days and these animals were also given 500 μg cloprostenol i.m. 7 days after insertion. Cows treated either with cloprostenol or with PRID that failed to show estrus signs during the 14 days after the treatment were returned to the weekly gynecological exam. The cows were finally inseminated after estrus had been confirmed by examination of the genital tract and vaginal fluid. Only cows showing estrus, whether natural or following cloprostenol or PRID treatment, and with strong uterine contractility (determined by uterine tone) and copious, transparent vaginal fluid were inseminated 6–15 h after the onset of behavioral estrus. Pregnancy diagnosis was performed 38–45 days post-insemination by palpation per rectum.

2.3. Data collection and analysis

In the geographical area of study there are only two clearly differentiated weather periods: warm (May to September) and cool (October to April) periods [13]. During the 18 years period, mean number of days in a month that the maximum temperature was higher than 25 °C ranged from 20 to 31 days and from 0 to 4 days, for the warm and the cool period, respectively. Reproductive variables are generally significantly impaired during the warm period [6,13]. For this reason, calving and conception dates were used to analyze the effect of the season (warm versus cool period) of calving and conception on the probability of high fertility. Date of conception was the date of insemination in which cows became pregnant.

Cows were classified as high or low fertile cows based on conception during the interval from 50 to 90 days in milk, or after this interval, respectively. The following data were recorded for each animal: herd, repeated animal (cows included two or more times within the study in which data were obtained from different lactational periods), lactation number, previous twinning, reproductive disorders following calving (retained placenta, primary metritis) and at postpartum gynecological examination (incomplete uterine involution, pyometra, ovarian cysts), days in milk at conception, previous estrous synchronization (cloprostenol or PRID for animals conceiving within 7 days after treatment) and season of calving and conception. In order to evaluate the possible effect of high production during the peak milk yield on subsequent fertility, daily milk production at Day 50 postpartum (the average of the previous 3 days) was also recorded and cows were classified as high ($\geq$50 kg) and low (<50 kg) producers. Because of their low incidence (0.8%), data on metabolic diseases were not included in the study.

The relative contribution of each factor to the probability of high fertility was determined by logistic regression methods. High fertility was considered as the dependent variable, and calving and conception season (warm period), repeated animal, twinning, parity (primiparous versus multiparous), high production at Day 50 postpartum and the reproductive disorders were considered independent factors and coded as dichotomous variables (where 1 denotes presence and 0 absence). Herd and previous estrous synchronization were considered as class variables.

Regression analysis (SAS software, logistic procedure [14]) was performed according to the method of Hosmer and Lemeshow [15]. Basically, this method consists of five steps as follows: preliminary screening of all variables for univariate associations; construction of a full model using all the significant variables resulting from the univariate analysis; stepwise removal of non-significant variables from the full model; comparison of the reduced model with the previous model for model fit and confounding; evaluation of interactions among variables; and assessment of model fit using Hosmer–Lemeshow statistics. Variables with univariate associations showing P values <0.25 were included in the initial model. Modeling was continued until all the main effects or interaction terms were significant according to the Wald statistic at $P < 0.05$.

Finally, relationships between milk production at Day 50 postpartum (factor included in the main final model) and days in milk at conception were explored using the regression analysis procedures implemented in SAS.

3. Results

Fifty-two pregnant cows were excluded of the study for different clinical disorders diagnosed during the interval from 50 days in milk to pregnancy diagnosis. One thousand and thirty-eight cows were culled during the study period, with a mean lactation number (mean $\pm$ S.D.; ranges within parentheses) of 3.4 ± 1.6 (1–11) lactations. Twinning and retention placenta rate for culled animals were 8.4% (87/1038) and 22.1% (229/1038), respectively. Of the 1038 culled animals, 778 were excluded after 50 days in milk, with a mean production at Day 50 postpartum of 47 ± 9.6 (18–70) kg. Of the 778 cows reaching 50 days in milk, 633 received at least one insemination and 334 became pregnant. The mean lactation number and milk production at Day 50 postpartum for the 444 (778–334) non-pregnant cows which were culled after 50 days in milk were 3.6 ± 1.6 (1–11) lactations and 45 ± 9.4 (18–63) kg, respectively. The mean insemination number for the 299 inseminated non-pregnant cows was 2.1 ± 1.6 (1–12) inseminations. Data from 330 culled pregnant animals which satisfied the established conditions were included in the study population: five were culled for different reasons and 325 after abortion. The final study population was constituted of 2756 pregnancies.

High fertility was recorded in 989/2756 (35.9%) pregnancies. The mean lactation numbers were 2.3 ± 1.4 (1–9), 2.2 ± 1.4 (1–7), and 2.4 ± 1.4 (1–9) lactations for total, high fertile and low fertile cows, respectively. The mean milk productions were 45.4 ± 9.5 (25–84), 49.5 ± 8.2 (28–83), and 43.2 ± 10.3 (25–84) kg for total, high fertile and low fertile cows, respectively. The mean number of inseminations and days in milk at conception were 2.4 ± 1.9 (1–16) inseminations and 132 ± 75 (50–622) days, respectively.

The variables possibly affecting high fertility are listed in Table 1. Factors which affected high fertility were high production at 50 days postpartum, parity and retention of placenta. Logistic regression analysis indicated no significant effects of herd, repeated animal, previous twinning, reproductive disorders such as primary metritis, incomplete uterine involution, pyometra and ovarian cysts, previous estrous synchronization and season of calving and insemination. Table 2 shows the variables finally included in the logistic model. Based on the odds ratio, the likelihood of high fertility increased in high-producing cows by a factor of 6.8. High fertility was less likely for multiparous cows (by a factor of 0.35) and for cows suffering placenta retention (by a factor of 0.65). No significant interactions were found.

Of the 197 cows which had twins in their previous parturition, 125 (63.5%) suffered placenta retention, whereas this disorder was registered in 391 (15.3%) of the 2559 cows which delivered singletons.

Fig. 1 shows changes in the conception date according to the milk production at Day 50 postpartum. Each 1 kg decrease in milk yield was associated with an increase of 1.8 days in milk at conception.

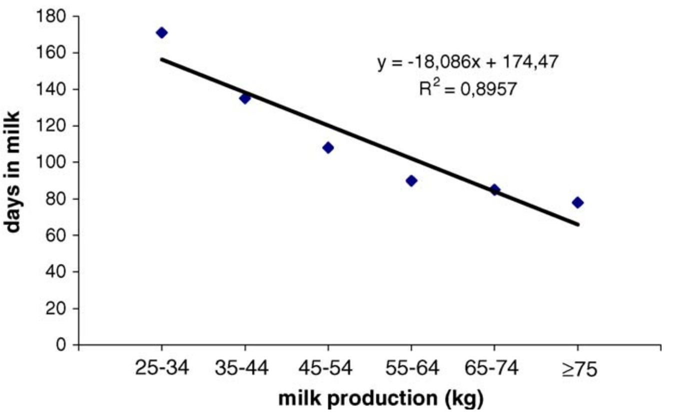

Fig. 1. Days in milk at conception according to the milk production at Day 50 postpartum.

Table 1
Risk factors assessed for their effects on high fertility[a] in pregnant cows

Risk factor	N classes	Total pregnancies[b] ($N = 2756$)	High fertile cows[c] ($N = 989$)	Low fertile cows[c] ($N = 1767$)
Herd	1	843	300 (35.6)	543 (64.4)
	2	1913	689 (36)	1224 (64)
Repeated animal[d]	0	1984	721 (36.3)	1263 (63.7)
	1	772	268 (34.7)	504 (62.3)
Calving season	Warm	1076	348 (32.3)	728 (67.7)
	Cool	1680	641 (38.2)	1039 (61.8)
Conception season	Warm	876	323 (36.9)	553 (63.1)
	Cool	1880	666 (35.4)	1215 (64.6)
Twins	0	2559	921 (36)	1638 (64)
	1	197	68 (34.5)	129 (65.5)
Retained placenta[e]	0	2240	848 (37.9)	1392 (62.1)
	1	516	141 (27.3)	375 (72.7)
Primary metritis	0	2591	930 (35.9)	1661 (64.1)
	1	165	59 (35.8)	106 (64.2)
Incomplete uterine involution	0	2682	959 (35.8)	1723 (64.2)
	1	74	30 (40.5)	44 (59.5)
Pyometra	0	2656	961 (36.2)	1695 (63.8)
	1	100	28 (28)	72 (72)
Ovarian cysts	0	2315	821 (35.5)	1494 (64.5)
	1	441	168 (38.1)	273 (61.9)
Estrous synchronization	Natural	2083	746 (35.8)	1337 (64.2)
	Cloprostenol[f]	449	165 (36.7)	284 (63.3)
	PRID[f]	224	78 (34.8)	146 (65.2)
Parity[e]	Primiparous	1009	386 (38.3)	623 (61.7)
	Multiparous	1747	603 (34.2)	1144 (65.5)
High production at Day 50 postpartum[e]	<50 kg	1935	509 (26.3)	1426 (73.5)
	≥ 50 kg	821	480 (58.5)	341 (41.5)

[a] Represented by cows becoming pregnant before 90 days in milk.
[b] Class description: N pregnancies per class, or pregnancy ranges within classes, or mean ± S.D. (ranges).
[c] Class description: N pregnancies per class (percentages), or mean ± S.D. (ranges).
[d] Cows included two ($n = 766$) or more ($n = 6$) times within the study, in which data were obtained from different lactational periods.
[e] Variables included as explanatory variables in the final model of the logistic regression.
[f] Cows conceiving within 7 days after cloprostenol or PRID treatment, respectively.

4. Discussion

Herein, we assessed for the first time factors affecting high fertility, paying particular attention to milk production at 50 days postpartum, in high-producing dairy cows. A

Table 2
Conception rates and odds ratios of the variables included in the final logistic regression model for high fertile cows[a]

Independent (explanatory) variables		*n*	Percent	Odds ratio	95% confidence interval	*P*
High producers at day 50 postpartum	<50 kg	1935				
	≥50 kg	821		6.8[b]	5.5–8.4	<0.0001
Parity	Primiparous	1009	38.8			
	Multiparous	1747	34.5	0.35[c]	0.28–0.43	<0.0001
Placenta retention	Absence	2240	37.9			
	Presence	516	27.3	0.65[d]	0.52–0.81	<0.0001

Likelihood ratio test = 3219, 3 d.f., $P = 0.0001$. Hosmer and Lemeshow goodness-of-fit statistics = 1.83; 8 d.f., $P = 0.77$ (the model fits).
[a] Represented by cows becoming pregnant before 90 days in milk.
[b] Odds of a high-producer cow becoming pregnant before Day 90, compared to low producer cows.
[c] Odds of a multiparous cow becoming pregnant before Day 90, compared to primiparous cows.
[d] Odds of a cow becoming pregnant before Day 90 suffering retention of placenta, compared to cows without this disorder.

positive correlation between milk production and reproductive performance was found. Milk yield usually peaks between 4 and 8 weeks postpartum, and the milk production peak is a good indicator of the total milk production throughout the lactation period [10]. Thirty-six percent of cows became pregnant before 90 days in milk (high fertile cows), and produced a mean of 49.5 kg milk within the peak yield period, in contrast to that 43.2 kg milk of cows becoming pregnant later. Based on the odds ratio, high-producing cows increased the probability of high fertility by a factor of 6.8. Furthermore, each 1 kg decrease in milk yield at 50 days postpartum was associated with an increase of 1.8 days in the parturition to conception interval. These findings question the negative effect of high production on fertility.

In two recent large-scale studies [16,17], we could not detect effects of milk production at AI on conception rate in high-producing dairy herds. The most important factors for conception were season of insemination, semen providing bull and lactation number. Neutral effect on the interval from parturition to conception for most levels of milk production in US Holstein cows was also reported in a study on the epidemiology of reproductive performance in dairy cows [18]. In the present study, we used information from only pregnant cows. Perhaps, the positive correlation between yield and fertility could not have been so clear if culled cows had been included in the analysis. It has been extensively assumed that the exclusion of cows is heavily influenced by owner decisions. Cows are often culled as a result of several factors so that management decisions can be confounded with biological factors. In contrast, pregnant cows are more likely to remain in a herd and data from their reproductive history could allow a more direct study on effect of yield on fertility.

It has been suggested that approximately 50% of the progress in milk yield can be attributed to genetics and the remaining 50% can be attributed to improved environmental factors such as better nutrition, housing, health and management [7]. The point is made that high milk production is related to high fertility. The question is why higher producing cows are more likely to conceive at the beginning of lactation? Is it because good management practices allow to better express genetic potential for these animals? If so, could lower producers receive inadequate care? If genetic progress is linked to fertility declining, low producers should have a major chance for becoming pregnant earlier, and this was not the case. Probably, highly fertile and producer cows had the highest genetic merit within the herd and suffered a low negative energy balance during the postpartum period. Conception rate and calving interval do not appear to be affected by the genetic merit of a herd [19], and the highest producing dairy cows in the herd are not necessarily those with the greatest negative energy balance or the lowest body condition score [2,18]. Genetic evaluation was not the objective of our study and, unfortunately, we had not complete information on body condition score changes in these farms. However, daily collection of weight measurements of lactating cows is becoming popular in our area and it should provide useful information in future studies on associations between milk production and fertility. More closely related to management practices, the association of delaying conception with lower producers could be explained as a response to stressful subclinical conditions (either environmental, social, nutritional, managerial or disease) in early lactation. Our results suggest that the percentage of high-producing cows becoming pregnant in the early lactation might be and indicator of the cow well being in high-producing herds. In fact, higher producing herds generally have better reproductive performance [8,19–22].

Primiparous cows were associated with a 2.9-fold (1/0.35) increase in the likelihood of high fertility compared to multiparous cows, supporting previous studies [16,17]. There is some disagreement about the effect of parity on conception failure in the literature. First, parity has been described as a risk factor [18,23], a preventive factor [4,24], and without effect [8] on delayed cyclicity or conception. Social status within the herd could explain this discrepancy. First-lactation cows from other herds are often used for herd expansion or for cow replacement in herds with fertility problems [25]. Therefore, reproductive efficiency may be compromised subsequent to stressor effects of transport or adaptation to a novel herd [26,27]. Both stresses were not existent in our study population, all animals were reared within the herd. On the other hand, animals which due to changes in the social structure of the herd display a decrease in their social status are less fertile [27]. As noted above, primiparous and multiparous lactating cows were placed into separated groups in these farms. Social stressor effect of older on younger cows was in this manner reduced.

Placenta retention was associated negatively (by a factor of 0.65) with high fertility in agreement with numerous studies that show the relationship between retention of placenta and a subsequent lower reproductive performance [28–30]. In fact, retained placenta was the only reproductive disorder included in the final model. The remaining postpartum disorders were probably solved efficiently by corresponding treatment before the insemination period. Interestingly, however, previous twinning was not a risk factor for reduced fertility. Similar percentage of cows with twins (34.5%) and singletons (36%) were registered as high fertile cows. Twin births are evidently in the etiology of most periparturient diseases [13,24,31–33], and placenta retention is greatly correlated with twin calvings [13,32,34–36]. Sixty-four percent of the cows which had twins suffered placenta retention in contrast to that the 15% of cows without this disorder. Because interactions were not found in the final model, the possible confounding relationship between twinning and placenta retention was analyzed with a further logistic regression analysis, without considering placenta retention as a fertility risk factor for cows with twins. This model (not presented) was practically the same from that presented in Table 2, slightly less adjusted ($P = 0.68$, the model fits). Twin pregnancies represent a management problem, and births with twins probably received a further attention respect to other disorders such as retained placenta. It is clear that under our work conditions treatment of placenta retention in cows with singletons needs to be reconsidered because injury to the intrauterine environment caused by retained placenta is largely healed by 60 days after parturition [37].

Neither calving season nor conception season was a significant risk factor for high fertility. Influences of season of year (notably heat stress) have been always found in other studies of this region, either concerning the interactions among reproductive disorders [13], fertility [6,16,17,38], or early fetal loss [39,40]. Reproductive variables were significantly impaired during the warm period reinforcing generally accepted conclusion from elsewhere [41,42]. For example, in a large scale retrospective study [6] over a 10-year period (1991–2000), average pregnancy rates to first AI were 44 and 27% for the cool and warm period, respectively. It is difficult to explain how high fertile cows could cope with season effects. Again, this point questions the negative effect of high production on fertility. High production at 50 days postpartum obviously reflects the cow genetic merit. High-producing dairy cows during peak milk yield are furthermore true survivors to metabolic, environmental and managerial stresses if they finally become pregnant during the early lactation period. Inclusion of fertility in breeding goals could undoubtedly improve genetic correlation between milk production and reproductive measures. However, it is not less important to learn more about how animals can respond to its variable environmental situations to better adjust management practices.

Overall, this study provided useful insight into risk factors for high fertility in high-producing dairy cows. Our results indicated that high individual cow milk production could be positively related to high fertility.

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Factors affecting the fertility of high producing dairy herds in northeastern Spain

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Abstract

Infertility has been often correlated to a rising milk yield in high producing dairy cattle. The aim of the present study was to evaluate, using logistic regression procedures, the effects of several management indicators on the fertility of four dairy herds in northeastern Spain. Data derived from 10,965 artificial insemination (AI). The factors examined were: herd, milking frequency (three versus two milkings per day), lactation number, previous twinning and disorders such as placenta retention and pyometra, milk production at AI, the inseminating bull, season (warm versus cool period) and year effects, AI technician and repeat breeding syndrome (cows undergoing four or more AI). Our findings indicated no effects on fertility of the herd, year of AI, previous twining, placenta retention and pyometra and milk production at AI. Based on the odds ratios, the likelihood of pregnancy decreased: in cows milked three times per day (by a factor of 0.62); for each one unit increase in lactation number (by a factor of 0.92); for inseminations performed during the warm period (by a factor of 0.67); in repeat breeder cows (by a factor of 0.73); and when 3 of the 45 inseminating bulls included in the study were used (by factors of 0.35, 0.43 and 0.44, respectively). Of the 13 AI technicians participating in the study, 3 were related to a fertility rate improved by odds ratios of 1.86, 1.84 and 1.30, respectively, whereas 2 technicians gave rise to fertility rates reduced by odds ratios of 0.64 and 0.49, respectively. Under our study conditions, management practices were able to compensate for the effects of previous twining and reproductive disorders such as placenta retention and pyometra. However, fertility was significantly affected by the factors milking frequency, AI technician, inseminating bull, repeat breeding syndrome, lactation number and AI season.

Keywords: Dairy cows; Fertility; Management; Milking frequency

1. Introduction

In the past 20 years, infertility has been often linked to a rising milk yield in high producing dairy cattle [1–5]. New management practices can improve the health and fertility of dairy cows [6], and there is a tendency towards a higher level of management in high producing compared to lower producing herds [7,8]. It is well known, that high milk production correlates negatively with fertility [9–11]. However, this relationship is difficult to demonstrate because of the interference of culling and the environment [12]. A high milk yield will only provoke a higher risk of infertility under suboptimal conditions, such as inadequate nutrition or environment [7,8,13]. Accordingly, we observed no

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relationship between milk production at artificial insemination (AI) and conception rates in two recent large-scale studies performed in high producing dairy herds [14,15]. Moreover, in another recent study [16], it was the high fertility cows – defined as those becoming pregnant before 90 days postpartum – that produced most milk on Day 50 postpartum.

These findings highlight the need to gain further knowledge on high producing management routines to improve both the production and fertility of dairy herds. This study was designed to evaluate, using logistic regression procedures, the effects of a series of management indicators on the fertility of four high producing dairy herds in northeastern Spain. The factors examined were: herd, lactation number, previous twinning and disorders such as retained placenta and pyometra, milk production at AI, the inseminating bull, season (warm versus cool period) and year effects, AI technician, milking frequency (three milkings/day versus two milkings/day) and repeat breeding syndrome (cows receiving four or more inseminations).

2. Materials and methods

2.1. Cattle and herd management

We analyzed data derived from 10,965 inseminations recorded for 4 well-managed, high producing Holstein–Friesian dairy herds in northeastern Spain, over a 3-year period (1 January 2002 to 31 December 2004). The four herds comprised a mean of/total number of 1735 mature animals (164, 450, 967 and 154, respectively). The cows were kept in open stalls. Two of the four herds were milked three times/day (8747 AI). The third herd had decreased the milking frequency, from three to two milkings/day in June 2004 (1025 AI under conditions of three milkings/day and 109 AI under conditions of two milkings/day), and the fourth herd underwent two milkings per day (1084 AI). Mean annual milk production of the herds for this period was 10,499 kg per cow in 2002, 10,584 kg in 2003 and 10,626 kg in 2004. The culling rate was 30% throughout the study period. The cows were fed complete rations. Feeds consisted of cotton seed hulls, barley, corn, soybean and bran and roughage, primarily corn, barley or alfalfa silage and alfalfa hay. Rations were in line with NRC recommendations [17]. All animals were tuberculosis and brucellosis free, as shown by yearly tests from 1985 to 2004. Vaccination programs were undertaken for the prevention of bovine virus diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) by applying modified live vaccines to calves between 6 and 8 months old, and killed vaccines to pregnant animals at 210 days of gestation.

Postpartum checks (daily) involved the treatment of the following puerperal diseases until resolved or until culling: metabolic diseases such as hypocalcemia and ketosis, a retained placenta (fetal membranes retained longer than 12 h after parturition), or primary metritis. The herds were maintained on a weekly reproductive health program. This involved examining the reproductive tract of each animal by palpation per rectum within 30–36 days postpartum to check for normal uterine involution and ovarian structures. Reproductive disorders diagnosed at this time such as incomplete uterine involution, pyometra or ovarian cysts were treated until resolved. Involution of the uterus was defined as incomplete when the uterine horns and/or cervix were larger than those in non-pregnant cows. Detectable intrauterine fluid was interpreted as pyometra. An ovarian cyst was diagnosed when a structure 20 mm diameter or larger in either or both ovaries persisted for at least 7 days in the absence of a palpable corpus luteum.

Boluses containing oxytetracycline were introduced into the uterus for cows suffering from a retained placenta or primary metritis. Cloprostenol (500 mg i.m.; Estrumate, Schering Plough Animal Health, Madrid, Spain) was the luteolytic agent used to treat incomplete uterine involution, pyometra and ovarian cysts. In the latter case, treatment was applied following manual rupture of the cystic structure per rectum. Since complete records from all farms were available only for animals suffering placenta retention and pyometra, these were the reproductive disorders included in the study.

In the geographical area of our study, there are only two clearly differentiated periods of weather: warm (May–September) and cool (October–April) periods [18]. Reproductive variables are generally impaired during the warm season period [5,18]. We therefore recorded the dates of pregnancy and insemination to analyze the effects of season on reproductive performance.

2.2. Artificial insemination and pregnancy diagnosis

The four herds were subjected to artificial insemination only. Cows were inseminated after estrus had been confirmed by examination of the genital tract and vaginal fluid. Only healthy cows (with no signs of mastitis, lameness or digestive disorders) with strong uterine contractility and copious, transparent vaginal fluid were inseminated. AI technicians number 5 and number 6 performed bicornual insemination of all cows (half seminal doses 4–5 cm deep into each uterine horn), whereas the remaining 11 AI technicians performed uterine body insemination.

If cows returned to estrus, their status was also confirmed by examination per rectum, and the animals were recorded as non-pregnant. In the remaining cows, pregnancy diagnoses were performed by palpation per rectum or by transrectal ultrasonography at 33–40 day post-insemination, when they were registered as pregnant or non-pregnant.

2.3. Statistical analysis

Logistic regression analyses were performed on data from each insemination using a positive pregnancy diagnosis as the dependent variable (0 or 1), and herd, year effect (1, 2 and 3 for years 2002, 2003 and 2004, respectively), lactation number, previous twinning, retained placenta, pyometra, milk production at AI (calculated as mean daily milk production for the 3 days preceding AI), inseminating bull, AI season (warm versus cool period), AI technician, milking frequency (three milkings/day versus two milkings/day) and repeat breeding (four or more AI) as independent factors. The factors previous twining and disorders, season of AI and milking frequency were coded as dichotomous variables, where 1 denotes presence and 0 denotes absence. The herd, season and year of AI, milking frequency, inseminating bull and AI technician were considered class variables. The mean pregnancy rate achieved by the AI technicians was 32%. For the logistic regression analysis, values corresponding to the AI technician attaining a 32.5% pregnancy rate were chosen as the reference. The variables possibly affecting fertility are listed in Table 1. These data were analyzed with the Proc GENMOD routine in the SAS statistical package [20], treating the variable cow as a repeated measure.

Regression analyses were conducted according to the method of Hosmer and Lemeshow [19] by the logistic procedure (SAS, [20]). Basically, this method involves five steps as follows: (i) preliminary screening of all variables for univariate associations; (ii) construction of a full model using all the variables found to be significant in the univariate analysis; (iii) stepwise removal of non-significant variables from the full model and comparison of the reduced model with the previous model for model fit and confounding; (iv) evaluation of interactions among variables, v assessment of model fit using Hosmer–Lemeshow statistics and either logistic regression diagnostic applied was one that examine the effect that deleting all subjects with a particular covariate pattern has on the value of the estimated coefficients and the overall summary measures of fit. In this way, we tried to identify covariate patterns that were poorly fit or have a great deal of influence on values of the estimated parameters. No patterns affecting the model were identified. Variables with univariate associations showing $P < 0.25$ were included in the initial model. We continued modeling until all the main effects or interaction terms were significant according to the likelihood-ratio at $P < 0.05$.

Table 1
Risk factors assessed for possible effects on fertility ($N = 10{,}965$ AI)

Risk factor	*N* classes	Class description (*N* AI per class or AI ranges within classes)	Mean ± S.D. (ranges)	Interquartile range limits for 25, 50, 75 and 100% AI, respectively
Herd	4	1 (6099), 2 (2647), 3 (1135), 4 (1084)		
Cow	1735	One AI (404), two or more AI (1291)		
Year effect	3	2002 (3592), 2003 (4796), 2004 (2577)		
Twinning	2	Absence (10,327), presence (638)		
Placenta	2	Absence (9079), presence (1886)		
Pyometra	2	Absence (10,286), presence (679)		
Season of AI	2	Cool (6543), warm (4422)		
AI technician	13	Range 39–2851 AI		
Bull	45	Range 38–1016 AI		
Repeat breeding syndrome	2	Presence (2074) absence (8891)		
Milking frequency	2	Two milkings (1193), three milkings (9772)		
Lactation number	Continuous		2.44 ± 1.47	1, 2, 3 and from 4 to11, respectively
Milk production at AI	Continuous		40.7 ± 9.4	From 7 to 34, 40, 47 and from 48 to 80 kg, respectively

Table 2
Percentage (and total number of inseminations) of positive pregnancy diagnosis of AI technicians per herd

Herd				
AI technician	1	2	3	4
1		34.1 (497)		
2	23.7 (234)	43.8 (14)		
3			30.9 (694)	
4			26.1 (66)	
5		42.2 (70)		
6		42.7 (244)		
7				27.8 (249)
8			33 (246)	
9		27.2 (110)		
10			34.2 (976)	
11	20.6 (57)			
12				18 (7)
13				30 (49)

3. Results

The mean lactation number was 2.4 ± 1.5 (mean $\pm$ S.D.; range: 1–9 lactations). Data on last parturition included: 1327 cows (94.1%) bore singletons and 638 (5.9%) carried twins, no triplets were recorded; 1886 cows (17.2%) and 679 (6.2%) presented placenta retention and pyometra, respectively. Mean daily milk production at AI was 40.7 ± 9.4 (range: 17–80 kg). The mean number of days in milk at AI was 162 ± 99 (range: 35–685). Four thousand four hundred and twenty two (40.3%) became pregnant during the warm and 6543 (59.7%) during the cool period. Table 2 shows the percentage of positive pregnancy diagnoses of AI technicians per herd.

Logistic regression analysis indicated no significant effects on fertility of herd, of year of AI, previous twinning, retained placenta, pyometra and milk production at AI. Table 3 shows the odds ratios of variables finally included in the logistic model. No significant interactions were found.

Based on the odds ratio, the likelihood of pregnancy decreased: in cows milked three times per day (by a factor of 0.62); for each one unit increase in lactation number (by a factor of 0.92); for inseminations performed during the warm period (by a factor of 0.67); in repeat breeder cows (by a factor of 0.73); and when three of the 45 inseminating bulls included in the study were used (by factors of 0.35, 0.43 and 0.44, respectively). Of the 13 AI technicians who participated in the study, three were related to fertilities improved by odds ratio of 1.86, 1.84 and 1.30, respectively, whereas two technicians were attributed fertility rates reduced by odds ratios of 0.64 and 0.49, respectively.

4. Discussion

This study identifies the most important management factors affecting fertility in high producing dairy cows in northeastern Spain. An increased milking frequency was found to be a risk factor for infertility, while milk

Table 3
Odds ratios of the variables included in the final logistic regression model for pregnancy rate

Factor	Class	*N*	%Pregnancy rate	Odds ratio	95%confidence interval	*P*-value
Lactation number	Continuous			0.92	0.89–0.95	<0.0001
Season	Cool	2342/6543	34.8			
	Warm	1234/4422	27.9	0.67	0.62–0.74	<0.0001
AI technician	13 classes					
	Technician 5	70/166	42.2	1.86	1.23–2.81	0.03
	Technician 6	244/572	42.7	1.84	1.36–2.5	<0.0001
	Technician 1	497/1458	34.1	1.30	1.018–1.68	0.036
	Technician 4	66/253	26	0.49	0.29–0.82	0.008
	Technician 11	57/277	20.6	0.64	0.43–0.95	0.029
Inseminating bull	45 classes					<0.0001
	Bull 16	88/298	29.5	0.44	0.24–0.99	0.033
	Bull 19	53/207	25.6	0.43	0.20–0.92	0.031
	Bull 40	95/409	23.2	0.36	0.13–0.92	0.033
Milking frequency	2 milkings/day	388/1193	32.5			
	3 milkings/day	2746/9772	28.1	0.58	0.37–0.91	0.01
Repeat breeding syndrome	<4 AI	3023/8891	34			<0.0001
	≥4 AI	566/2074	27.3	0.73	0.640-0.827	

Likelihood-ratio test = 424.6; 61d.f., $P = 0.0001$; Hosmer and Lemeshow Goodness-of-fit test = 4.20; 8 d.f., $P = 0.84$ (the model fits).

production at AI did not affect the pregnancy rate. Management practices seem to be able to cope with the effects of previous twining and reproductive disorders such as placenta retention and pyometra, yet traditional risk factors for fertility such as AI season, lactation number, AI technician, the inseminating bull and repeat breeding syndrome were also identified here as factors affecting fertility.

The effects of milking frequency have been extensively explored in high producing dairy herds [21–24]. However, it remains to be established if milking dairy cows three times/day instead of twice/day affects fertility. Some studies conclude that reproductive performance is unaltered by milking three times per day [21,25,26], while others have related this milking frequency to reduced fertility [27–29]. The general consensus is therefore that further studies on a larger number of animals and studies in the field will be needed to clearly define the influence of a three times/day milking regime on reproductive performance. Kruip et al. [26] added that results could be influenced by herd management. In our study, the three times daily milking regime reduced fertility by an odds ratio of 0.58. To our knowledge, this is the first investigation performed on a large number of animals analyzing the effects of milking frequency on pregnancy rate along with other management factors. The strong negative effect of the three times daily milking routine observed in our study add more fuel to the controversy over the effects of milking frequency on reproductive performance. Despite 10,965 inseminations were analyzed, because of only four herds were used in the present study the probability that herd and milking frequency did not both fit in the model was assessed by a further analysis in which herd was not included as a factor, and the same results were obtained.

The increase in milk production produced when switching from a twice daily to thrice daily milking regime is more obvious [24,25]. This increased yield is the result of a lengthened yield peak and a reduced rate of subsequent decline. A carryover effect has also been shown when changing from three to two milkings per day [30]. The association between high milk production and reduced fertility in dairy cattle has long been acknowledged [11,31,32]. In our study, milk production determined on the day of insemination had no effect on fertility. These findings are in agreement with those reported in our last two recent large-scale studies [14,15], in which we were unable to detect any effects of milk production at AI on the conception rate in high producing dairy herds. Moreover, in a recent study [16], we noted that high fertility cows produced more milk on Day 50 postpartum than lower fertility cows. The results of this last study suggested that the percentage of high producing dairy cows becoming pregnant before Day 90 of gestation could serve as indicator of the herd's well-being. Other authors have also found that high producing herds tend to show better reproductive performance [6,31–36].

Milking frequency is a risk factor for infertility, whereas milk production at AI is not. This indicates that the fertility decrease associated with milking frequency seems not to be directly related to the increased milk yield. It is likely that cows milked three times per day will be more influenced by the luteolytic effects of oxytocin release in response to udder massage at each milking [37]. An equally plausible explanation is the additional stress induced by the extra milking event each day.

The health testing protocols used by the US Certified Semen Services (CSS) ensure that the semen of the tested bull is well identified and disease free. Our ever increasing knowledge of diseases and continued updating of CSS databases means that the health status of commercial donor bulls is better today than ever before. This added to evermore discriminating semen evaluation procedures prevents subfertile semen reaching the dairy industry. Thus, today the dairy industry receives the highest quality semen ever produced. In spite of this, we identified 3 out of the 45 inseminating bulls included in our study, responsible for a reproductive performance reduced by odds ratios of 0.35, 0.43 and 0.44, respectively. According to DeJarnette et al. [38], we should consider whether the decline of fertility of high producing dairy herds can be attributed to the male, as a logical question. The problem is that many environmental and herd management factors will affect fertility estimates in an inseminating bull [39]. In the present study, we included management and environmental interferences as risk factors in the analysis. These bulls clearly affected fertility and should be eliminated from the insemination program.

The AI technician was a drastic risk factor for the fertility of our high producing dairy cows. Two of the 13 AI technicians diminished reproductive performance by odds ratios of 0.64 and 0.49, respectively, whereas three of the technicians were responsible for pregnancy rates increased by odds ratios of 1.86, 1.84 and 1.30, respectively. In effect, the different AI technicians caused great variation in pregnancy rates and odds ratios in this study. This could be an important practical limitation for the success of AI and also for herd fertility. There has been a tendency to adopt routine insemination procedures, and clearly some AI technicians inseminate cows less efficiently than others. Two of the three inseminators with the highest fertility performed bicornual insemination of all cows in the present study. Deep cornual AI probably requires a better training of inseminators and therefore favours better results [40]. Although insemination effects have been extensively documented, our results suggest that the AI technician should be correctly trained and retrained as an incentive for constant improvement.

Repeat breeding cows led to a fertility rate reduced by an odds ratio of 0.73. These results reinforce numerous previous studies on this multifactorial disorder. It is difficult to determine the possible causes of infertility in these cows. It has been suggested that this syndrome could be the result of a hormonal imbalance [42], abnormalities in the gametes [43], inadequate luteal function [44,45] or management causes [45,46].

Other management factors found to affect fertility in our study were the lactation number and AI season. This finding is consistent with those of previous studies performed in our geographical area [14,15] and elsewhere [47,48].

As an overall conclusion, we could emphasis the importance of adequate management practices for the economic success of dairy farms and the need for balanced management programmes in high producing dairy herds. On the farms examined here, the level of management was sufficient to offset the potential negative effects of milk production at AI, previous twining and reproductive disorders on the fertility of these high producing dairy cows. However, the factors milking frequency, AI technician, inseminating bull, repeat breeding syndrome, lactation number and AI season did compromise fertility. Improving management practices should be one of the main focuses of dairy herds, even those with a high level of management.

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Climate factors affecting conception rate of high producing dairy cows in northeastern Spain

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Abstract

Summer heat stress is a main factor related to low conception rate in high producing dairy herds in warm areas worldwide. We assessed the impact of several climate variables on conception rate in high producing dairy cows in northeastern Spain by examining 10,964 inseminations. The temperature–humidity index (THI) was compared with maximum temperature in terms of its efficiency at predicting conception rate. The following data were recorded for each animal: herd, lactation number, insemination number, insemination date, inseminating bull, and AI technician along with climate variables such as mean and maximum temperatures, rainfall, mean and maximum THI for individual time points Days 7 to 1 before insemination, the day of insemination and 1, 2 and 3 days after insemination. Averages were also established for the following periods: from 7 days before insemination to the insemination day, from 3 days before insemination to the insemination day and from the insemination day to 3 days postinsemination. Based on the odds ratios, the likelihood of conception rate increased significantly by factors of 1.48, 1.47, 1.5, and 1.1 for the respective maximum THI classes <70, 71–75, 76–80, and 81–85 only on Day 3 before AI, while on the insemination day, it increased by factors of 1.73, 1.53, 1.11, and 1.3 for the respective maximum THI classes <70, 71–75, 76–80, and 81–85. In a subsequent logistic regression excluding mean and maximum THI, the effectiveness of temperature at predicting conception rate was evaluated. Although high, the fit of the second logistic regression model was slightly lower than that of the full model ($P = 0.88$ versus $P = 0.98$, respectively) and the information provided by the THI model. The likelihood of conception rate increased significantly by factors of 1.5, 1.2, 1.0, 1.0 for the respective maximum temperature classes <20, 21–25, 26–30, 31–35 °C on Day 1 after AI. The choice of the THI or temperature to monitor the farm environment would have to depend on the particular farm and situation. In our study conditions, the use of maximum temperature alone gives a new point of view regarding the information provided by the THI variables.

Keywords: Dairy cows; Heat stress; Fertility; Climate factors; Temperature

1. Introduction

The profitability of dairy herds greatly depends on fertility. Yet despite rapid worldwide progress in genetics and management of high producing dairy herds, reproductive efficiency has suffered a dramatic decrease since the mid 1980s [1–4]. The reasons for the

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decline in fertility are multifactorial and cannot be solely attributed to an increase in milk production [3]. We propose that summer heat stress is likely to be a major factor related to low fertility in high producing dairy herds, especially in countries with warm weather.

Thermal stress before insemination has been associated with decreased fertility [5,6]. The intrauterine environment is also compromised in cows that suffer heat stress including alterations such as diminished uterine blood flow and an increased core body temperature [7,8]. These changes have been linked to early embryonic loss and to unsuccessful inseminations [9].

The environmental temperature, radiant energy, relative humidity, and wind speed all contribute to the degree of heat stress [10]. Heat stress may be defined as any combination of environmental variables that give rise to conditions that are higher than those of the temperature range of the animal's thermal neutral zone. The temperature–humidity index (THI) incorporates the effects of both ambient temperature and relative humidity (RH) in an index [11]. This index is widely used in hot areas worldwide to assess the impact of heat stress on dairy cows [12,13]. Despite this, there has been little research into the direct effects of the THI and temperature on the conception rate of dairy herds [14].

In this study, we examined the impact of several climate variables such as temperature, rainfall, and THI on the conception rate of high producing dairy cows in northeastern Spain. Since no studies exist on the effects of the THI on large series of dairy cattle, the efficiency of the THI at predicting conception rate was compared to the use of mean and maximum temperatures.

2. Materials and methods

2.1. Cattle and herd management

We analyzed data derived from 10,964 inseminations that were used in a previous study in which management factors affecting conception rate were assessed [15]. These artificial inseminations were recorded in four well-managed, high producing Holstein–Friesian dairy herds in northeastern Spain (41.13 latitude, −2.4 longitude), over a 3-year period (1 January 2002–31 December 2004). The four herds comprised a total number of 1735 lactating cows (164, 450, 967, and 154, respectively). The cows were kept in open stalls. Mean annual milk production of the herds for this period was 10,499 kg per cow in 2002, 10,584 kg in 2003, and 10,626 kg in 2004. Herd variation in annual milk yield/cow ranged from 9500 to 12,800 kg. The culling rate was 27–34% throughout the study period. The cows were fed complete rations and don't have access to pasture. Feeds consisted of cottonseed hulls, barley, corn, soybean, and bran, and roughage, primarily corn, barley or alfalfa silage and alfalfa hay. Rations were in line with NRC recommendations [16]. All animals were tuberculosis and brucellosis free, as shown by yearly tests from 1985 to 2004. Vaccination programs were undertaken for the prevention of bovine virus diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) by applying modified live vaccines to calves between 6 and 8 months old, and killed vaccines to pregnant animals at 210 days of gestation.

Post partum checks (daily) involved the treatment of the following puerperal diseases until resolved or until culling: metabolic diseases such as hypocalcemia and ketosis, a retained placenta (fetal membranes retained longer than 12 h after parturition), or primary metritis. The herds were maintained on a weekly reproductive health program. This involved examining the reproductive tract of each animal by palpation per rectum within 30–36 days post partum to check for normal uterine involution and ovarian structures. Reproductive disorders diagnosed at this time such as incomplete uterine involution, pyometra, or ovarian cysts were treated until resolved. Involution of the uterus was defined as incomplete when the uterine horns and/or cervix were larger than those in non-pregnant cows. Detectable intrauterine fluid was interpreted as pyometra. An ovarian cyst was diagnosed when a structure 20 mm diameter or larger in either or both ovaries persisted for at least 7 days in the absence of a palpable corpus luteum. Boluses containing oxytetracycline were introduced into the uterus for cows suffering retained placenta or primary metritis. Cloprostenol (500 mg i.m.; Estrumate, Schering Plough Animal Health, Madrid, Spain) was the luteolytic agent used to treat incomplete uterine involution, pyometra and ovarian cysts. In the latter case, treatment was applied following manual rupture of the cystic structure per rectum. Since complete records from all farms were available only for animals suffering placenta retention and pyometra, these were the reproductive disorders included in the study.

All animals were bred by artificial insemination (AI). The cows bred all year around and were inseminated by 11 different professional technicians. The voluntary waiting period was 60 days. Cows were inseminated after estrus had been confirmed by examination of the genital tract and vaginal fluid. Only healthy cows (with no signs of mastitis, lameness or digestive disorders) with strong uterine contractility and copious, transparent vaginal fluid entered in the study and were inseminated by uterine body insemination. If cows returned to estrus, their status was also confirmed by examination per rectum. Only healthy cows (with no signs of mastitis, lameness or digestive disorders) with strong uterine contractility and copious, transparent vaginal fluid entered in the study. Inseminations of cows with estrus return interval lower than 8 days were removed of the study. Pregnancy detection was performed by the same veterinary technician by palpation per rectum or by transrectal ultrasonography at 33–40 days post insemination.

2.2. Climate variables

Climate data such as daily mean and maximum temperature mean and minimum relative humidity, and rainfall were obtained from a meteorological station located less than 6 km away within the herds. Temperatures were collected in the shade. Temperature–humidity indices (THI) were calculated. The mean and maximum THI indices were fitted to the following equations [12,17,18]:

$$\text{Mean THI} = (0.8 \times \text{mean}\, T + (\text{mean RH}(\%)/100) \times (\text{mean}\, T - 14.4) + 46.4);$$

$$\text{Maximum THI} = (0.8 \times \text{maximum}\, T + (\text{minimum RH}(\%)/100) \times (\text{maximum}\, T - 14.4) + 46.4)$$

where T is the temperature, and RH is the relative humidity.

The four herds have shade and fans. Fans are activated when temperature arise at 23–27 °C.

2.3. Statistical analysis

The following data were recorded for each animal: herd, lactation number, number of inseminations, insemination date (cool – October to April versus warm – May to September), inseminating bull, and climate variables such as mean and maximum temperature, rainfall, mean and maximum THI for each day from days 7 to 1 before insemination (−7 to −1), AI day (Day 0), and 1, 2, and 3 days after insemination. Averages were also established for the following periods: from 7 days before insemination to the AI day, from 3 days before insemination to the AI day and from the AI day to 3 days postinsemination. These periods were chosen because only acute effect of heat stress wanted to be analyzed.

In the previous study noted above [15], management factors such as herd, the inseminating bull (46 bulls), insemination period (cool versus warm), and lactation and insemination number were analyzed. These factors were therefore included in the logistic regression analysis as control variables.

Logistic regression analyses were performed on data from each insemination, using pregnancy detection as the dependent variable (0 or 1), and herd, lactation number, insemination number, insemination date (cool versus warm period), the inseminating bull, AI technician and climate variables as independent factors. Herd, insemination date, inseminating bull, AI technician, rainfall, mean and maximum temperatures, and mean and maximum THI were considered as class variables. Five rainfall classes were defined: <30, 31–40, 41–50, 51–60 and >60 mm rainfall. Four mean temperature classes were defined: <15, 16–20, 21–25, and >25 °C. Finally, five maximum temperature classes were defined: <20, 21–25, 26–30, 31–35, and >35 °C. Five mean and maximum THI variables were defined: <70, 71–75, 76–80, 81–85, >86.

A further logistic regression excluding mean and maximum THI variables was performed to compare the effectiveness of THI and temperature at predicting conception rate in dairy herds. Logistic regression analyses were performed using the same data from each insemination.

Regression analyses were conducted according to the method of Hosmer and Lemeshow [19] by the logistic procedure (SAS, [20]). Basically, this method involves five steps as follows: (i) preliminary screening of all variables for univariate associations; (ii) construction of a full model using all the variables found to be significant in the univariate analysis; (iii) stepwise removal of non-significant variables from the full model and comparison of the reduced model with the previous model for model fit and confounding; (iv) evaluation of plausible two-ways interactions among variables; and (v) assessment of model fit using Hosmer–Lemeshow statistics. Variables with univariate associations showing $P < 0.25$ were included in the initial model. We continued modelling until all the main effects or interaction terms were significant according to the Wald statistic at $P < 0.05$.

3. Results

Mean monthly temperature, humidity, and rainfall conditions for the study period are given in Table 1. Mean annual rainfall was 131.5 mm, with 88 days of rain per year.

Table 1
Mean monthly temperatures, humidity, and rainfall conditions for the study period (3 years; 2002–2004)

Month	Mean T (°C)	Maximum T (°C)	Mean RH (%)	Minimum RH (%)	Mean THI	Maximum THI	Rainfall (mm)	CR (%)
January	5.6	10.8	91	74	42.9	52.2	4.7	34.0
February	6.2	11.6	86	67	44.1	52.2	14.6	34.7
March	10.3	17.2	82	56	51.1	61.4	8.5	35.5
April	12.7	19.4	80	53	55.0	64.1	13.6	32.0
May	16.5	23.5	76	51	61.0	69.3	14.3	31.2
June	23.2	31.0	72	47	71.2	78.9	8.7	29.3
July	23.7	31.4	79	52	72.8	80.3	7.1	26.1
August	23.8	31.5	81	54	73.0	80.7	9.2	26.0
September	19.5	26.8	89	64	66.7	75.8	13.1	25.1
October	14.3	20.2	91	72	57.7	66.4	19.0	32.3
November	10.1	15.1	92	80	50.4	58.7	12.5	31.1
December	6.8	11.1	94	85	44.6	52.4	6.2	37.5

T, temperature; RH, relative humidity; THI, temperature–humidity index; CR , conception rate.

The study population conception rate was 32% (25.7%, 35.5%, 32.5%, and 27.7% for the herds, respectively). Conception rate for the warm and cool period was 27.9% and 35%, respectively.

Logistic regression analysis indicated no significant effects on conception rate of rain nor other climate factors recorded for individual Days 7–4, 2–1 before AI, on the AI day, and Days 1– 3 postinsemination, and herd. Table 2 shows the adjusted odds ratios of the variables finally included in the logistic model. No significant interactions were found. Based on the odds ratios, the likelihood of conception rate increased significantly by factors of 1.48, 1.47, 1.5, and 1.1 for the respective maximum THI classes <70, 71–75, 76–80, and 81–85 on Day 3 before AI (using the class >86 THI units as reference), and increased by factors of 1.73, 1.53, 1.11, and 1.3 for the respective maximum THI classes <70, 71–75, 76–80, and 81–85 on the AI day (using the class >86 THI units as reference).

The logistic regression excluding mean and maximum THI variables showed slightly worse fit than the full model, which only included THI variables in the results ($P = 0.88$ versus $P = 0.98$). Table 3 shows the odds ratios for the variables finally included in this logistic model. Based on the odds ratios, the likelihood of conception rate increased significantly by factors of 1.42, 1.59, 1.51, and 1.08 for the respective mean maximum temperature classes <20, 21–25, 26–30, and 31–35 °C from 3 days before AI to the AI day, (using the class >36 °C as reference) and by 1.49, 1.17, 1.03, 1.00 for the respective maximum temperature classes on Day 1 after AI (using the class >36 °C as reference).

4. Discussion

Efforts were made to study the effects of specific individual climate factors on conception rate by examining the period 7 days before AI to 3 days after

Table 2
Odds ratios of the variables included in the final logistic regression model for conception rate

Factor	Class	*n*	% Conception rate	Odds ratio	95% Confidence interval	*P*-value
Maximum	<70	2292/6659	34.4	1.48	1.1–1.9	0.005
THI	71–75	464/1407	33.0	1.47	1.1–1.9	0.005
Day 3 before	76–80	392/1279	30.6	1.50	1.2–1.9	0.002
AI	81–85	246/1071	23.0	1.10	0.9–1.1	0.464
	>86	119/548	21.7	Reference		
Maximum	<70	2316/6630	34.9	1.73	1.1–2.3	<0.001
THI on the AI	71–75	466/1400	33.3	1.53	1.2–2.0	0.002
Day	76–80	225/1306	25.7	1.11	0.9–1.4	0.446
	81–85	278/1047	26.6	1.30	1.0–1.7	0.045
	>86	121/581	20.8	Reference		

Likelihood ratio test = 491.07; 70df, $P = 0.0001$.
Hosmer and Lemeshow Goodness-of-fit test = 2.12; 8 df, $P = 0.98$. Adjusted $R^2 = 0.1$.

Table 3
Odds ratios of the variables included in the final logistic regression model for conception rate (model excluded mean and maximum THI)

Factor	Class	*n*	% Conception rate	Odds ratio	95% Confidence interval	*P*-value
Mean	<20	1965/5696	34.5	1.42	0.9–2.0	0.053
Maximum	21–25	494/1430	34.5	1.59	1.1–2.2	0.008
Temperature	26–30	619/1994	31	1.51	1.1–2.0	0.009
From 3 days	31–35	341/1436	23.7	1.08	0.8–1.4	0.588
Before AI to AI (°C)	>36	94/408	23	Reference		
Maximum	<20	1956/5562	35.2	1.49	1.07–2.07	0.018
Temperature	21–25	566/1716	33.0	1.17	0.86–1.6	0.298
One day after	26–30	521/1810	28.8	1.03	0.77–1.3	0.808
AI	31–35	339/1334	25.4	1.00	0.77–1.29	0.979
(°C)	>36	131/542	24.2	Reference		

Likelihood ratio test = 479.60; 70df, $P = 0.0001$.
Hosmer and Lemeshow Goodness-of-fit test = 3.63; 8 df, $P = 0.88$. Adjusted $R^2 = 0.1$.

AI. Our assessment of the conception rate of high producing dairy cows by climate factors proved to be very valuable ($P = 0.98$).

Our findings indicate that when the maximum THI units for Day 3 before AI increased, conception rate decreased [14,21]. Heat stress can affect many reproductive variables in cattle including follicular dynamics [22]. Environmental and stress factors promote ovulation failure, cows that had failed to ovulate by Day 50 postpartum following either natural or synchronized estrus, which is considered the major cause of infertility in dairy cattle by some authors [23–25]. In our geographical area of study, López-Gatius et al. [26] described that the only factor that dramatically affected ovulation was the AI season. Ovulation failure was 3.9 times higher in cows inseminated during the warm period (May–September) compared to the cool period (October–April). High producing dairy cows commonly suffer metabolic stress and this added to another stressful effect such as a hot environment could cause an endocrine imbalance and finally compromise folliculogenesis [3,10].

In our study, when maximum THI 3–1 days pre-AI values were higher than 80, conception rate decreased from 30.6 to 23%. The odds ratios for THI 3–1 days pre-AI classes <70, 71–75, and 76–80 were similar at 1.5, whereas for class 81–85 THI units, the odds ratio was 1.1. It is thought that >72 THI units causes heat stress in cows [27]. In our study, the negative effects of heat stress on conception rate seem to appear from 75 THI units, although the effects of heat stress become more obvious from 80 units of THI. Moreover, in the second logistic regression in which THI was excluded, the conception rate decrease appeared from a mean maximum temperature of 31 °C recorded from 3 days before AI to the AI day, although it has been proposed that heat stress commences at temperatures of 25 °C [28]. Heat stress may be defined as any combination of environmental variables that give rise to conditions that are higher than those of the temperature range of the animal's thermal neutral zone (5–20 °C [29]). Heat stress before AI has been associated with decreased fertility in countries with warm weather [30]. High producing dairy herds are usually subjected to more complete management practices than other herds, and it could be that the high producing cow's environment is more comfortable than that of lower producers because of the use of fans, shades, air-conditioning and other management measures. The four herds of the study have routine hygiene measures such as provision of clean and frequent removal of bedding, appropriate stocking densities in buildings, good drainage, adequate ventilation and appropriate building design. Moreover, all cows are in the shade, and fans opened at 25 °C. More studies are needed to control real farm climate conditions and to establish the impact of stress on cows [31].

A high maximum THI at AI was found here to be related to low conception rate. The likelihood of conception rate increased by factors of 1.73, 1.53, 1.11, and 1.3 for the respective maximum THI classes. Once again, the decrease in conception rate was evident, from 35–33% to 21–27%, at THI values higher than 75. Due to our retrospective data collection, however, it was not possible to establish whether this heat stress affected the oocytes, zygotes, or even spermatozoa. It could be that exposure of spermatozoa to elevated temperatures after AI in the uterus or oviduct of a hyperthermic female could compromise sperm survival, fertilizing capacity or both. Howarth et al. [32] described that rabbit spermatozoa washed from the uterus of heat-stressed females and used to inseminate females maintained in thermoneutral conditions resulted in similar fertilization rates but lower embryonic survival than pregnancies arising from inseminations with spermatozoa retrieved from females maintained in thermoneutrality. The oviductal or intrauterine environment could thus be compromised in heat stressed cows owing to decreased blood flow to the uterus and an increase in uterine temperature under heat stress conditions [8].

Given the multiple factors affecting conception rate and their interactions, we performed two logistic regressions, one including mean and maximum THI and temperature variables and the other one only including the mean and maximum temperature variables. Although temperature and THI are related variables, two different results were observed. However, the two logistic regression models showed similar fits and the Hosmer–Lameshow index indicated a good model fit in both analyses ($P = 0.88$ versus $P = 0.98$). Thus, we could not conclude that the temperature–humidity index is a better predictor of conception rate than temperature alone. More studies on the direct effects of temperature and THI are needed to determine which index is more appropriate to predict the reproductive performance of high producing dairy cows.

A high maximum temperature on day 1 after AI was found to reduce conception rate. Although it has been described that hyperthermia in lactating dairy cows can occur at air temperatures as low as 27 °C [33], only maximum daily temperatures <20 °C led to a significant increase in conception rate (using the class >36 °C as reference) ($P < 0.05$). It should be stressed that in our geographical area of study, the maximum temperature was only <20 °C for 170 days per year. Other classes (21–25, 26–30, 31–35 °C) rendered no significant increases in conception rate. Probably, heat stress on the day after AI affected embryo viability. A 1 day embryo is more sensitive to high environmental temperatures in vivo than 3-, 5- and 7-day old embryos [34].

In our previous study on the same study population [15], management factors such as the herd, inseminating bull, AI technician, insemination season (cool period versus warm period) and lactation and insemination number were analyzed. Here, the above mentioned factors were included in the logistic regression analysis as control variables and finally entered in the logistic regression results except the insemination period (cool period versus warm period). Obviously, season was not entered in the final model because more specific climate factors were analyzed.

As an overall conclusion, climate factors seem to be highly relevant for conception rate, especially during the period 3 days before to 1 day after AI. The use of maximum temperatures gives more information that the one provided by THI variables. Thus, the use of the THI or temperature to control a farm environment would depend on the individual farm and on each environmental situation, but it is important to check temperature and humidity to know when to adopt cooling measures. Practical implications are for example, implement of fans that open at 70–75 THI units and increase management practices around AI day. We obtained our climate data from a meteorological station 6 km within the herds. It is likely that the different management systems applied in these high producing dairy farms changed the environmental conditions. For example, fans, cool water, correct ventilation could have changed environment of dairy herds. Further studies should try to establish real climate conditions at the farm level.

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Cystic ovaries and low milk production as risk indicators of decreased fertility in high producing dairy herds under warm conditions

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Abstract

Numerous studies have described associations between reproductive disorders and fertility from the individual cow but not by using summary records. The present study was conducted to examine possible relations between production, reproductive disorders and the fertility of high producing dairy herds under warm conditions by using annual and monthly records. Data derived from a total of 8649 lactations including 26645 AI and 7705 positive diagnosed pregnancies on four high producing dairy herds in a four year study. High monthly conception rate was defined as the percentage of pregnant cows of the total AI per month higher than the mean conception rate of the four herds during the four years (29%). Logistic regression analysis indicated no significant effects of herd, lactation number, days in milk, relation of protein/fat, anovulatory follicles, season (warm versus cool period) and year on high monthly fertility. Based on the odds ratio, high monthly fertility was 0.14 and 0.55 times less likely for mean daily milk production per month <30 kg and 30-35 kg, respectively, than for values >35 kg used as reference. The likelihood of high monthly fertility decreased for each one unit % increase in the monthly incidence of ovarian cysts by an odds ratio of 0.62. An annual rising trend of milk production was observed besides a reduced incidence of uterine disorders such as placenta retention and pyometra, and increased conception and anovulatory follicle rates. Fluctuations of different reproductive parameters during and within the years were also registered. In conclusion, our results indicate that monthly summary records can provide useful risk markers of fertility variations. Monthly values of decreased milk production and increased incidence of ovarian cysts were associated with a decreased fertility.

Keywords: High fertility; Milk production; Ovarian cysts; Heat stress; Dairy cattle

1. Introduction.

Reproductive performance of dairy cattle influences profitability of herds because of its affects on milk production and culling rates [1]. Some studies have reported an increase of infertility worldwide [2-5], and for that reason the main objective in recent years was to preserve fertility of dairy herds [6]. Dekkers [7] reported that an improvement in fertility increases profit, not only by reducing culling cost but also by increasing incomes from milk sale and shorter calving intervals. Recently, in many countries, female fertility has been included in breeding goals to place emphasis on genetic aspects of reducing fertility costs in dairy cattle [8].

Infertility is a disorder of multifactorial origin not always associated with high milk production [5, 9, 10]. Numerous management practices and environmental factors influence reproductive performance of dairy herds. Nutrition and management changes have tried during the last decades to adapt the cow to high production. However, the long-term consequence on the basic reproductive physiology of the animal has not been often considered. Moreover, summer heat stress is a major factor affecting fertility in high producing dairy herds, especially in countries with warm weather as Spain [9, 11-13].

Optimal reproductive efficiency begins with proper use of records. Annual and monthly records provide commonly access to the reproductive production performance results of management on the dairy herd, either to the dairy owner as to the veterinarian. However, although records are a very valuable source of information and diagnosis when problems arise, possible events related to a disorder or to a deviation of goal levels occurred often in a too distant past. Risk indicators of fertility variations within a monthly summary record would be a valuable tool in elaborating plans and performing actions in the day-to-day dairy farm management. Numerous studies have described associations between reproductive disorders and fertility from the individual cow but not by using summary records. Therefore, the present study was conducted to examine possible associations between production, the incidence of several reproductive disorders and the fertility of high producing dairy herds under warm conditions by using annual and monthly records.

2. Material and methods

2.1. Cattle and herd management

The data examined were obtained from a reproductive control programme conducted at the University of Lleida on 4 well-managed, commercial, high producing dairy herds in northeastern Spain over the period January 2003 to December 2006. The four herds were selected on the basis of similar management practices, the use of only their own heifer replacement, as indicator of a good reproductive performance level, and the fact that all gynecological exams following day 30 post partum had been performed by the same veterinarian. Management practices included the use of pedometers in 3 of the four herds, housing in free stalls with cubicles, fans and water sprinklers for the warm season and a concrete slatted floor, a rigorous postpartum revision control, application of the same reproductive health program, confirmation of estrus at AI, and the fact that most AI (more than 90%) were performed by veterinarians.

The herds comprised a mean of 2348 mature cows (120, 523, 694 and 1012, respectively), with a mean annual culling rate of 30%. Mean annual milk production of the herds for the study period was 11,232 kg per cow. The cows were grouped according their milk production, milked three times daily and fed complete rations. Feeds consisted of cotton seed hulls, barley, corn, soybean, and bran, and roughage, primarily corn, barley or alfalfa silage and alfalfa hay. Rations were in line with NRC recommendations [14]. All animals were tuberculosis and brucellosis free, as shown by yearly tests from 1985 to 2007. Vaccination programs were undertaken for the prevention of bovine virus diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) by applying modified live vaccines to calves between 6 and 8 months old, and killed vaccines to pregnant animals at 210 days of gestation. Dry cows were kept in a separate group and transferred, depending on their body condition score and age, 7-25 days prior to parturition to a “parturition group”. An early postpartum group was established “fresh cows group” for postpartum nutrition and controls, and 7-20 days postpartum primiparous and multiparous lactating cows were transferred to separate groups. All cows were artificially inseminated. The voluntary waiting period for the herds was 45 days.

2.2. Reproductive health management

Postpartum checks (daily control) involved the treatment of the following puerperal diseases until resolved or until culling: metabolic diseases such as hypocalcemia and ketosis (the latter, diagnosed during the first or second week post partum), retained placenta (fetal membranes retained longer than 12 h after parturition), or primary metritis (diagnosed during the first or second week post partum in cows not suffering placenta retention).

The herds were maintained on a weekly reproductive health program. This involved examining the reproductive tract of each animal by palpation per rectum within 30 to 36 days postpartum to check for normal uterine involution and ovarian structures. Reproductive disorders diagnosed at this time such as pyometra or ovarian cysts were treated until resolved. Detectable intrauterine fluid was interpreted as pyometra. An ovarian cyst was diagnosed when a structure larger than 20 mm in diameter was detected in either or both ovaries in absence of a corpus luteum and uterine tone.

Boluses containing oxytetracicline were always administered into the uterus for cows suffering retained placenta or primary metritis. Cloprostenol (500 µg i.m.; Estrumate, Schering Plough Animal Health, Madrid, Spain) was the luteolytic agent used to treat pyometra and ovarian cysts. In the latter case, treatment was applied following manual rupture of the cystic structure per rectum.

Cows 60 days in milk and not detected to be in estrus in the last 21 days were examined weekly until estrus synchronization or until AI was performed during a natural estrus. At the weekly visit, ovarian structures and uterine status or contents were recorded. If a cow had a corpus luteum estimated to be at least 15 mm (mean of the maximum and minimum diameter), the animal was treated with cloprostenol. If a cow was diagnosed to have an ovarian cyst, the treatment was the same than that during the postpartum period. Cows with anovulatory follicles (anestrous cows) were also synchronized for estrus. A cow was considered to suffer follicular anovulation when a follicular structure of at least 8 to 15 mm was detected in two consecutive examinations in the absence of a corpus luteum or cyst, and no estrous signs were noted during the 7-day period between the exams [15, 16]. A follicular structure of this size was always detected in anestrous cows. These cows were fitted with a progesterone releasing intravaginal device (PRID, containing 1.55 g of progesterone, without the estradiol benzoate capsule; CEVA Salud Animal, Barcelona, Spain). The PRID was left in the animal for 9 days and these animals were also given 500 µg cloprostenol i.m. 7 days after insertion. Cows treated either with cloprostenol or with PRID that failed to show estrus signs during the 14 days after treatment were returned to the weekly gynecological exam. The cows were finally inseminated after estrus had been confirmed by examination of the genital tract and vaginal fluid. Only cows showing estrus, whether natural or following cloprostenol or PRID treatment, and with strong uterine contractility (determined by uterine tone) and copious, transparent vaginal fluid were inseminated 6-15 h after the onset of behavioral estrus. Pregnancy diagnosis was performed 39-45 days post-insemination by ultrasound or palpation per rectum.

2.3. Survey

Since complete records from all farms were only available for the conception rate, milk production and reproductive disorders, metabolic (hypocalcemia and ketosis) and clinical disease (mastitis, lameness and digestive disorders) were not included in the study. Metabolic and/or clinical disease was reason for culling in approximately 30% of culled animals for all farms. Tables 1 and 2 show mean annual and month values, respectively, of production and reproductive parameters included in the study.

2.4. Data collection and analysis

We used monthly summary records including: herd, mean values of lactation number, days in milk, milk production and milk relation of protein/fat, and total numbers of parturitions, twins, reproductive disorders (retained placenta, primary metritis, pyometra, ovarian cysts and anovulatory follicles), inseminations and positive pregnancy diagnoses. The conception rate was evaluated in terms of total number of pregnant cows as a percentage of the total inseminations, and each uterine disorder (placenta retention, primary metritis and pyometra) as a percentage of the total parturitions. The incidence of ovarian cysts and anovulatory follicles was evaluated on number of inseminating cows (number of cows not diagnosed pregnant with more than 45 days in milk). Average milk yield per cow and day was obtained by dividing the total annual marketed milk by 365 days and the mean daily number of milked and milked plus dry cows.

Climate data such as daily mean and maximum temperature means, minimum relative humidity and rainfall were obtained from a meteorological station located less than 6 km away within the herds. Temperatures were collected in the shade.

Possible significant fluctuations ($P<0.05$) of the different parameters over the years or over the months were explored with Tukey-Kramer multiple comparison test procedure of the SPSS package, version 14.0 (SPSS Inc., Chicago, IL, USA).

High monthly conception rate was defined as the percentage of pregnant cows of the total AI per month higher than the mean conception rate of the four herds during the four years (29%). Logistic regression methods were performed to determine the influence of herd, mean monthly values (40 months) of lactation number, days in milk, milk production per day, milk relation of protein/fat, reproductive disorders following day 45 post partum (anovulatory follicles and ovarian cysts), season, and year in which cows were inseminated or reproductive disorders diagnosed, and possible plausible interactions of paired factors on high monthly conception rate (dependent variable). Herd, mean daily milk production per month (<30, 30-35 and > 35 kg), season (cool-October to April- or warm- May to September) and year were considered as class variables. Monthly values of lactation number, days in milk, milk relation of protein/fat and of the incidence of reproductive disorders (percentages) were considered as continuous variables.

Regression analyses were conducted according to the method of Hosmer and Lemeshow [17] by the logistic procedure of the SAS package, version 9.1 (SAS Intitute Inc., Cary, NC, USA). Basically, this method involves five steps as follows: (i) preliminary screening of all variables for univariate associations; (ii) construction of a full model using all the variables found to be significant in the univariate analysis; (iii) stepwise removal of non-significant variables from the full model and comparison of the reduced model with the previous model for model fit and confounding; (iv) evaluation of plausible two-ways interactions among variables and (v) assessment of model fit using Hosmer–Lemeshow statistics. Variables with univariate associations showing $P < 0.25$ were included in the initial model. We continued modelling until all the main effects or interaction terms were significant according to the Wald statistic at $P < 0.05$.

3. Results.

3.1. Reproductive performance

The study was performed on a total of 8649 lactations including 26645 AI and 7705 positive diagnosed pregnancies (29% conception rate). The total number of mature cows increased from 2257 to 2370 during the four years. All cows were inseminated or diagnosed to have any reproductive disorder before Day 70-77 postpartum. The mean number (mean ± S.D.) for the four herds of days from calving to first AI, calving to conception and of the calving interval were 77. 4 ± 13, 150 ± 25, and 430 ± 41 days, respectively. The culling rate was 30 % ± 3.

3.2. Year effect

Significant differences throughout years were also detected for days in milk, milk production, ovarian cysts, anovulatory follicles, pyometra, retained placenta and conception rate using the Tukey Kramer test (Table 1).

Table 1. Mean or total annual values for production and reproductive parameters included in the study[a].

	2003	2004	2005	2006
Mean monthly values per year				
Number of milking plus dry cows	2258	2342	2418	2374
Number of milking cows	1977	2050	2125	2074
Ppostpartum cows[b]	434	455	438	409
Inseminating cows[c]	720	692	689	659
Pregnant cows[d]	1030	1085	1153	1125
Lactation number	2.44	2.35	2.27	2.33
Days in milk	200[k]	199[k]	192[l]	191[l]
Milk production (Kg/day/cow)	29.9[k]	29.8[k]	31.4[k,l]	31.8[l]
Milk production (Kg/day/milking cow)	34.1[k]	34.1[k]	35.7[l]	36.2[l]
Protein/fat[e]	0.88	0.88	0.89	0.90
Environmental temperatures (ºC)				
Cool season (October to April)				
Mean daily temperature	12.1	10.2	9.0	7.8
Mean maximum daily temperature	14.5	17.2	10.5	16.7
Warm season (May to September)				
Mean daily temperature	16.7	13.1	17.2	18.5
Mean maximum daily temperature	30.5	27.3	34.1	31.3
Reproductive parameters				
Total parturitions	2058	2227	2144	2220
Twins (%)[f]	5.2	7.3	6.8	6.2
Placenta retention (%)[g]	17[k]	16[k]	13[l]	12[l]
Primary metritis (%)[g]	17	17	18	16
Pyometra (%)[g]	3.7[k]	2[l]	1.4[m]	1.1[m]
Ovarian cysts (%)[h]	28[k]	24[k]	20[l]	28[k]
Anovulatory follicles (%)[h]	23[k]	26[k,l]	31[m]	32[m]
Total AI	6622	6724	6558	6741
Total diagnosed pregnancies	1706	1919	2074	2006
Conception rate (%)[i]	26[k]	29[l]	32[m]	30[l,m]
Percentage of culled cows	30	31	30	31

[a]Values with different superscript differ within columns according Tukey-Kramer Tests ($P<0.05$)

[b]Number of cows with less than 45 days in milk (waiting period).
[c]Number of cows not diagnosed pregnant with more than 45 days in milk.
[d]Number of diagnosed pregnant cows.
[e]Relation of protein and fat expressed as a proportion.
[f]Number of twin parturitions as a percentage of the total number of parturitions per year.
[g]Number of cases for each disorder as a percentage of the total number of parturitions per year.
[h]Number of cases for each disorder as a percentage of the mean number of inseminating cows per month.
[i]Number of pregnant cows as a percentage of the total number of inseminations per year.

The incidence of ovarian cysts was significantly reduced in the year 2005. The incidence of anovulatory follicles and milk production increased significantly in time, whereas uterine disorders (placenta retention and pyometra), and productive parameters such as days in milk and milk production improved with time.

3.3. Month effect

Tukey Kramer tests indicated month differences on milk production, twinning, ovarian cysts, anovulatory follicles and conception rate (Table 2). Milk production and anovulatory follicles increased and twinning decreased during the warm months, whereas the incidence of ovarian cysts presented a peak of incidence from September to December months.

3.4. High monthly fertility

Logistic regression analysis indicated no significant effects of herd, lactation number, twinning, days in milk, relation of protein/fat, anovulatory follicles, season and year on high monthly fertility. Table 3 shows the variables finally included in the logistic model. Based on the odds ratio, high monthly fertility was 0.14 and 0.55 times less likely for mean daily milk production per month <30 kg and 30-35 kg, respectively, than for values >35 kg, used as reference.

The likelihood of high monthly fertility decreased for each one unit % increase in the monthly incidence of ovarian cysts by an odds ratio of 0.62.

Table 2. Mean or total month values for production and reproductive parameters included in the study[a].

	January	February	March	April	May	June	July	August	September	October	November	December
Mean daily values per month												
Number of milking plus dry cows	2304	2289	2296	2336	2374	2375	2359	2348	2338	2384	2377	2386
Number of milking cows	2006	2023	2040	2078	2101	2088	2079	2047	2019	2052	2066	2074
Postpartum cows[b]	440	442	435	421	431	439	409	402	433	452	460	443
Inseminating cows[c]	707	680	637	675	635	621	674	716	720	701	743	727
Pregnant cows[d]	1069	1077	1107	1150	1181	1191	1135	1086	1052	1045	1035	1045
Lactation number	2.33	2.35	2.35	2.30	2.29	2.30	2.35	2.35	2.36	2.37	2.36	2.35
Days in milk	205	206	209	209	206	201	200	195	194	190	187	190
Milk production (Kg/day/cow)	30.1^{k}	31.2^{k,l}	31.6^{k,l}	32.3^{l}	32.3^{l}	31.9^{k,l}	30.7^{k}	29.5^{k}	29.3^{k}	29.2^{k}	29.8^{k}	30.5^{k}
Milk production (Kg/day/milking cow)	33.6^{k}	36.5^{l}	34.7^{k,l}	35.7^{l}	35.4^{l}	35.9^{l}	34.5^{k,l}	32.9^{k}	32.6^{k}	33.4^{k}	32.8^{k}	34.1^{k}
Protein/fat[e]	0.86	0.86	0.86	0.88	0.91	0.90	0.90	0.89	0.89	0.90	0.88	0.87
Reproductive parameters												
Total parturitions	731	729	720	658	681	626	665	748	802	744	767	778
Twins (%)[f]	5.1^{k}	4.3^{l}	3.4^{m,n}	1.8^{o}	1.8^{o}	3.1^{n}	3.1^{n}	2.8^{n}	3.7^{l,m}	6.4^{p}	3.8^{l,m}	8.8^{q}
Placenta retention (%)[g]	14	11	11	16	12	16	16	18	8	16	13	11
Primary metritis (%)[g]	18	17	17	16	17	16	17	17	16	17	17	18
Pyometra (%)[g]	2.2	2.2	1.7	2.3	1.6	1.4	1.4	1.3	1.9	2.4	3.3	2.3
Ovarian cysts (%)[h]	13^{k}	10^{k}	22^{k,l}	26^{k,l}	20^{k,l}	23^{k,l}	32^{l,m}	30^{l,m}	41^{n}	52^{n}	41^{n}	39^{m,n}
Anovulatory follicles (%)[h]	8^{a}	11^{a}	15^{a,b}	25^{b}	33^{b}	57^{c}	48^{c}	21^{a,b}	22^{a,b}	22^{a,b}	8^{a}	17^{a,b}
Total AI	2196	1988	1982	1855	1969	2038	2213	2581	2525	2551	2370	2377
Total diagnosed pregnancies	727	609	526	516	519	536	532	681	754	779	762	764
Conception rate (%)[i]	33^{k}	31^{k,l}	27^{m}	28^{m,l}	26^{m}	26^{m}	24^{n}	26^{m}	30^{l}	30^{k,l}	32^{k}	32^{k}
Percentage of culled cows	2.8	2.8	2.4	2.3	1.8	1.7	2.5	2.4	2.5	2.8	2.8	2.8

[a]Values with different superscript differ within columns according Tukey-Kramer Tests (P<0.05)
[b]Number of cows with less than 45 days in milk (waiting period).
[c]Number of cows not diagnosed pregnant with more than 45 days in milk.
[d]Number of diagnosed pregnant cows.
[e]Relation of protein and fat expressed as a proportion.
[f]Number of twin parturitions as a percentage of the number of parturitions per month.
[g]Number of cases for each disorder as a percentage of the number of parturitions per month.
[h]Number of cases for each disorder as a percentage of the number of inseminating cows per month.
[i]Number of pregnant cows as a percentage of the total number of inseminations per month

Table 3. Odds ratios of the variables included in the final logistic regression model for factors affecting high monthly conception rate (> 29%).

Factor	Class	% high month fertility	Odds ratio	95% Confidence Interval	P
Milk production	< 30	22.2	0.14[a]	0.08-0.5	0.01
per milking (kg)	30-35	47.1	0.55[a]	0.35-1.1	0.07
	>35	57.7	Reference		
Ovarian cysts	continuous		0.62[b]	0.44-0.86	0.004

Likelihood ratio test = 243.60; 70df, P=0.0001

Hosmer and Lemeshow Goodness-of-fit test = 21.6; 3 df, P = 0.86. Adjusted R2=0.15

[a]Odds of a month with low or medium milk production, respectively, reaching high conception rate, compared to months with high production.

[b]Odds of a month with high conception rate, compared to months with 1% less ovarian cysts.

4. Discussion

The goal of our study was to determine possible associations of reproductive disorders with the fertility of high producing dairy herds under warm conditions by using annual and monthly records. Analyses of annual and monthly records proved valuable to establish risk indicators of decreased fertility in the present study. Low milk production and cystic ovaries were associated with decreased fertility by analyzing monthly summary records. The annual rising trend of milk production was furthermore accompanied by a reduced incidence of uterine disorders such as placenta retention and pyometra, and increased conception and anovulatory follicle rates. Fluctuations of different reproductive parameters during and within the years were also registered.

There is a long history of associating high milk production with reduced fertility in dairy cattle [18, 19], but to counter these negative effects, new management tools have been advised [20]. In the present study, a positive relationship between milk production and fertility was noted, in agreement with previous studies in our geographical area [10, 12]. Better reproductive performance has also been reported elsewhere in higher producing herds [21, 22].

Cystic ovarian follicles are one of the most frequent and important ovarian disorders in modern high yielding dairy herds since they are an important cause of subfertility in dairy cattle [23, 24]. It has been reported that ovarian cysts increase the time to first insemination and the interval from parturition to conception. In addition, ovarian cysts decrease the pregnancy rate after first insemination and increase the number of services per conception [25, 26]. In our study, the increase of 1% in the monthly incidence of ovarian cysts affected negatively the chance of high monthly conception rate by an odds ratio of 0.62. High milk production has been frequently associated with ovarian cysts [27], but herein we found that milk production and ovarian cysts were associated positively and negatively, respectively, with fertility. Probably, the level of management on farms examined was sufficient to offset the negative effects of milk production on both parameters. Milk production is the main economic return to ownership. Therefore, it is logical to think that the risk of fluctuations in milk production is reduced to the minimum. Thus, in high producing dairy herds, either a decrease in milk production as an increase in the incidence of ovarian cysts could be both markers of any management or nutrition problem and risk indicators of decreased fertility.

It should be noted the fact that although season was not a factor affecting high monthly fertility, when the reproductive parameters were studied month by month, seasonality clearly emerged (Tukey-Kramer test, Table 2). The conception rate decreased besides an increase of the ovarian disorders during the warm season. Probably, the hard effect in this study of milk production and ovarian cysts masked the seasonal effects on fertility extensively described in our geographical area [9, 13]. Despite of relative low conception rate values during the warm months, anti heat-stress systems installed in the herds such as fans and water sprinklers probably allowed a high production in the farms during the warm season. The clear increase of the ovarian cyst incidence from July to December could be the result the partial failure of the anti-stress systems, when cows are under continuous heat stress conditions during long time. The incidence of ovarian cysts drastically decreased in the year 2005, the year with the highest conception rate registered. It would interesting to asses if during this year the continuous effect of prolonged heat stress during the warm season was softened due to a dripping of cool days, for example raining days. Unfortunately, we have not information to asses this point. These facts reinforce the idea that ovarian cysts can be an indicator of the management situation in a herd.

Probably, the remaining reproductive disorders follow an evolution of improvement or worsening more independent of the global temperature of a specific year.

Anovulatory follicles presented a high incidence during the warm season, since April to September, especially in June, 57% of inseminating cows. Wiltbank et al [28] described this disorder as anovulation with follicle growth to deviation but not ovulatory size. Ovulation and follicular atresia of the dominant follicle is associated with a rapid and slow LH pulse frequency, respectively. However, intermediate LH pulse frequencies (one pulse per 1–2 h) have been related to follicular persistence [28, 29]. Hot climate and so heat stress could be related to the higher incidence of anovulatory follicles during the summer months since several stress factors are known to reduce the frequency and amplitude of LH pulses [30]. The progesterone based treatment for anovulatory follicles was probably the reason why this disorder did not affect high monthly fertility. We found similar conception rates in cows suffering anovulatoy follicle condition following treatment than that in cows inseminated at natural estrus in previous large extensive studies [10, 31].

Twinning is an undesirable reproductive outcome in dairy cattle production systems and reduces profitability through negative effects on calves born as twins as well as on cows delivering twins [32]. The twinning rate in dairy cattle seems to have increased recently, and this may have important practical implications [33]. In the present study, an increase in the twinning rate was found during the autumn-winter months, whereas the lowest twining rate was found in spring-summer. Thus, double ovulations rate would be higher during the winter-spring months, in agreement with a previous study where double ovulation decreased during the warm season [34]. Cows could be more comfortable, less metabolically stressed and already did not present delayed effects of heat stress on winter or spring. A well nutrition management would favour double ovulation in cattle.

The four herds used in the study had different number of cows but demonstrated being similar in reproductive and productive parameters since herd was not a factor affecting high monthly fertility. Uterine disorders such as placenta retention and pyometra diminished over time with the milk yield increase in agreement with previous findings [9]. Improved management practices during the puerperal period could explain this decline in these farms.

As an overall conclusion, results presented here indicate that monthly summary records can provide useful risk markers of fertility variations. Monthly values of decreased milk production and increased incidence of ovarian cysts were associated with a decreased fertility.

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General Discussion

The main objective of this thesis was to study environmental and management factors that affect fertility and pregnancy losses in high producing dairy cattle in northeastern Spain, a country with a seasonal warm weather. Although heat stress is not a new problem affecting animals, there is a lack of studies of the effect of high temperatures in the “modern” high producing dairy herds. Moreover, management practices have suffered significant changes during the last decades besides an increase in milk production.

THI versus Temperature.

Temperature-humidity index is known as one the best indicator of heat stress in dairy cows nowadays. This index reflects not only the effect of temperature on the body of animals, but also the thermal sensation of warm, combining temperature and humidity in the same index. There are other indexes to quantify heat stress by complex formulas (Igono et al, 1992; Linvill and Padue, 1992), but THI can estimate heat stress by a simple formula, probably for that reason it has become one of the most used worldwide.

Under our work conditions, we considered that THI was a better predictor of early fetal loss than just temperature alone. That is probably because late embryos or fetuses are much less sensible to direct temperatures than early embryos, since sensitivity decreases as embryo develops (Edwars and Hansen, 1997; Parks and Yang, 1999; Krininger et al, 2002). But, obviously, they can be affected by changes in metabolism of their mother. The problem appears with fertility. Which factor has more influence in the decrease of fertility: direct effects of temperature on oocytes, embryos or spermatozoa, or changes in the metabolism of the mother and the resulting not ideal environment for pregnancy? There are several studies on the effect of high temperatures on oocytes and embryos (Ulberg and Sheean, 1973; Lenz et al, 1983; Baumgartner and Chrisman, 1987; Edwards and Hansen, 1996, 1997; Rivera and Hansen, 2001). Thus, it is known that high temperature *per se* can cause damage in oocytes and embryos. In one of the studies, we couldn’t conclude that THI was better predictor for infertility than temperature alone. Probably there is not only one solution to this question. Each herd, in its particular environment, should analyze with proper use of records, which indicator could be more helpful more to control heat stress.

It is known that heat stress can appear when 72 units THI or 27ºC are exceeded (Johnson, 1980; Berman et al, 1985). However, we have established that when THI exceeds 75, but especially when it is higher than 80, around AI day, fertility decreases significantly. The physiological conditions of the cows analyzed in the earlier studies probably are not the same than those of the present “modern” high producing dairy cows. Moreover, management practices have suffered drastic changes nowadays. On the other hand, only maximum daily temperatures lower than 20º C led to a significant increase in conception rate. Sensitivity of dairy cows to stressful agents can change depending on their physiological and reproductive status or even their productivity, that is why try to delimit when heat stress appears is a hard task. New investigations should be focused on methods to improve dairy cattle environment, otherwise milk production would not be able to continue increasing in high producing dairy herds. In countries with warm weather, it would be interesting to analyze possible management practices than can help to alleviate the negative effects of heat stress such as fans, shadows, and cool water in high producing dairy herds with very intensive management. Embryo transfer has been proposed to by-pass effects of heat stress in dairy cows (Zeron et al, 2001; Al-Katanami et al, 2002), and one possible helpful practice would be maintain several cows under air-conditioned environment to obtain not heat-stressed embryos, and transfer them to other cows. Block et al (2007) also proposed the treatment of bovine embryos with IGF-I after superovulation, and posterior embryo transfer of these embryos to heat stressed dairy cows. There is lack of studies on the economical success of applying air-conditioning during warm periods in large herds. Studies should be performed in order to know whether or not air conditioning is a feasible solution for heat stressed dairy cows

Management practices.

Management practices are important tools to improve animal health, and in turn, animal health factors greatly affect economic success of dairy herds. Optimal herd management is indispensable for maintaining high milk production (de Kruif and Opsomer, 2002) and actually high producing dairy herds tend to have better management practices than low producing dairy herds. As long as higher production per cow was profitable, more and more dairy producers breed and manage cows to produce more. There is little reason to believe that the herds have been adversely affected by genetic and management change. On the contrary, economic efficiency of the population would appear to be increasing as production increases.

High milk production has long been associated with reduced fertility in dairy cattle (Oltanacu et al, 1991; Dematawewa and Berger, 1998). In our geographical area of study we have found not only that milk production didn’t have negative effects on fertility, but also a positive relationship between them have been noted. Good management practices would cope the negative effects of selection for milk production. Moreover, in an adequate environment, high producing dairy cows can express their potential and present better conception rates. Probably the “problem” of high producing dairy cows consists in something else nowadays. These cows have more metabolic demands than lower producers, and therefore can be more sensitive to other stressful factors. Increasing milking frequency to 3 times per day or high temperatures during the warm period could be enough to impair their metabolic system. For that reason, more analysis should be done in the future to know if the increase of milk production compensates the decrease in fertility in 3 times milking dairy herds.

Management practices have improved enough these years to cope with negative effects of milk production, but disorders still remain. AI technicians and inseminating bulls were a dramatic risk factor for conception rate. The question is why dairy farms have been capable to improve their level of management to cope negative effects of milk production, but at the same time incapable to improve the AI procedure. Regarding AI technician, probably as AI technique improved, training of AI technicians would also have to increase. More emphasis has to be done in the AI technique especially in high producing dairy herds. On the other hand, the inseminating bull showed also a dramatic effect in conception rate. Usually, male is forgotten of possible causes affecting conception rate because AI bulls have passed several health test and less fertile bull have been eliminated. But in fact, fertility of semen can be influenced by the environment and management practices (Foote, 2003). One possible solution is to analyze fertility of the AI bulls and AI technicians periodically in each dairy herd. Thus, an optimal record and proper analysis of data is necessary to improve both factors. A computerized on-farm individual cow record system has several advantages, particularly when there is a need to know details about individual cows or individual inseminations. Computerized records are a great convenience and nearly a necessity to manage data effectively for herd size greater than l00 cows (Spahr, 1993). Maintenance of an on-farm database encourages more detailed records. High producing dairy herds would have to improve the use of data to improve their management practices.

One critical period for dairy cows is the periimplantational period (Corner, 1923; Hanly, 1961; Bulman and Lamming, 1979). Implantation requires complex signaling interaction between the fetus and mother (Tatcher et al, 1995; Wathes and Lamming, 1995; Hansen, 2002), and probably any stress factor can disrupt it. We found that heat stress during this period can increase subsequent pregnancy losses during the early fetal period in dairy cattle, but probably another stress factor could have the same effect. Management practices should be directed on make this period more comfortable for dairy cows. Moreover, supplementation with progesterone during pregnancy should be studied in detail for practical application. Progesterone supplementation has the potential to reduce the incidence of pregnancy loss during the early fetal period (López-Gatius et al, 2004a). Thus, it can help dairy cows to maintain pregnancy during periods of heat stress or other stressful periods.

Twinning has negative effects on pregnancy. We found that twins increase pregnancy losses in dairy cattle according to previous reports (López-Gatius et al, 2002, 2004b; López-Gatius and Hunter, 2005). On the other hand, in our geographical area, the lowest twining rate was found during spring-summer. That means that double ovulations rate would be higher during the winter-spring months, according to other studies performed in the same area (López-Gatius et al, 2005). This fact might reflect the well-being of cows during winter and spring months, when cows can express their genetic potential without environmental interferences. According to López-Gatius et al (2005), risk factors for double ovulation are cool season, lactation number, low milk production and DIM between 90 and 150 days. Then, again, high milk producers (and high fertile cows) will show less twinning rate than lower producers. Twinning rate and possible actions to diminish them should be analyzed in each herd. One reliable option might be the deletion of one of the embryos. It has been demonstrated that administration of progesterone after manual rupture of the amnion on Day 34 of gestation may provide a satisfactory way for twin reduction in dairy cattle (López-Gatius, 2005).

Markers of good management practices in dairy herds.

To increase reproductive performance of high producing dairy herds, it is necessary to monitor periodically their status in order to correct punctual deficiencies of the system. For that reason, markers of the efficiency of management practices should be found.

High producing dairy cows, cows that become pregnant before 90 days in milk, might be a good indicator of the cow well-being. An increase of ovarian cysts and a decrease in milk production could indicate that some problems appeared in the farm. Thus, these parameters should be analyzed in dairy herds in order to improve knowledge of real situation of each herd. Milk production is the main focus of dairy farmers because milk is the main economic imputes and it is logical that main efforts are done to maintain production constant. But, why ovarian cysts are a good marker of management or nutritional status? Is that because they are more sensitive to external effects or because dairy herds still don't known how to treat this disease properly? Some researchers have reported an effect of season on ovarian cysts (Seguin et al, 1976; Mantysaari et al, 1993; López-Gatius et al, 2002) and others have not (Barlett et al, 1986; Scholl et al, 1990). Probably it will depend on whether management practices are able to cope with negative effects of environmental or management stressors.

Reproductive and productive parameters of dairy herds are improving these last years: DIM and placenta retention are decreasing, while milk production and conception rate are increasing. The only parameters that are getting worse are ovarian diseases. Anovulatory follicles have increased 39% in the last 4 years. At the same time, this ovarian disease did not affect fertility of dairy cows, and this means that treatment for anovulatory follicles is the appropriate. Probably, anovulatory follicles are telling us that something is not going well. Is it the incessant increasing of environmental temperatures? Or are there other stressors capable to reduce frequency and amplitude of LH pulses and then, anovulatory follicles remain in the ovary? More studies are needed to understand the importance and the significance of its increase during the last years.

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Conclusions

The main conclusions of this thesis are:

- High values of THI during the periimplantational period and around AI period (especially before AI) were a risk factor for pregnancy loss and conception rate of high producing dairy cows, respectively.

- High temperatures between 3 days before to 1 day after AI had negative effects on fertility of high producing dairy cows.

- As in countries with seasonal warm weather, warm season demonstrated to impair fertility and increase early fetal loss in dairy cows.

- High milk production on day 50 postpartum can show a positive correlation with high fertility. Cows that get pregnant before 90 days postpartum were those who produce more milk on 50 days postpartum. Management practices of these herds offset the negative effects of milk production.

- Three times per day milking, inseminating bull and AI technician were a risk factors for infertility.

- Monthly ovarian cyst rate had a negative correlation with the monthly conception rate in high producing dairy herds.

- A monthly decrease in milk production had a negative correlation on the monthly conception rate.

- Anovulatory follicles, ovarian cysts and twinning present a great seasonality in Northeaster Spain.

- Monthly summary records can provide useful risk markers of fertility variations.

ANEX

Literature Review

The European Union (EU) is a major player on world markets for most dairy products. It produces 22% of world milk production, which is the biggest of the global market. In 1998, 15 countries of the EU produced and exported approximately 121 million tones and 12 million tones in milk equivalent, respectively (Burrell, 2000). EU dairy production continues to follow a trend towards increased intensification on a smaller number of larger, more specialized production units. For all producers this effectively focuses attention on producing maximum amount of milk per cow at the lowest possible cost. Intensification of dairy farms for maximizing production of cows has been the result in the majority of EU. There are nevertheless still a large number of registered dairy farms with relatively low cow numbers. These farms are probably less specialized and have other economical input than high producing dairy herds.

This intensification of production has culminated in a great selection for milk production of dairy cows. This selection has been associated with the decreased reproductive performance worldwide. Dekkers (1991) reported that an improvement in fertility increases profit, not only by reducing culling cost but also by increasing incomes from milk sale and shorter calving intervals (CI). Recently, in many countries, female fertility has been included in breeding goals to place emphasis on genetic aspects of reducing fertility costs in dairy cattle (Kadarmideen and Simm, 2002). Reproductive performance is a multifactorial problem and not totally associated with increase of milk production (Lucy, 2001; López-Gatius, 2003; López-Gatius et al, 2006). Many management practices and environmental factors influence reproductive performance of dairy herds. Many efforts have been made to improve reproductive performance in recent years. For that reason, nutrition and general management of dairy cows have both changed considerably in the past 40 years.

Heat stress

Temperature gradients have special relevance in the mammalian reproductive system. Domestic ruminants maintain homeothermy or deep body temperature at about 38.6ºC, within a wide thermoneutral range where nutrition and genetic interactions can proceed independent of air temperature.

A satisfactory environment for any farm livestock is one that ensures optimal productivity but also ensure the animal health of the animal. Environment could be defined as (Webster, 1981):

1. Thermal comfort. The environment must not be so hot nor so cold as to significantly affect production or cause discomfort.

2. Physical comfort. The space available to the animal and the surfaces with which it makes contact should be such as to prevent acute injury or chronic discomfort.

3. Disease control. The environment should be such as to minimize disease, either by restricting the spread of infection or by avoiding stresses liable to decrease resistance to infection, or both.

4. Behavioral satisfaction. The animal should not be impeded from performing most socially acceptably patterns of spontaneous behavior. It should also be reasonably free from fear.

If these are the criteria for a satisfactory environment, then environmental stress becomes anything that departs from these criteria as measured by a disturbance to normal physiology, performance, health or behavior.

The impact of environmental temperatures on animals has been alluded to since antiquity. In *On Airs, Waters and Places*, Hippocrates in the 5th century B.C. saw that cattle raised in the Near East were more prolific than European cattle because of the temperate climate. The first reports of decreasing fertility of this century started approximately 60-70 years ago (Erb et al., 1940; Seath and Staples, 1941). And probably trends will make the problem of heat stress even greater in the future than at present.

The climate plays an important role in reproductive and productive function (Bitman et al, 1984; De Rensis and Scaramuzzi, 2003). Temperature gradients have special relevance in the mammalian reproductive system. Male gonads need a cooler than abdominal location, the scrotal area, to become functional. A consistent lower temperature in the isthmus than the proximal ampulla seems to facilitate the relatively prolonged period of sperm storage in the distal portion of the isthmus during the preovulatory period (Hunter and Nichol, 1986). Graafian follicles are cooler than neighbouring ovarian tissues and deep rectal temperatures (Hunter et al., 1997). Domestic ruminants maintain homeothermy, or deep body temperature at about 38.6ºC, within a wide thermoneutral range where nutrition and genetic interactions can proceed independent of air temperature. Heat stress has become an important problem in countries with warm weather as Catalonia or other parts of Spain. Herein, there are only two clearly differentiated climates: warm (May–September) and cool (October–April) periods. Reproductive parameters are significantly impaired in the warm period (López-Gatius, 2003) and the season of insemination and parity have been significantly correlated with pregnancy loss (Labèrnia et al., 1996; López-Gatius et al., 2002). Heat stress can appear in any combination that causes higher effective environmental temperature than comfort temperature of the cow (5º-25ºC) (McDowell et al, 1972). Homeotherms have optimal temperature zones for production within which no additional energy above maintenance is expended to heat or cool the body. In this comfort temperature zone, minimum physiological cost and maximum productivity arises (Folk, 1974). Over the critic temperature of 25ºC, cows increase respiratory rate and start decreasing milk production and dry matter intake (DMI) (Johnson et al, 1963; Fuquay, 1981; Ronchi et al, 2001). Moreover, reproductive performance of cows undergoing heat stress is impaired (Bitman et al, 1984; De Rensis and Scaramuzzi, 2003; López-Gatius, 2003).

Hormonal changes have also been described. The problem is that these changes could be directly caused by high temperature or by the decrease of dry matter intake (Fuquay 1981, Drew, 1999). These changes are higher in Holstein dairy cows than in other breeds less specialized in milk production as Brown Swiss (Johnson and Vanjonack, 1976). Different breeds exhibit different reactions to unfavorable environmental thermal conditions. At high temperatures, *Bos indicus* animals perform better than *Bos taurus* ones, due to their higher tolerance to heat stress. Their lower metabolic rate and their more efficient heat loss mechanisms are the most important traits explaining the better heat tolerance and productivity of zebu type cattle under hot conditions (Colditz and Kellaway 1972; Frisch and Vercoe 1984; Spiers et al. 1994). However, within the less heat tolerant European cattle breeds (*Bos taurus*), there are anatomic and physiological differences which could explain different levels of thermal tolerance (Titto et al. 1998).

It is well documented that heat stress can cause a reduction in dry mater intake which prolongs the period of negative energy balance (Hansen, 1997; Wilson, 1998; Drew, 1999) and decreased plasma concentrations of insulin, glucose and IGF-I, and increased plasma concentrations of GH and non-esterified fatty acid (Lucy et al, 1992; Jolly et al, 1995; Butler, 2001). To this fact, a direct effect of heat stress on FSH (increased) and estradiol (decreased) plasma concentrations should be added (Wolfenson et al, 1995, 1997; Wilson et al, 1998). This causes not only poor expression of estrus (Gwazdauskas et al, 1981; Younas et al, 1993), but also delayed follicle selection and thus has potentially adverse effects on the oocytes quality (Roth et al, 2001a,b). The effects of heat stress may be exacerbated in high producing dairy cows compared with low producers, because metabolic demands placed upon high-yielding dairy cows are greater and should be added to the metabolic stress related to high temperatures.

Heat stress may influence the uterine environment by decreasing blood flow to the uterus with consecutive increase of uterine temperature (Gwazdauskas et al, 1975; Roman-Ponce et al, 1978). These changes inhibit embryonic development, increase early embryonic loss and reduce the proportion of successful inseminations (Rivera and Hansen, 2001). The magnitude of the effect decreases as embryos develop (Edwards and Hansen 1997; Paula-Lopes et al, 2003). Heat stress can also increase early fetal loss if high producing dairy cows suffer heat stress during peri-implantation period and a figure as high as 54% of losses were registered in cows carrying twins during the warm period (López-Gatius et al, 2004b).

There are 4 factors that can affect effective temperature (Bufimgton et al, 1981) as has been said: air temperature, solar radiation, air movement and relative humidity. Combine the four parameters in an index has not been possible since nowadays, although very recently index emerged to combine all four variables (Graughan et al, 2007), but more studies should be performed concerning the effectiveness of this new index. One of the most important indexes to analyze heat stress in cows is temperature-humidity index (THI) (Hahn, 1969; Fuquay, 1981). This index was created by Thom (1958) to analyze the thermal comfort sensation in humans, and later the Livestock Conservation Institute observed that this index was a good heat stress predictor also for dairy cows. This index combines both, temperature and relative humidity in one index. The mean THI was fitted to the following equation (Thom, 1959; McDowell et al, 1973):

Mean THI= (0,8x mean T + (mean RH (%)/100)x(mean T-14,4)+46,4)

Where T is temperature and RH is relative humidity

There is also a maximum THI, defined as maximum temperature and minimum humidity. This is one of the most efficient indexes to detect heat stress in dairy cows, because high temperature is always accompanied by low relative humidity (normally in the midday). On the other hand, hours with lower temperatures are always accompanied by high relative humidity concentrations (dew). Maximum THI is considered to be one of the most efficient indexes to detect heat stress in dairy cows (Hahn, 1969; McDowell et al, 1979; Ravagnolo et al, 2000)

Maximum THI (maxTHI)= (0,8x maximum T+ (minimum HR (%)/100) x(maximum T-14,4)+46,4)

When THI exceeds 72 units, it is considered that dairy cows undergo heat stress (Johnson, 1980). Then, production systems can improve fertility during high THI periods.

1.Temperature and humidity control → use of shade, fans, air-conditioning and sprinkler systems to cool animals. The most widely used methods are cooling systems that mist the cows with water from overhead sprays and cool the air. The use of these systems has produced some improvement of fertility but they were still unable to match the level of normal winter fertility (Armstrong, 1994).

2.Supplements of mineral and vitamins in the diet → Heat stress is associated with reduced total antioxidant activity in blood plasma (Harmon et al, 1997) and there is some evidence that the depression in embryo survival following exposure to elevated temperatures involves increased free radical production (Ealy et al, 1992). Long-term β-carotene supplements had a beneficial effect on fertility in lactating cows.

3.Embryo transfer → Embryo transfer can be used to bypass the harmful effects of heat stress on oocytes quality that limit embryonic development (Zeron et al, 2001; Al Katanami et al, 2002a). Al Katanami et al (2002b) showed that timed embryo transfer improved pregnancy rates under heat stress conditions but only when fresh embryos were transferred.

4.Hormonal therapy → In summer, the administration of GnRH to lactating dairy cows at estrus increased the conception rate from 18 to 29% (Ullah et al, 1996). The use of fixed time insemination (AI) has the distinct advantage of not requiring the detection of estrus and effective synchronization methods for fixed time AI have been developed. These programs did not increase the number of cows pregnant to the fixed time insemination but they did increase the number of cows pregnant by 120 days postpartum and reduced the number of days open (De la Sota et al, 1998; Cartmill et al, 1999).

Management of high producing dairy cows

Good management is one of the most important factors for cow fertility. Methods of farming have changed considerably the last 25 years, at least in European countries. It is demonstrated that good management can overcome with adverse effects of high milk production of dairy cows (Fahey et al, 2002). Some farmers manage high genetic merit cows, so as to achieve acceptable conception rates. For that reason, good management practices are indispensable to high milk production of dairy herds (de Kruif and Opsomer, 2002).

- **Milking frequency**

Milking cows three times per day has become a common milking frequency in recent years. From 1920 to 1950 milking 3 times per day was only done in purebred registered herds to increase milk production of these selected cows. Nowadays, the rising cost of the staff and facilities per cow, together with the increasing selection for milk production, have increased the interest in milking three times per day to improve the profitability of dairy herds (Table 1).

Increasing milking frequency increases milk production in cattle and in other species (Table 1) (Stelwagen, 2001). A response percentage of 3 to 39% for cows changed from 2 times milking per day to 3 times milking per day has been reported in research literature (Lush and Shroede, 1950; Pelissier et al, 1978; Pearson and Foulton 1979; Barner et al 1990). Management and facilities certainly have an important role in the percentage response to 3 times milking per day. Nutrition requirements for any potential increase in milk production must also be met, with 3 milkings per day herds being fed three times or more each day. Milking management and milking systems must be of top quality to assure udder health. High producing dairy cows are normally subjected to a higher lever of management than others. For that reason, nowadays high producing dairy herds normally milk their cows 3 times per day.

Table 1. Difference in milk yield with various milking frequency (Erdman and Varner, 1995.)

Milking frequency	Milk yield (Kg/cow/day)
2 vs 3	3.5
2 vs 4	4.9
1 vs 2	-6.2

Increasing milking frequency to 4 times per day has important practical limitations. Even though, the practice of milking cows 4 times per day for a short period of time, one to two weeks, is a common practice in registered herds. There are also studies of 4 times milking per day the entire lactation (Erdman and Varner, 1995) with increases of 5-12% of total milk compared with 3 times per day milking.

High producing dairy herds are not normally milked 4 times per day in our countries. Increasing milking frequency to 4 times per day needs extra money inversion due to increased routine work. Moreover, protein and fat decrease as milking frequency increase (Erdman and Varner, 1995).

- **Animal health**

Animal health is one of many factors that affect the economic efficiency of a dairy herd. Economic efficiency in turn is an important criterion to use in judging the desirability of alternative methods for enhancing animal health. Probably for that reason Animal health interest has increased in the last years. Changes in housing and mechanization have had a profound impact on management of dairy cows, especially in high producing dairy cows. For maximum comfort and milk yield in dairy cows, they must stand to eat, stand to be milked, and lie down to ruminate and rest. Standing to be mounted also is an important activity. Cows should spend time lying down when not eating or being milked to maximize blood flow to the mammary glands. This is the limiting factor to maximize milk yield and is greater when cows are lying down that when standing up (Metcalf et al., 1992; Rulquin and Caudal, 1992). Therefore, tie-stall or free-stall comfort is critical to increased milk yields and acceptable conception rates.

Many advances related to herd health are economical for large herds but might not be economical for small herds. Health monitoring and the automation of milking procedures are prominent and important examples. As indicated by Etgen and Reaves (1978) larger herds mean increased concentration of cattle. Increased concentration means that management must be more aware of and responsive to the basics of herd health care or health problems may increase dramatically. This can occur because larger number of animals in closer proximity increases opportunity to spread of a disease. Despite this problem, it has become apparent that with a good overall management, large herds can have an excellent health (American Society for Agricultural Engineers, 1983).

A good reproductive and productive management system starts with an efficient individual cow record system. Electronic records provide a rapid, efficient, and convenient way to predict reproductive events. Records should be updated at least weekly to utilize fully the schedules of cows expected to be in estrus and those that are due to be checked for pregnancy. Lists of calving, cows that are due to calve, and cows that are ready to dry off , AI technician, inseminating bull per AI have long been used on well-managed dairy farms. However, record systems only predict events; they do not monitor a cow's current status.

- **Artificial insemination**

Nowadays the modern industry of artificial insemination (AI) looks very different of the industry of 25 tears ago. In the 1980's there was a period of tremendous industry growth, driven by an enormous exportation of Holstein semen. The importation of elite North American genetics into developed dairy countries, especially Western Europe, led to increased international competition among the AI companies in the 1990s. In many cases, elite bulls being progeny tested in the United

States had brothers being progeny tested in Europe. The heavy use of a few key sire lines internationally also led to a global Holstein population that is now quite closely related. The health testing protocols used by the US Certified Semen Services (CSS) ensure that the semen of the tested bull is well identified and disease free. Nowadays the dairy industry receives the highest quality semen ever produced.

Not only is possible to buy semen of different proved characteristics, but also AI technique has changed these years. After 1960 almost all inseminations are performed with frozen semen, 0.25 ml straws containing 10-20 millions of spermatozoa. One of the most significant contributions to the successful commercial application of AI in dairy cattle breeding has been from the highly trained inseminator (Foote, 1996). Nevertheless, there has been a tendency to adopt routine techniques for semen deposition and ignore numerous factors unrelated to deposition side. Presently the pregnancy rate after a single AI service is normally not higher than 40% which is far removed from the 60% or higher rate commonly recorded in the 1960s (Olds, 1978).

Evolution of the site of semen deposition (based on Foote, 1996; López-Gatius, 2000):

- 1930s. Brownell demonstrated that AI method had similar conception rates to natural services.

- 1940s. Vaginal insemination was replaced by deep cervical insemination (standard AI method).

- 1950s. Deep cervical, uterine and cornual insemination showed only minor differences between these deposition sites on fertility (Knight et al, 1951; Salisbury and VanDemark, 1951; Weeth and Herman, 1951; Stewart and Melrose, 1952; Olds, 1953).

- 1960, 1970s. Deposition of semen into the uterine body resulted in higher fertility than deposition into the cervix. Based on these latter studies semen deposition into the uterine body became the established procedure (Macpherson, 1968 ; Moller et al, 1972).

- 1980s. Peters et al, (1984) demonstrated a lack of precision in the technique used by inseminators for depositing semen into the uterine body.

- 1988. Senger et al, demonstrated that bicornual insemination have better results than unicornual. Others researchers still question the efficiency of this method (Williams et al, 1988; McKenna et al, 1990; Graves et al, 1991).

- 1990s. Higher pregnancy rates were recorded when the inseminate was deposited into the uterine horn ipsilateral to the side of impending ovulation than for contralateral or uterine body inseminations (López-Gatius et al, 1988; López-Gatius, 1996).

The long history of uterine semen deposition in artificial insemination probably has limited the development of other techniques. However, the increasing trend in infertility in dairy cattle industry would justify effort to the development of other strategies.

Main reproductive disorders

The prevention and treatment of reproductive tract disorders in the dairy cow have been the basis of regularly scheduled veterinary programs for herd health. During the 1960s to 1970s, the emphasis has been mainly the control of infectious diseases and nonspecific infections affecting the reproductive tract, to improve estrus detection, monitoring of breeding techniques, and diagnosis and treatment of hormonal imbalances. Despite the programs developed by veterinarians to improve reproductive herd health, the past five decades there has been an increase of reproductive disorders worldwide, accompanied with decreasing fertility and increasing milk production, at least in countries with intensive dairy industry (Foote, 1996; López-Gatius, 2003).

- **Ovarian cysts**

Ovarian cysts are an important cause of subfertility since they increase calving interval (Lee et al, 1988; Borsberry and Dobson, 1989; Fourichon et al, 2000) and are one of the most frequent disorders in dairy cattle. Approximately 6-19% of cows develop ovarian follicular cysts (Jordan and Fourdraine, 1993; Garverick, 1997). Each occurrence of ovarian follicular cysts has been estimated to add between 22 and 64 additional days open (Borsberry and Dobson, 1989).

Cystic ovarian follicles develop when one or more follicles fail to ovulate and subsequently do not regress but maintain growth and steroidogenesis. They are defined as follicle-like structures with a diameter of 20 mm or more in either or both ovaries that persisted for at least 7 days in absence of a palpable corpus luteum. Ovarian cysts have been classified as follicular or luteal. Follicular cysts are usually thin-walled and secrete little progesterone; luteal cysts generally have thicker walls and secrete varying amounts of progesterone. Follicular cysts are more common than luteal cysts. Cysts have a multifactorial origin in which environmental, genetic and phenotypic factors are involved.

  - Endocrine imbalance

- Heredity
- Milk production
- Seasonality

The diagnosis of ovarian cysts are usually performed the first 60 days postpartum (Kesler and Garverich, 1982; Bosu and Peters, 1987; Day, 1991a,b). The self-recovery of these cysts is 60-65% (Refsdal, 1982; Barlett et al, 1986). The clinical signs for this pathology are variable are anoestrus, irregular estrus intervals, nymphomania and relaxation of the broad pelvic ligaments and development of masculine physical traits (Kesler and Garverick, 1982; Youngquis, 1986).

- **Anovulatory follicles**

Anovulatory follicles are defined as follicular structure of at least 8 to 15 mm detected in two consecutive examinations in the absence of a corpus luteum or cyst, and no estrous signs during the 7-day period between the exams (López-Gatius et al., 2001; López-Gatius et al., 2004a). Fertility of cows is reduced after ovulation of anovulatory follicles.

- **Twinning**

Cattle are uniparous species meaning that, normally, females produce one offspring per female. In dairy herds the incidence of twinning is approximately 4-5% of all parturitions. In high producers, the twinning rate can exceed 9% (Kinsel et al, 1998) and the rate of double ovulation may be over 20% (Fricke and Wiltbank, 1999) and it is really influenced by the age of the cow.

Twinning rate in cattle is closely related to the ovulation rate. The monozygotic, genetically identical twins, due to the spontaneous single embryo division, were estimated to comprise less than 10% of all double births (Erb and Morrison, 1959; Cady and Van Vleck, 1978). A majority of bovine twins are of the dizygous type, resulting from the ovulation and fertilization of two oocytes.

The most important problems of twin pregnancy in dairy cows are that it increases the risk of:

- Early fetal loss.
- Freemartinism.
- Dystocia
- Premature calving
- Placenta retention

"Freemartin" is the term used to describe the infertile females born as a co-twin to the males. Masculinization of the internal reproductive tract of these animals results from the exposure of the female fetus to the blood of the male twin in the uterus. In cattle, shortly after the implantation of twin pregnancies, placental vascular anastomoses of the two fetuses usually occur. This allows for the exchange of blood constituents between twins. In most cases, freemartin gonads develop into ovotestes that contain both ovarian and testicular tissues.

- **Uterine disorders**

  - Retained placenta

Loss of the placenta in the cow occurs during the third stage of parturition, the process of separation usually taking less than 6 hours (Roberts 1986). Retained placenta is defined as the retention of fetal membranes longer than 12 h after parturition.

The main consequences of retained placenta are:

- Reduced appetite
- Reduced milk production
- Increased risk of metritis
- Reduced fertility

In many cases the predisposing cause of retained placenta is uncertain and probably multifactorial. There are three ways in which a retained placenta can be induced: myometrial dysfunction, retention of the feto-maternal union and mechanical obstruction. The relative importance of the three is unclear. However, mechanical obstruction is apparently rare with estimates ranging from 2 per cent (Hindson 1976).

Many factors can be associated with retained placenta in dairy cows, some are shown in table 2 (based on Laven and Peters, 1996).

Table 2. Main risk factors for retained placenta in dairy cows.

Factor	Effect	
Year	+	Tendency to reduce during years
Season (cool versus warm period)	+	High temperatures increase the incidence
Herd	+/-	Great inter herd variability
Length of gestation	-	Prolonged and shortened increases the incidence
Lactation number	-	
Twins	-	
Dystocia	-	
Abortion	-	If abortion occurs after 120 days of gestation
Hypocalcaemia	-	
Nutrition	+/-	Low magnesium, copper, zinc and iron increases retained placenta

The methods for preventing the condition are at the moment limited to reducing the prevalence of some of the predisposing factors, for example by providing balanced nutrition and clean calving areas, because of the lack of knowledge of its etiology.

- Incomplete uterus involution

Involution of the uterus was defined as incomplete when the uterine horns and/or cervix were larger than those in nonpregnant cows. Slow recovery of reproductive competence during the post-partum period is a major limitation to the success of subsequent reproductive management programs that are implemented for inseminations beginning at the start of the voluntary waiting period. It was proposed initially that early and frequent occurrences of estrus following calving were associated with increased reproductive performance due to a more optimal restoration of the uterine environment (Tatcher and Wilcow, 1973).

During the first 4 weeks post-partum, the cow's immune system is challenged severely (Guff and Horst, 1997). Most cows develop a mild non pathological endometritis during the early puerperal phase of the post-partum period (Lewis, 1997), and uterine fluids (lochia) are usually voided during the first 2 weeks post-partum.

- Pyometra

The bovine pyometra is an inflammatory disease, which develops after the first ovulation postpartum in presence of an active (sometimes persistent) luteal tissue, usually from about the 20th–21st day onwards. Due to the luteal progesterone production the cervix is closed and not permeable, consequently the mucopurulent or purulent exudates accumulates in the uterine cavity (BonDurant, 1999; Sheldon and Dobson, 2004; Sheldon et al., 2006).

As potent chemotactic signals stimulating the influx of PMN cells into the inflamed, uterus, the local production of TNF-α, leukotriens and also other eicosanoids (PGF2-α and PGE2) may be relevant in pathogenesis (Kindahl et al., 1992, 1996; BonDurant, 1999; Seals et al., 2002). However, the local release of these inflammatory products, and/or their absorption from the uterus remains limited. Consequently, usually neither systemic signs are generated nor production of acute phase proteins is induced. So cows affected by endometritis or pyometra are rarely systemically ill, as a direct consequence of the uterine condition (BonDurant, 1999),

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