Abortion studies in Iranian dairy herds: I. Risk factors for abortion

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Abortion studies in Iranian dairy herds:

I. Risk factors for abortion

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ABSTRACT

Abortions, especially those occurring during late pregnancy, lead to considerable economic losses. To estimate the financial losses related to pregnancy loss, at first the influencing factors on abortion need to be identified. Thus, the objective of this study was to determine and quantify the risk factors and their interactions for abortion in Iranian dairy herds. Based on data from 6 commercial herds, logistic regression was used to identify the risk factors for abortion. The basic time unit used in the study was a 3-week period corresponding to an estrus cycle. Thus, stage of lactation is measured as number of 3-week periods in milk (3-WIM) and stage of pregnancy accordingly as number of 3-week periods in pregnancy. After removing the records with missing information, the analysis included 482,071 3-WIM records for 26,289 pregnant cows collected between 2005 and 2014. The investigated factors were herd effect, pregnancy stage, previous abortion, calving month, cumulative fat corrected milk (FCM) yield level, mastitis in current 3-weeks in milk, accumulated number of mastitis and all 2-way interactions. Pregnancy tests were performed between 35 and 50 days after insemination. Abortion was defined as fetal death or return to estrus after confirmed pregnancy between 63 and 252 days in pregnancy. The overall rate of abortion, calculated as the number of aborted cows divided by the number of pregnant cows, was 15.4% ranging from 13.6% to 17.4% at herd level. The results of the logistic regression analysis showed that the risk of abortion differs between herds. Furthermore, all other investigated factors interacted significantly with herd thus illustrating that the effects of risk factors also differ between herds. Other significant risk factors included parity (interacting with pregnancy stage, mastitis, lactation stage and previous abortion), calving month, mastitis (interacting with pregnancy stage), pregnancy stage (interacting with previous abortion and mastitis), lactation stage (interacting with mastitis) and previous abortion. Milk yield was not a significant risk factor for abortion, but due to significant interaction with mastitis it was kept in the final model. In general, it is concluded that inclusion of significant

interactions in a risk factor analysis as the present is of paramount importance for a correct quantification of the risk factors for a cow with given characteristics.

Key words: dairy herds, abortion, risk factors, logistic regression

Highlights:

- We have identified risk factors for abortion in Iranian dairy herds
- Estimates on probability of abortion will be used to determine financial losses
- Risk of abortion differs between i.e. herds, parities, pregnancy and lactation stage
- Inclusion of interactions in a risk factor analysis was essential

1. Introduction

Abortion is a major problem for dairy producers in Iran, like in many other countries. In addition to loss of fetus, abortion imposes re-breeding costs, veterinary care, decreased milk yield, and replacement costs to farmers (Peter, 2000). For example, in California incidents of abortion resulted in $US 640 loss per case (Thurmond and Picanso, 1990). In the United States in general abortion cost was estimated at $US 555 on average (De Vries, 2006), while in Iran these costs ranged from only $US 82 to even as much as $US 1,302 (Kalantari et al., 2008).

Compared to other countries (9.8% in USA (Ettema and Santos, 2004) or 1.2% in New Zealand(Moller et al., 1967)), the rate of abortion is high in Iran (10-20% for abortions caused by non-infectious agents in Tehran, (Kalantari et al., 2008) or even 30% in Tabriz region due to Leptospirosis occurrences (Hassanpour et al., 2007)). A higher incidence rate of abortion in Iran might be because of different affecting risk factors such as hygiene levels, climate condition and high density of cows in a barn .However, reported rates of pregnancy loss may also differ as a result of the applied definition of abortion. For instance, Thurmond et al. (1990) defined abortion as fetal death between 52-260 days in pregnancy and reported the rate of 11% for abortion whereas Norman et al. (2012) considered abortion cases only for cows which are more than 150 days in pregnancy and reported abortion rate at the level of 1.3%.

The overall objective of these abortion studies was to estimate the costs of abortion under different conditions. The cost of abortion can, to some extent, be compensated by farmer decisions - particularly about culling. Probably, in some cases, it will be beneficial to cull a cow after abortion whereas in other cases it will be beneficial to keep the cow and inseminate it again. Therefore, the interaction with the culling policy needs to be included. Thus, along the lines of previous studies by Bar et al. (2008) and Cha et al. (2011) regarding costs of mastitis, a dynamic programming model with emphasis on abortion is under development. The dynamic programming model is constructed as a multi-level hierarchical Markov decision process (Kristensen and Jørgensen, 2000) and it will in the following be referred to as the *economic model*. State variables of the model are – in addition to standard properties like milk yield, lactation number, stage of lactation and stage of pregnancy – also relevant risk factors for abortion. The basic time unit of the model (also known as the *stage length*) is a 3-week period. Thus stage of lactation is measured by number of 3-week periods in milk (**3-WIM**) and stage of pregnancy is measured by the number of 3-week periods in pregnancy (**3-WIP**). A detailed description of the model will be given in a subsequent paper.

In order to build the economic model and, particularly to parameterize it, relevant risk factors for abortion need to be identified and quantified in terms of a probability of abortion for a given stage (3-week periods). Therefore, in order to improve the precision for modeling abortion in Iranian dairy herds, the state of a cow for production and health was described during 3-WIM periods and consequently pregnancy status was assigned to 3-WIP periods. Thus, as the first step, factors related to loss of pregnancy for cows with different properties in the herd need to be identified.

Abortion can happen for several reasons including infectious agents (caused by bacteria, viruses, protozoa and fungi), non-infective agents (such as nutritional factors, management, genetic disorders, etc.) and unknown factors (Peter, 2000). Since as much as 57.3% of all cases of abortion are caused by unknown factors (Jamaluddin et al., 1996), elimination of an abortion problem from a herd is a difficult task. Even when we know the source of the problem (37.1% of all cases of abortion were due to infectious diseases and 5.5% related to non-infectious factors (Jamaluddin et al., 1996)) eradication of abortion cases is not getting any easier. Mastitis with both infectious and non-infectious etiology (Bradley, 2002), can substantially increase the risk of abortion (e.g. Risco et al., 1999). However, treating mastitis by frequent administration of antibiotics may result in antimicrobial resistance of mastitis pathogens (Oliver and Murinda, 2012) rather than eliminating the problem.

The probability of abortion may differ between herds (Rafati et al., 2010), calving seasons (Norman et al., 2012), insemination season (Rafati et al., 2010), parity (Gädicke and Monti, 2013; Norman et al., 2012; Rafati et al., 2010), pregnancy stage (Forar et al., 1996; Norman et al., 2012; Thurmond et al., 2005), milk yield (Gröhn et al., 1990; Melendez and Pinedo, 2007; Norman et al., 2012; Silva-del-Río et al., 2009) and DIM (Norman et al., 2012). Moreover, occurrences of previous abortions (Rafati et al., 2010) or mastitis cases (Chebel et al., 2004; Risco et al., 1999) may increase the risk of pregnancy loss.

Broad studies cited above tackle the problem of estimation of the probability of abortion. However, so far there has been a lack of a comprehensive study which would take into account all mentioned factors simultaneously (and interactions among them) in order to estimate the probability of abortion for a cow.

Thus, the objective of this study was to estimate the risk of abortion based on herd information, parity, calving month, milk yield, pregnancy stage, lactation stage, incidents of mastitis and previous abortions. In agreement with the economic model, the probability of abortion was estimated for 3-WIM and 3-WIP periods. Adopting 3-week stages both for lactation and pregnancy variable allowed obtaining the probability of abortion for cows successfully inseminated during different estrus cycles as well as very detail representation of abortion risk during lactation and pregnancy.

2. Material and methods

2.1. Data and herds

Data used in this study were collected from 6 industrial dairy herds located at 3 provinces of Iran during 2005-2014. The criteria for selection of the farms were an accurate recording system and herd size (farms with > 600 milking cows). The herds studied were under a similar management practice namely receiving regular veterinary service, heat synchronization, artificial insemination and vaccination. All cows also were raised in intensive production systems with free stall barns and fed with a balanced total mixed ration. Cows were milked 3 times a day. Pregnancy diagnosis in the studied farms was done between 35 and 50 days after insemination by ultrasonography or rectal palpation and then repeated in the 3rd, 4th and 7th month of gestation.

Data on herd and cow identification, parity, calving date, milk yield, milk fat percentage, insemination date, diagnosed pregnancy, abortion and mastitis were recorded. Data editing was carried out by SQL Server software (Microsoft, 2012) and R (R Core Team, 2015). The original dataset was edited to eliminate non-pregnant cows, cows with missing parity number or calving dates, and cows without mastitis and milk yield records (cows with only one fat percentage record during lactation or cows with lactation records only during open days were removed). Records were assigned to periods of 3-WIM with 27 levels for pregnant cows. Consequently, also the whole length of pregnancy was divided into 3-week periods (with 13 levels). According to the abortion definition, if a cow conceived at second 3-WIM (21-42 days in milk (DIM)), the first abortion can happen at the 5th 3-WIM (84-105 DIM) or the 4th 3-WIP (63-84 days in pregnancy). Therefore, all records before the possible occurrence of first abortion were removed from the dataset. The final dataset included 482,071 3-WIM records for 26,289 pregnant cows. The number of cows, number of 3-WIM records and the percentage of cows per parity for all studied herds are presented in Table 1.

2.2. Definition of traits

Pregnancy: Pregnancy stage (PS) was calculated as the interval between successful insemination and the time of the three week periods observed.

Lactation: In this study cows in parity 1, 2, 3 and ≥4 were considered. To estimate the risk of abortion during the lactation, 4 stages of lactation (expressed in DIM) were distinguished: Early (84-189 DIM), Mid (190-294 DIM), Late (295-399 DIM), and Extended (≥400DIM).

Abortion: Abortion was considered as fetal death between 63 and 252 day in pregnancy (from the 4th 3-WIP until the 12th 3-WIP). The reason for using this definition was due to the performed pregnancy test and the defined stages (with 3-week intervals). Visual signs of abortion or a return to service after confirmed pregnancy were recorded as abortion. In the dataset, abortion was coded as a binomial variable (1=abortion and 0 =no abortion) indicating whether or not a cow aborted during a specific 3-WIP. Also, preceding abortions of a cow, either in present or in previous lactation (PAIPP) were included in the dataset as a binary variable (1 = preceding abortion in present or previous lactation and 0 = no preceding abortion in present or previous lactation). For cows in parity 1, abortions which took place during the heifer's lifespan were counted as preceding abortions.

Milk yield: The milk yield expressed as fat corrected milk (FCM) was calculated using Gaines's formula (Giance, 1928):

$$FCM\ (kg) = (0.4\ \times kg\ of\ milk\ yield) + (15\ \times kg\ of\ fat\ yield)$$

The cumulative FCM yield from calving until the end of each defined 3-WIM period was calculated according to the guidelines described in Section 2 of ICAR (ICAR, 2014). To eliminate outliers, cumulative milk yields greater than 3 standard deviations from their unadjusted means were deleted. Afterwards, measurements associated with each period were categorized into 5 levels based on the corresponding mean and standard deviation given lactation and herd information.

Mastitis: Clinical and subclinical mastitis records were available as any abnormalities of milk or any quarter reported by the farm veterinarian or milking personnel during periodic checks, milking, check for other disorders, and check immediately after calving. In this study, mastitis cases in current 3-WIM (MAS) as well as accumulated number of mastitis incidences during the lactation until a particular 3-week periods (ACM) were considered. For MAS only 2 levels were possible (0 – no mastitis, 1- mastitis), while for ACM 4 levels were defined (0- no mastitis, 1-one case, 2-two cases, and ≥3 – three and more cases). This way of accounting for mastitis history is directly inspired by a Dutch study (Houben et al., 1993).

Month of calving: For each record, the month of most recent calving was included.

2.3. Statistical analysis

According to the binary nature of abortion, a logistic regression model was used to investigate the association between abortion and the studied risk factors. The model was constructed using the glm function with a binomial distribution in R stats package (R Core Team, 2015). The model specification was based on the backward elimination method. At first a full date analysis including the main factors and all 2-way interaction between variables was done. Interaction terms which did not significantly contribute to the regression model for abortion were removed from the model one-by-one. Different models were compared using the Akaike information criteria (AIC). The final model was as follow:

$$
\begin{aligned}
\mathrm{Logit}(\pi) = {} & \alpha + \mathrm{Herd_i} + \mathrm{Parity_j} + \mathrm{CM_k} + \mathrm{CFCML_l} + \mathrm{MAS_m} + \mathrm{ACM_n} + \mathrm{PS_o} + \mathrm{CDIM_p} \\
& + \mathrm{PAIPP_q} + \mathrm{Herd_i} \times \mathrm{Parity_j} + \mathrm{Herd_i} \times \mathrm{MAS_m} + \mathrm{Herd_i} \times \mathrm{ACM_n} + \mathrm{Herd_i} \times \mathrm{PS_o} \\
& + \mathrm{Herd_i} \times \mathrm{CDIM_p} + \mathrm{Herd_i} \times \mathrm{PAIPP_q} + \mathrm{Parity_j} \times \mathrm{PS_o} + \mathrm{Parity_j} \times \mathrm{MAS_m} \\
& + \mathrm{Parity_j} \times \mathrm{ACM_n} + \mathrm{Parity_j} \times \mathrm{CDIM_p} + \mathrm{Parity_j} \times \mathrm{PAIPP_q} + \mathrm{PS_o} \times \mathrm{PAIPP_q} \\
& + \mathrm{PS_o} \times \mathrm{MAS_m} + \mathrm{CFCML_l} \times \mathrm{ACM_n} + \mathrm{CDIM_p} \times \mathrm{ACM_n}
\end{aligned}
$$

Where π = the probability of abortion in current 3-week period (given that the cow was still pregnant at the beginning of the period), α= model intercept, = fixed effect of herd (i=1,…,6), = parity effect (j=1,…,4where 4 referred to 4 or more parities), = calving month (k=1,…,12), = cumulative FCM yield level(l=1,…,5), = mastitis in current 3-WIM (m={0,1} where 0 was no mastitis, 1 was mastitis), = accumulated number of mastitis (n =0,…,3 where 3 referred to 3 or more cases of mastitis), = pregnancy stage (o=4,…, 12 expressed as 3-WIP), =class of DIM (p={Early, Mid, Late, Extended}, where Early was between 84 and 189DIM, Mid was between 190 and 294 DIM, Late was between 295 and 399 DIM, and Extended was above 399DIM), and = previous abortion in preceding pregnancy in either present or previous lactation (q={0,1}where 0 was no abortion, 1 was abortion).

The association between abortion incidence and each potential risk factor was investigated by odds ratio (OR) and its Wald 95% confidence intervals. The predicted probability of abortion was calculated and plotted based on the results from the final logistic regression model.

3. Results and discussion

3.1 Descriptive statistics

A summary of descriptive statistics of the studied herds is presented in Table 1. Abortion rate, calculated as the number of aborted cows divided by the number of pregnant cows, ranged from 13.6% in Herd 1 to 17.4% in Herd 5, while the mean abortion rate across all examined herds was 15.4%. The reported rate of abortion was greater than the rate reported in the studies conducted with a similar definition of abortion on data from the United States (11%;Thurmond et al., (1990)). However, in our study, the rate of abortion was lower than that reported earlier in Iranian dairy herds (Hassanpour et al., 2007; Kalantari et al., 2008). Different definition of abortion may contribute to these differences. Overall 1.9% of cows in the studied herds aborted with an experience of previous abortion. The variation among herds was from 1.3% to 2.3% (Table 1). Overall, 25.8% of pregnant cows (ranging from 19.2% to 34.7%) were exposed to mastitis during the lactation (Table 1). The average of 105-day cumulative FCM yield ranged from 2,467 kg in Herd 5 to 2,953 kg in Herd 3.

The distribution of pregnancy, abortion and mastitis incidence during the lactation for the study herds is presented in Fig. 1. As expected, only 3.9% of all cows got pregnant during the first 42 DIM. However, until 105 DIM almost 49% of cows were successfully inseminated. On the other hand, as many as 20.3% of all mastitis cases in the herds took place during the first 42 DIM when only marginal numbers of the cows were pregnant. Incidence of abortion was most frequent between 169 and 189 DIM and accounted for 8.7% of all cases between 0 and 567 DIM.

3.2. Logistic regression model

The results of the final logistic regression model for risk factors on abortion are shown in Table 2. The results of logistic regression analysis showed that the risk of abortion was significantly

(P<0.01) different between studied herds. Moreover, the interactions of herd with parity, pregnancy stage, previous abortion, class of DIM, MAS and ACM were significant (P< 0.05).

The statistically significant differences among herds were hardly any surprise. Especially since preliminary herd statistics presented above revealed that the difference between a herd with the highest (Herd 5) and the lowest (Herd 1) rate of abortion was 3.8%. Also, OR obtained from the final logistic regression model followed a similar tendency (Herd 1 had the lowest while Herd 5 had the highest odds of abortion).

However, the probability of a particular cow experiencing pregnancy loss is not necessarily equally high for all cows in Herd 5. That is simply due to the fact that the probability of abortion depends on the level of the herd factor as well as on the levels of other factors included in the model (such as DIM, MAS or PAIPP). As demonstrated in Fig. 2a, if a cow was in parity 2, was from Herd 5, calved in September, had cumulated FCM yield at level 2, was pregnant after 63 DIM and had no experience of mastitis or abortion in previous pregnancy then indeed, that cow was in the highest risk of abortion compared to cows with the same conditions regarding lactation and pregnancy but coming from other herds.

If we would change conditions on incidents of abortion or mastitis (a cow would have abortion in last pregnancy (Fig. 2b), experience mastitis in current 3-WIM (Fig. 2c) or have mastitis twice during current lactation (Fig. 2d)) then cows from Herds 6, 3 and 6, respectively, for most of the pregnancy stages would be in highest risk of abortion. Therefore, the presented results suggest that pregnancy losses in Herd 5 are to a smaller degree due to mastitis cases and more related to some other unknown factors.

The obtained result might also be influenced by different herd management strategies regarding repeated abortions. For example, in one herd, a manager might decide to replace a cow immediately just after a single case of abortion, while in another that decision might be postponed and depend on milk yield level and parity number. In our study, Herd 6 and Herd 4 had the highest rate of repeated abortion with respect to general abortion rate (Table 1). These results suggest that changes to applied strategies regarding cows with abortion incidents might be necessary to prevent repeated pregnancy losses and related economic losses. The difference in climate type, herd size, herd parity structure, ownership of farms (by government or private), and milk yield in the studied herds are the other management factors that can result in different rates of abortion (Table 1). Furthermore, there is a direct relationship between the mastitis and abortion rate in studied herds and those with higher mastitis rate have higher abortion rate, as shown in Table 1. The different rates of mastitis may be due to differences in bedding hygiene, milking machine brand, use of gloves, and teat disinfection type, as reported by Sadeghi-Sefidmazgi and Amer (2015),which can in turn lead to different abortion incident rate.

According to our results, the odds of abortion increased with parity number (Table 2). Since the final model included significant interactions of parity with herd, MAS, ACM, class of DIM, PAIPP and pregnancy stage, the final probability of abortion will depend on the selected levels of the factors mentioned. For example, as demonstrated on Fig. 3a, the probability of abortion will be the highest for cow which was in parity 4, was from Herd 2, calved in September, had cumulated FCM yield at level 3, and got pregnant after 168 DIM, however only from 63 to 147 day of pregnancy. For cows in the same herd, and having the same conditions regarding milk production level, calving season as well as time of pregnancy initiation, advanced pregnancy (after 147 days) for parity 1 was connected with the highest risk of abortion.

As explained by Markusfeld-Nir (2005), the risk of abortion depends on type of agent attacking a herd. With a new agent, both old and young cows are similarly in risk of pregnancy loss. However, when a herd is attacked by an endemic agent which produces a long lasting immunity, abortion rate will be higher in a young population. Contrarily, the rate of abortion will be higher in an older population, when cows are vaccinated once in their lifetime (for example against brucellosis).

Therefore, it is not surprising that existing studies on risk factors for abortion are reporting contradictory results. For instance, Rafati et al.(2010) reported higher risk of abortion for older cows. Thurmond et al., (1990) also found that abortion rate increased after cows reached 5 year of age. On the other hand, Waldner (2014) reported the lowest risk of abortion in cows pregnant with their third calf. Other studies reported a decrease in risk of abortion by lactation number (Gädicke and Monti, 2013; Norman et al., 2012; Pinedo et al., 2009). Moreover, two studies found no association between parity and abortion (Gröhn et al., 1990; Risco et al., 1999). The higher probability of abortion for parity≥4 found in our study may suggest that some of the abortion cases are due to decreased immunity to for example brucellosis for older cows.

The results of the logistic regression analysis showed the odds of pregnancy loss were significantly different for pregnancy stages, with the highest odds of abortion for the $5^{th}$and the 7^{th} 3-WIP, respectively (Table 2). According to the obtained results, odds of pregnancy loss decreased from the 8^{th} 3-WIP (168 day of pregnancy).

Previous studies reported higher risk of abortion in the first (Forar et al., 1996; Gädicke and Monti, 2013; Rafati et al., 2010) and the second trimester of pregnancy (Hanson et al., 2003; Thurmond et al., 2005). Abortions, especially those caused by pathogens, can be time specific. For example, bovine viral diarrhea virus (BVDV) causes abortions during early stages of fetal development (Moennig and Liess, 1995), while abortions due to *Neospora caninum* occur in the second trimester (López-Gatius et al., 2004).

Since, the interactions of pregnancy stage with parity, PAIPP, and MAS were significant ($P <$ 0.01), the predicted probability of abortion for different pregnancy stages depended on parity (Fig. 3a), previous incidents of abortion (Fig. 3b) and incidences of mastitis (Fig. 3c and Fig. 3d).

In our study, previous abortion in preceding pregnancy in either present or previous lactation (PAIPP) increased odds of abortion (Table 2). Previous reports also have identified previous abortion as a risk factor for abortion (Rafati et al., 2010; Thurmond et al., 2005). Repeated abortions are expected in cattle with *Neospora caninum* infections (Corbellini et al., 2006), brucellosis and leptospirosis infection (Markusfeld-Nir, 1997). Previous abortion in previous pregnancy, as mentioned, had significant effect on risk of abortion across the pregnancy ($P < 0.001$) (Fig.3b).

The predicted probability of abortion during the pregnancy increased if an incidence of mastitis was detected in current 3-WIM with the highest probability of pregnancy loss between 7 and 10th 3-WIP for an example cow (Fig.3c). For cows exposed 2 or ≥ 3 times to mastitis during a lactation, the predicted probability of abortion was higher than for an example cow without or with a single case of mastitis during the lactation until a particular 3-WIM (Fig.3d). Surprisingly, the predicted probability of abortion for an example cow with one case of mastitis during lactation was lower compared to a cow without mastitis incidence during lactation (Fig. 3c). The explanation of the obtained result could be the fact that as much as 13.1% of all mastitis cases in the examined herds were registered during the first 21 days of lactation. At the same time (during the first 21 DIM) none of the cows in examined herds were pregnant (Fig. 1a). Mastitis during such an early lactation could not have any effect on developing fetus. However, commonly used mastitis treatment strategy, such as antibiotic therapy, at early stage of lactation could have reduced number of other infectious agents present at the herd and indirectly help to reduce the abortion rate.

The authors are not aware of any previous studies with the same definition of accumulated number of mastitis cases on abortion. However, existing studies agree that, in general, mastitis cases during pregnancy are increasing the probability of abortion. For example, Risco et al. (1999) reported a 2.7 times higher risk of abortion within the next 90 days for cows having clinical mastitis during the first 45 days of gestation compared with those without this experience. Also Chebel et al.(2004) found that cows experiencing clinical mastitis between artificial insemination and pregnancy diagnosis are 2.8 times more likely to lose their pregnancy during early pregnancy than those without such a history.

Cumulative fat corrected milk yield level (CFCML) was not a significant factor in the model (P=0.07). However, due to significant interaction of CFCML with ACM we have decided to keep CFCML in the final model. In agreement with our finding, Gädicke and Monti (2013) reported that 120-days cumulative milk production did not have a statistically significant association with abortion, although our study differs by dividing the state space of lactation to 3-WIM periods. Moreover, Risco et al., (1999) concluded that 305-days mature equivalent milk yield also was not associated with abortion incidence. In contrast, the risk of abortion increased due to high milk yield during the lactation (Norman et al., 2012), high previous milk yield (Gröhn et al., 1990) or high milk yield near the artificial insemination time (Melendez and Pinedo, 2007; Silva-del-Río et al., 2009).

Concerning stage of lactation, odds of abortion was greater for Extended classes of DIM but lower for Late and Mid class compared with Early class (Table 2). Norman et al. (2012) evaluated risk of abortion during different DIM periods. They reported that risk of abortion for each pregnancy stage decreased as lactation progressed. DIM class together with ACM affected risk of abortion ($P<0.01$). As an example, predicted probability of abortion for cows 2 times exposed to mastitis decreased when DIM for pregnancy increased (Fig. 4).

We have observed increased odds of abortion during warm months (July through October). As compared with cows calving in April, cows calving in July and January had higher and lower odds of abortion, respectively (Table 2). Several studies investigated the effect of season on abortion. Norman et al. (2012) have found higher and lower risk of abortion for cows calving in April through August and September through March, respectively. Some studies have not observed a significant association between breeding season and abortion (López-Gatius et al., 2004; Risco et al., 1999), and some have reported no seasonal patterns of risk for abortion (Gädicke and Monti, 2013; Gröhn et al., 1990).The higher incidence of abortion in warm seasons can be explained by heat stress and decrease in progesterone level as shown in literature cited by Geary (2005).

4. Conclusion

Abortion is a complex trait and several factors can influence its prevalence. In this study, the risk of abortion was explained by many variables (herd, parity, pregnancy stage, calving month, cumulative fat corrected milk, days in milk, occurrence of previous abortion, occurrence of mastitis, accumulated number of mastitis) and their interactions. The probability of pregnancy loss will therefore depend on the level of selected factor. Generalizing obtained results (for example to the statement that the risk of abortion is higher for cows in higher parities) can lead to misleading conclusions regarding e.g. replacement strategies. It is, therefore, concluded that inclusion of interactions in a risk factor analysis as the present is of paramount importance for a correct quantification of the risk factors for a cow with given characteristics. Detailed knowledge on the probability of abortion will allow proper estimation of economic losses in dairy herds and in the future will help in making optimal decisions by farm managers.

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References

Bar, D., Tauer, L.W., Bennett, G., González, R.N., Hertl, J.A., Schukken, Y.H., Schulte, H.F., Welcome, F.L., Gröhn, Y.T., 2008. The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. J. Dairy Sci. 91, 2205–2214. doi:10.3168/jds.2007-0573

Bradley, A.J., 2002. Bovine Mastitis: An Evolving Disease. Vet. J. 164, 116–128. doi:10.1053/tvjl.2002.0724

Cha, E., Bar, D., Hertl, J.A., Tauer, L.W., Bennett, G., González, R.N., Schukken, Y.H., Welcome, F.L., Gröhn, Y.T., 2011. The cost and management of different types of clinical mastitis in dairy cows estimated by dynamic programming. J. Dairy Sci. 94, 4476–4487. doi:10.3168/jds.2010-4123

Chebel, R.C., Santos, J.E.P., Reynolds, J.P., Cerri, R.L.A., Juchem, S.O., Overton, M., 2004. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim. Reprod. Sci. 84, 239–255. doi:10.1016/j.anireprosci.2003.12.012

Corbellini, L.G., Pescador, C.A., Frantz, F., Wunder, E., Steffen, D., Smith, D.R., Driemeier, D., 2006. Diagnostic survey of bovine abortion with special reference to Neospora caninum infection: Importance, repeated abortion and concurrent infection in aborted fetuses in Southern Brazil. Vet. J. 172, 114–120. doi:10.1016/j.tvjl.2005.03.006

De Vries, A., 2006. Economic value of pregnancy in dairy cattle. J. Dairy Sci. 89, 3876–3885. doi:10.3168/jds.S0022-0302(06)72430-4

Ettema, J.F., Santos, J.E.P., 2004. Impact of age at calving on lactation, reproduction, health, and income in first-parity Holsteins on commercial farms. J. Dairy Sci. 87, 2730–2742. doi:10.3168/jds.S0022-0302(04)73400-1

Forar, A.L., Gay, J.M., Hancock, D.D., Gay, C.C., 1996. Fetal loss frequency in ten Holstein dairy herds. Theriogenology 45, 1505–1513. doi:10.1016/0093-691X(96)00118-5

Gädicke, P., Monti, G., 2013. Factors related to the level of occurrence of bovine abortion in Chilean dairy herds. Prev. Vet. Med. 110, 183–189. doi:10.1016/j.prevetmed.2012.11.022

Geary, T., 2005. Management strategies to reduce embryonic loss, in: The Range Beef Cow Sym Posium XIX. Rapid City, South Dakota, pp. 1–11.

Giance, W.L., 1928. An efficiency formula for dairy cows. Scince 67, 353–354.

Gröhn, Y., Erb, H.N., McCulloch, C.E., Saloniemi, H.S., 1990. Epidemiology of reproductive disorders in dairy cattle: associations among host characteristics, disease and production. Prev. Vet. Med. 8, 25–39. doi:10.1016/0167-5877(90)90020-I

Hanson, T., Bedrick, E.J., Johnson, W.O., Thurmond, M.C., 2003. A mixture model for bovine abortion and foetal survival. Stat. Med. 22, 1725–1739. doi:10.1002/sim.1376

Hassanpour, A., Fartashvand, M., Abdollahpour, G., Mogaddam, G., Nadalian, A., Sattari, G.S., 2007. Seroprevalence of leptospiral infection in dairy herds in Tabriz – Iran. Pajohesh-Va-Sazandegi 67–77.

Houben, E.H., Dijkhuizen, A.A., Van Arendonk, J.A., Huirne, R.B., 1993. Short- and long-term production losses and repeatability of clinical mastitis in dairy cattle. J. Dairy Sci. 76, 2561–2578. doi:10.3168/jds.S0022-0302(93)77591-8

ICAR, (International Committe for Animal Recording), 2014. Page 37 in ICAR rules, standards and guidelines for dairy production recording. Section 2. Accessed May 2014. http://www.icar.org.

Jamaluddin, A.A., Case, J.T., Hird, D.W., Blanchard, P.C., Peauroi, J.R., Anderson, M.L., 1996. Dairy cattle abortion in California: evaluation of diagnostic laboratory data. J. Vet. Diagn. Investig. Off. Publ. Am. Assoc. Vet. Lab. Diagn. Inc 8, 210–218.

Kalantari, A.S., Mehrabani-Yeganeh, H., Moradi, M., 2008. Evaluation of economic losses due to abortion and decision making process for culling aborted cow. Master thesis in Animal Science, University of Tehran, Iran.

Kristensen, A.R., Jørgensen, E., 2000. Multi-level hierarchic Markov processes as a framework for herd management support. Ann. Oper. Res. 94, 69–89. doi:10.1023/A:1018921201113

López-Gatius, F., Pabón, M., Almería, S., 2004. Neospora caninum infection does not affect early pregnancy in dairy cattle. Theriogenology 62, 606–613. doi:10.1016/j.theriogenology.2003.11.002

Markusfeld-Nir, O., 2005. Chapter 10 Abortions, in: Herd Health. Ruppin Institute, Israel. Handbook written to support international dairy herd health courses in Israel.

Markusfeld-Nir, O., 1997. Epidemiology of bovine abortions in Israeli dairy herds. Prev. Vet. Med. 31, 245–255.

Melendez, P., Pinedo, P., 2007. The association between reproductive performance and milk yield in Chilean Holstein cattle. J. Dairy Sci. 90, 184–192. doi:10.3168/jds.S0022-0302(07)72619-X

Microsoft, 2012. Microsoft SQL Server Management Studio. http://www8.cs.umu.se/education/examina/Rapporter/EsquiviasFlarup.pdf.

Moennig, V., Liess, B., 1995. Pathogenesis of intrauterine infections with bovine viral diarrhea virus. Vet. Clin. North Am. Food Anim. Pract. 11, 477–487.

Moller, K., Newling, P.E., Robson, H.J., Jansen, G.J., Meursinge, J.A., 1967. A survey of abortions and long return intervals in dairy herds in the Huntly district. N. Z. Vet. J. 15, 137–142. doi:10.1080/00480169.1967.33713

Norman, H.D., Miller, R.H., Wright, J.R., Hutchison, J.L., Olson, K.M., 2012. Factors associated with frequency of abortions recorded through Dairy Herd Improvement test plans. J. Dairy Sci. 95, 4074–4084.

Oliver, S.P., Murinda, S.E., 2012. Antimicrobial resistance of mastitis pathogens. Vet. Clin. North Am. Food Anim. Pract. 28, 165–185. doi:10.1016/j.cvfa.2012.03.005

Peter, A.T., 2000. Abortions in Dairy Cows: New Insights and Economic Impact. Adv. Dairy Technol 12, 233–244.

Pinedo, P.J., Melendez, P., Villagomez-Cortes, J.A., Risco, C.A., 2009. Effect of high somatic cell counts on reproductive performance of Chilean dairy cattle. J. Dairy Sci. 92, 1575–1580. doi:10.3168/jds.2008-1783

R Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rafati, N., Mehrabani-Yeganeh, H., Hanson, T.E., 2010. Risk factors for abortion in dairy cows from commercial Holstein dairy herds in the Tehran region. Prev. Vet. Med. 96, 170–178. doi:10.1016/j.prevetmed.2010.05.008

Risco, C.A., Donovan, G.A., Hernandez, J., 1999. Clinical mastitis associated with abortion in dairy cows. J. Dairy Sci. 82, 1684–1689. doi:10.3168/jds.S0022-0302(99)75397-X

Sadeghi-Sefidmazgi, A., Amer, P.R., 2015. Milk losses and quality payment associated with somatic cell counts under different management systems in an arid climate. Can. J. Anim. Sci. 95, 351–360.

Silva-del-Río, N., Colloton, J.D., Fricke, P.M., 2009. Factors affecting pregnancy loss for single and twin pregnancies in a high-producing dairy herd. Theriogenology 71, 1462–1471. doi:10.1016/j.theriogenology.2009.01.013

Thurmond, M.C., Branscum, A.J., Johnson, W.O., Bedrick, E.J., Hanson, T.E., 2005. Predicting the probability of abortion in dairy cows: a hierarchical Bayesian logistic-survival model using sequential pregnancy data. Prev. Vet. Med. 68, 223–239. doi:10.1016/j.prevetmed.2005.01.008

Thurmond, M.C., Picanso, J.P., 1990. A surveillance system for bovine abortion. Prev. Vet. Med. 8, 41–53. doi:10.1016/0167-5877(90)90021-9

Thurmond, M.C., Picanso, J.P., Hietala, S.K., 1990. Prospective serology and analysis in diagnosis of dairy cow abortion. J. Vet. Diagn. Investig. Off. Publ. Am. Assoc. Vet. Lab. Diagn. Inc 2, 274–282.

Waldner, C.L., 2014. Cow attributes, herd management, and reproductive history events associated with abortion in cow-calf herds from Western Canada. Theriogenology 81, 840–848. doi:10.1016/j.theriogenology.2013.12.016

TABLES

Table 1. Characteristics and descriptive statistics of the studied herds.

	Herds					
Items	1	2	3	4	5	6
Dataset and management factors						
Total number of cows	5,582	7,067	2,855	3,865	3,771	3,149
Total 3-WIM[a] records for all cows	97,123	134,305	51,554	75,817	59,614	63,658
Herd parity structure						
Parity 1 (%)	41.0	38.5	42.8	40.9	41.2	38.3
Parity 2 (%)	28.1	28.5	29.0	28.8	29.1	32.0
Parity 3 (%)	17.9	19.4	17.7	18.1	17.6	19.4
Parity ≥4 (%)	13.0	13.6	10.5	12.2	12.1	10.3
Ownership of farms [b]	G	G	P	G	G	P
Climate type[c]	1	3	3	3	1	2
Binomial variables						
Abortion rate[d] (%)	13.6	16.6	14.5	15.4	17.4	14.6
Repeated abortion rate[e] (%)	1.6	2.3	1.3	2.0	2.1	2.3
Mastitis rate [f] (%)	21.4	25.2	27.7	26.6	34.7	19.2
Milk yield data						
Mean cumulative FCM[g](kg)	2,540	2,455	2,904	2,521	2,467	2,862
SD[h] in cumulative FCM[g](kg)	694	635	720	611	618	763

[a]3-weeks in milk.

[b]G= ownership by government, and P= private ownership.

[c]Climate type: 1= very cold, 2= cold, and 3= mild based on the humidity and temperature in the cold seasons. These climates have mild, relatively warm, and warm summers, respectively.

[d]The rate of abortion during 63-252 days of pregnancy calculated based on data for all cows as number of aborted cows divided by all number of pregnant cows.

[e]Repeated rate of abortion was calculated as number of cow with an abortion after a preceding abortion in either present or previous lactation divided by all number of pregnant cows.

[f]The rate of mastitis during the lactation for pregnant cows.

[g]Cumulative FCM (Fat Corrected Milk) was calculated based on milk records from the beginning of lactation until 105 day of lactation assuming 4% fat correction.

[h]standard deviation

Table 2. The results of the final logistic regression model for risk of abortion per three week periods in pregnant cows by herd, parity, pregnancy stage, calving month, milk yield, mastitis, and previous abortion in Iranian dairy herds.

Variable[a]	Parameter	OR[b]	95%CI for OR	*P*-value[c]
Intercept	-3.75			<0.01
Herd				<0.01
Herd1	Referent	1	-	
Herd2	0.34	1.41	1.19-1.68	
Herd3	0.46	1.58	1.28-1.97	
Herd4	0.54	1.71	1.41-2.08	
Herd5	0.62	1.58	1.52-2.24	
Herd6	0.23	1.26	1.03-1.55	
Herd × Parity				0.01
Herd × MAS				0.02
Herd × ACM				0.01
Herd × Pregnancy stage				<0.01
Herd ×Class of DIM				<0.01
Herd ×PAIPP				<0.01
Parity group				<0.01
Parity 1	Referent	1	-	
Parity 2	0.18	1.19	1.00-1.41	
Parity 3	0.21	1.23	1.02-1.49	
Parity ≥4	0.60	1.82	1.52-2.18	
Parity × MAS				<0.01
Parity × ACM				<0.01
Parity ×Class of DIM				<0.01
Parity ×PAIPP				<0.01
Pregnancy stage				<0.01
4^{th} 3-WIP	Referent	1	-	
5^{th} 3-WIP	0.15	1.16	0.97-1.38	
6^{th} 3-WIP	-0.08	0.92	0.76-1.12	
7^{th} 3-WIP	0.05	1.05	0.86-1.29	
8^{th} 3-WIP	-0.01	0.99	0.80-1.23	
9^{th} 3-WIP	-0.31	0.73	0.58-0.93	
10^{th} 3-WIP	-0.38	0.69	0.54-0.87	
11^{th} 3-WIP	-0.82	0.44	0.33-0.58	
12^{th} 3-WIP	-2.31	0.10	0.06-0.16	
Pregnancy stage ×PAIPP				<0.01
Pregnancy stage × MAS				<0.01
Pregnancy stage × Parity				<0.01
Calving month				0.01
April	Referent	1	-	
May	-0.02	0.98	0.88-1.09	
June	-0.07	0.94	0.84-1.03	
July	0.09	1.09	1.00-1.21	
August	0.05	1.05	0.95-1.15	

September	0.04	1.04	0.95-1.15	
October	0.03	1.03	0.93-1.14	
November	-0.07	0.94	0.85-1.03	
December	-0.02	0.98	0.89-1.09	
January	-0.12	0.89	0.80-0.98	
February	-0.01	0.99	0.89-1.09	
March	0.03	1.03	0.93-1.14	
CFCML				0.07
Level 1	Referent	1	-	
Level 2	-0.02	0.97	0.90-1.04	
Level 3	-0.03	0.96	0.90-1.03	
Level 4	-0.04	0.96	0.89-1.02	
Level 5	-0.04	0.96	0.90-1.03	
CFCML × ACM				0.01
Class of DIM				<0.01
Early	Referent	1	-	
Mid	0.06	0.90	0.78-1.04	
Late	-0.03	0.97	0.81-1.16	
Extended	-0.10	1.06	085-1.32	
Class of DIM× ACM				<0.01
PAIPP				<0.01
No	Referent	1		
Yes	0.20	1.22	0.98-1.53	
MAS				<0.01
No	Referent	1		
Yes	-0.66	0.51	0.32-0.82	
ACM				<0.01
No case	Referent	1		
1 case	-1.37	0.25	0.18-0.36	
2 cases	1.41	4.12	3.11-5.44	
≥3 cases	1.48	4.41	3.10-6.25	

[a]MAS = mastitis in current 3-weeks in milk; ACM = accumulated number of mastitis; class of DIM was defined as Early=84-189 days, Mid=190-294 days, Late=295-399 days, and Extended =≥400 days; PAIPP= previous abortions in preceding pregnancy either in present or previous lactation; 3-WIP= 3-weeks in pregnancy; CFCML = cumulative fat corrected milk yield level.

[b]Odds ratio (OR)of having a higher risk of abortion.

[c]*P*-value shows the statistical significance for entire parameter.

LIST OF FIGURES

Fig.1. Distribution of pregnancy rate (a), abortion rate (b), and mastitis rate (c) for 6 study herds based on data from all lactations, given for three week periods (each three week periods is marked with the maximum number of days in milk (DIM)).

Fig.2. The difference between predicted probabilities of abortion in studied herds during 3-weeks in pregnancy (3-WIP) periods presented on an example cow in parity 2, calved in September, had cumulated fat corrected milk yield at level 2, and conceived at 63 days in milk, moreover; a) was without experience of mastitis in current 3-week in milk (MAS=0) and current lactation (ACM=0) and was without previous abortion in preceding pregnancy either in present or previous lactation (PAIPP=0), b) experienced previous abortion in preceding pregnancy either in present or previous lactation (PAIPP=1, MAS=0, ACM=0), c) experienced mastitis in current 3-week in milk (PAIPP=0, MAS=1 and ACM=1), and d) was 2 times exposed to mastitis during the lactation (PAIPP=0, MAS=0 and ACM=2).

Fig.3. Predicted probability of abortion for different 3-weeks in pregnancy (3-WIP) periods for an example cow which was in Herd 2, calved in September, had cumulated fat corrected milk yield at level 3, and conceived at 168 days in milk, moreover a) was in different parity and without experience of mastitis in current 3-week in milk (MAS=0) and current lactation (ACM=0) as well as was without previous abortion in preceding pregnancy either in present or previous lactation (PAIPP=0), b) was in parity 2 and experienced (PAIPP=1, MAS=0, ACM=0) or not (PAIPP=0, MAS=0, ACM=0)previous abortion in preceding pregnancy either in present or previous lactation, c) was in parity 2 and experienced (PAIPP=0, MAS=1, ACM=1) or not (PAIPP=0, MAS=0 and

ACM=0) mastitis in current 3-week in milk, and d) was in parity 2 and had different number of mastitis cases during current lactation (PAIPP=0, MAS=0, ACM={0,1,2,3}).

Fig.4. Predicted probability of abortion during the 3-weeks in pregnancy (3-WIP) periods for cows conceived at different stages of lactation (at 63, 105, 147 and 189 days in milk (DIM)) and 2 times exposed to mastitis. Plot was drawn for example cows from Herd 2 which were in parity 2, calved in September, had cumulated fat corrected milk yield at level 2, and didn’t have experience of preceding abortion and mastitis in current lactation as well as in current 3 weeks in milk.

*Conflict of Interest Statement

Conflicts of interest:

The authors declare that there are no conflicts of interest.

Figure 1
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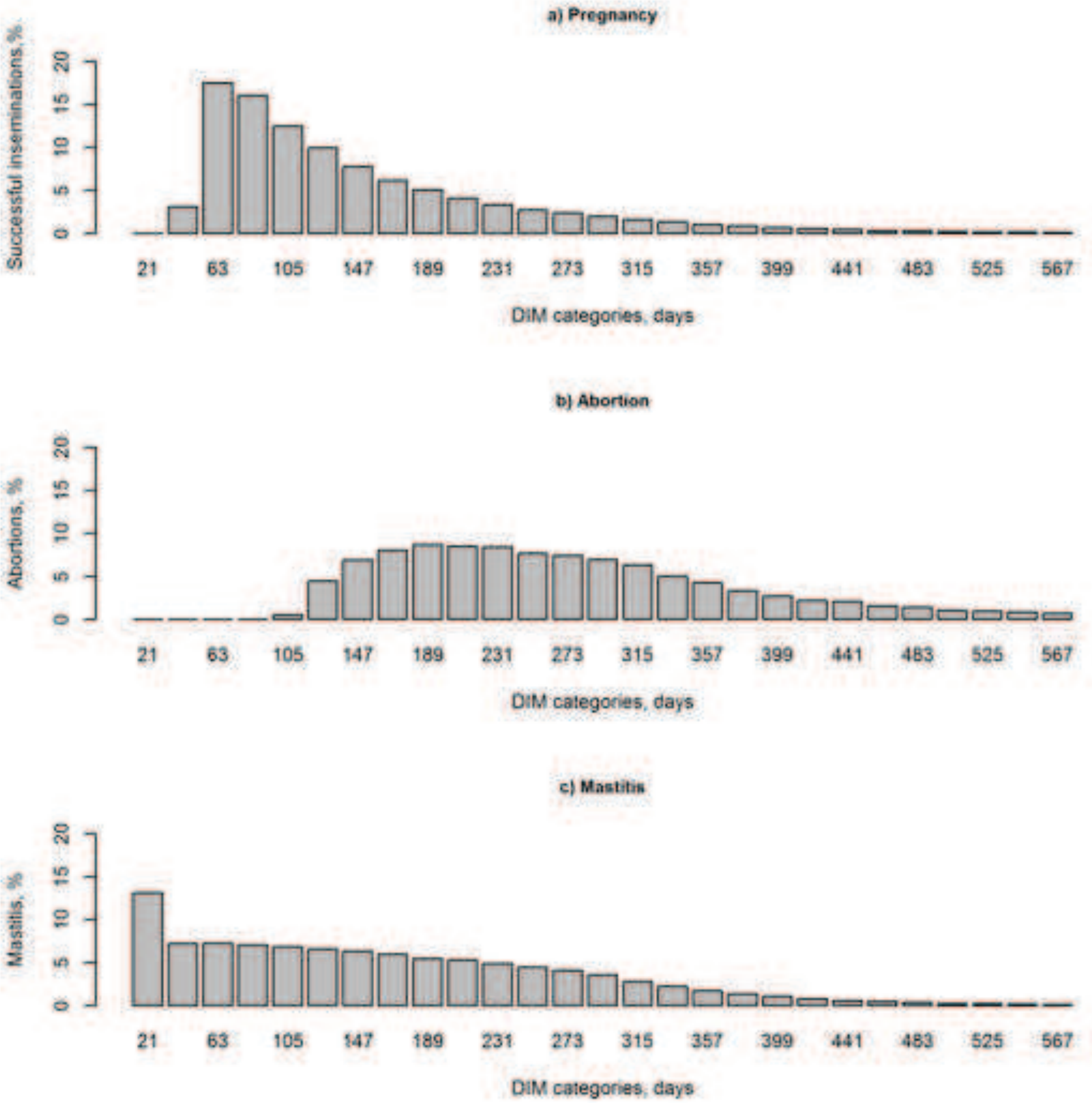

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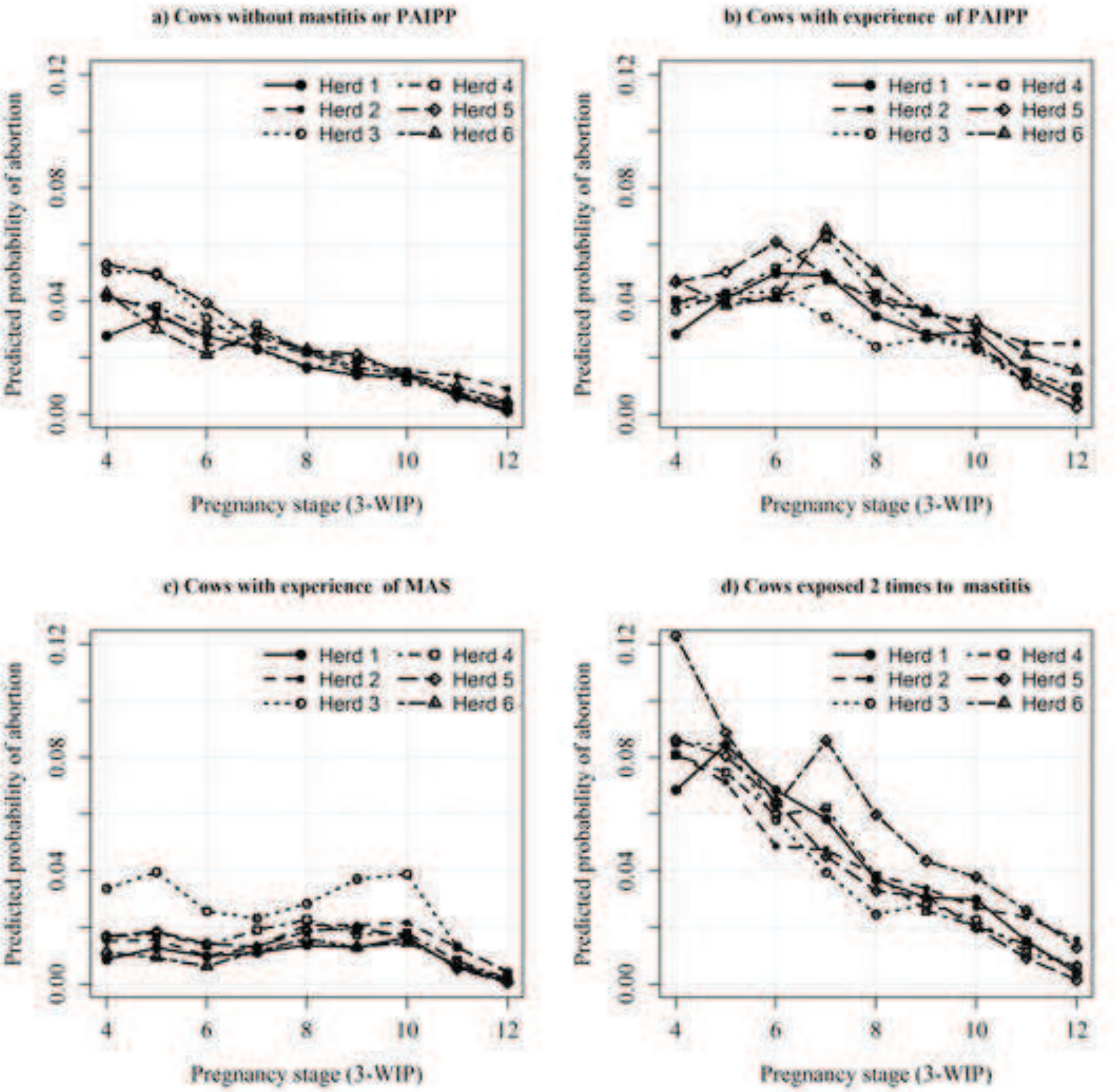

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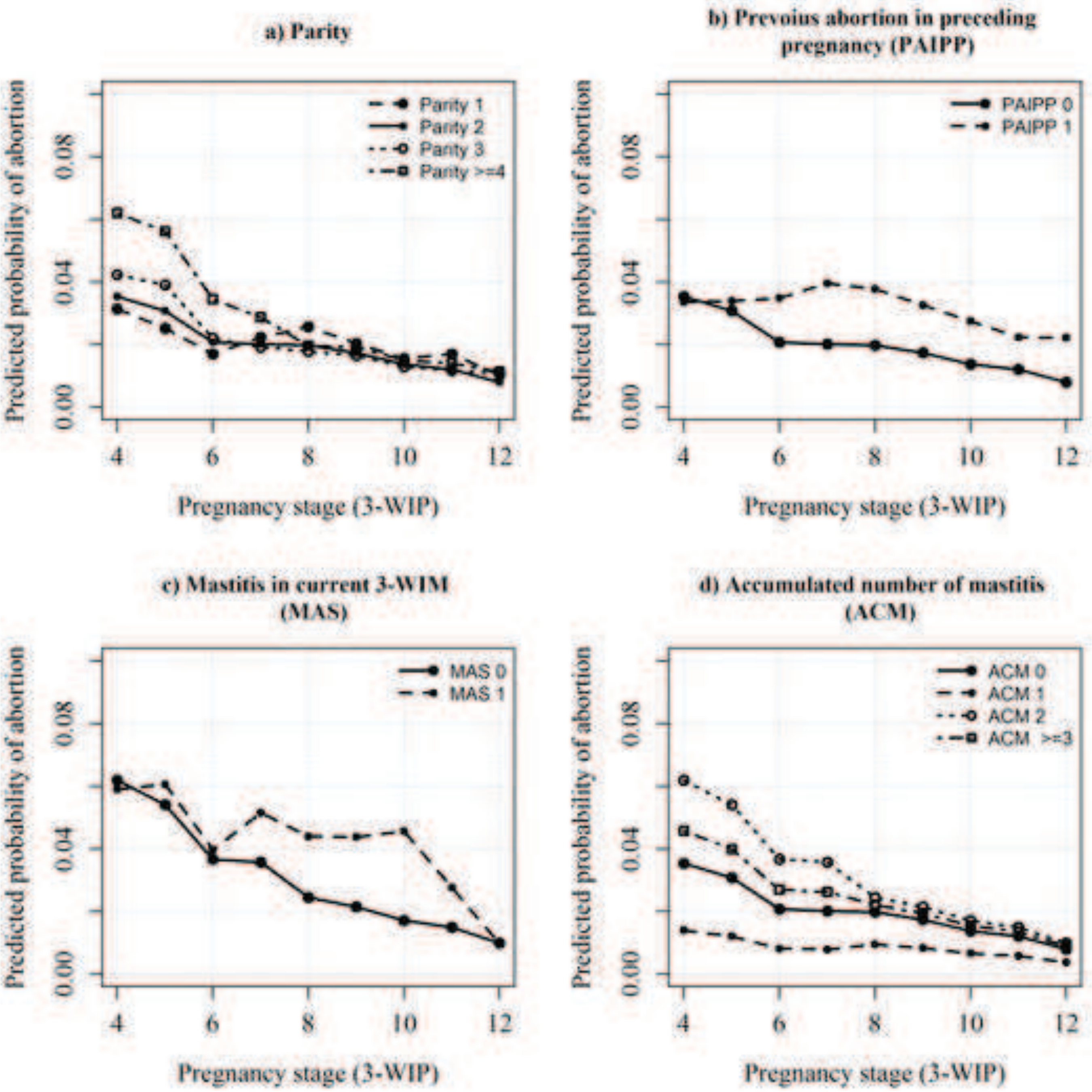
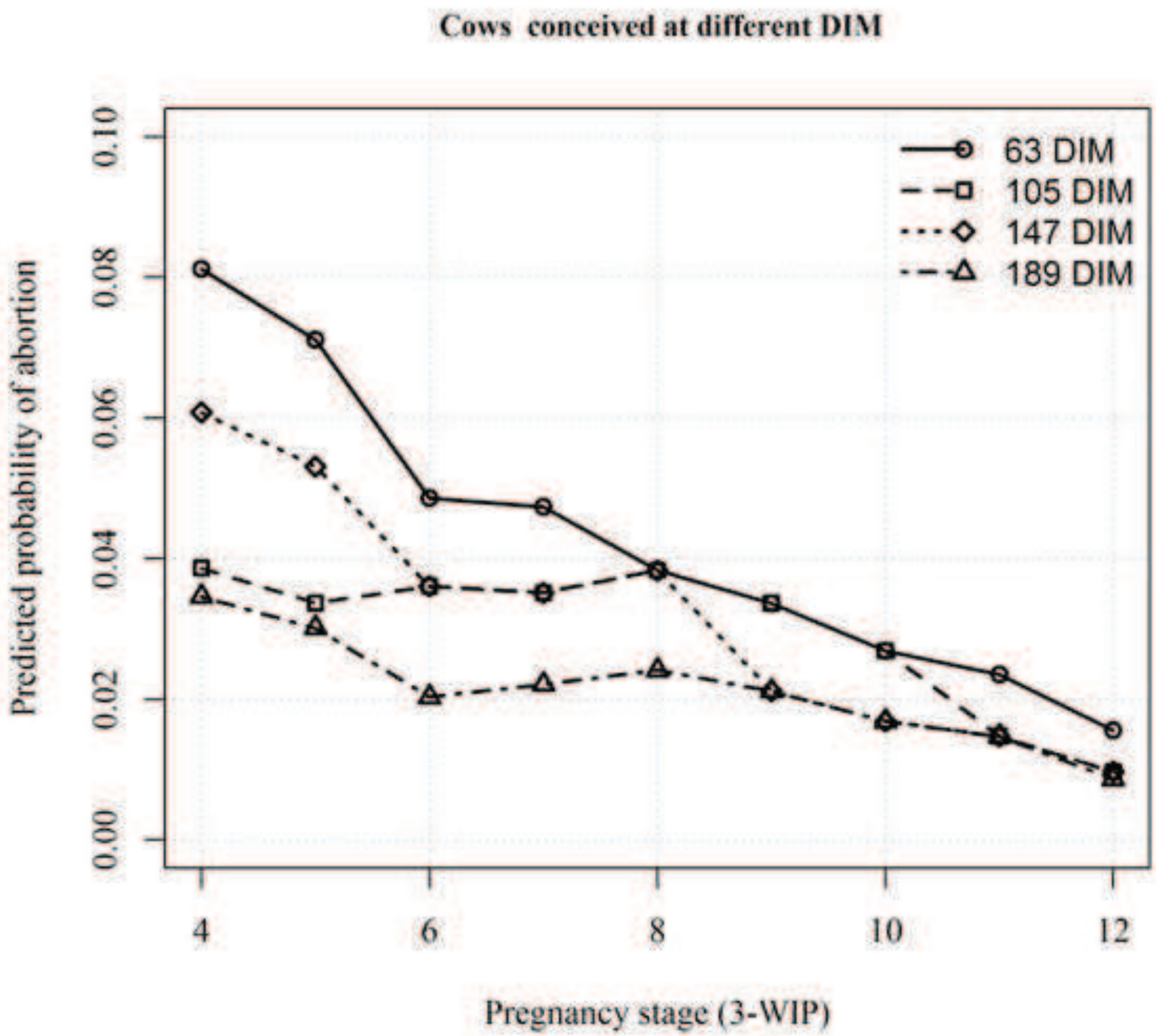


Figure 4


Cows conceived at different DIM
0.00
0.02
0.04
0.06
0.08
0.10
Predicted probability of abortion
4
6
8
10
12
Pregnancy stage (3-WIP)
63 DIM
105 DIM
147 DIM
189 DIM