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Impact and mechanisms of inflammatory diseases on embryonic development and fertility in cattle

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Abstract

Inflammatory diseases are prevalent in cattle and impair fertility. Cows affected by inflammatory disease from parturition to the day before breeding have reduced fertilization of oocytes, reduced survival of zygotes to the morula stage, impaired development to early stages of conceptus elongation, reduced secretion of interferon during the period of pregnancy recognition, altered transcriptome of preimplantation conceptus cells, and increased pregnancy loss. Consequently, these cows have reduced pregnancy and calving per breeding. Reduced oocyte competence is a likely reason for the carryover effects of diseases on developmental biology, but impaired uterine environment is also involved. Effects on pregnancy survival are observed up to 5 months after the diagnosis and treatment of disease, and effects on developmental biology seem to be extended into postnatal life in pregnancies that survive until term. Although the biological mechanism mediating the effects of inflammatory diseases are still not completely understood, control of inflammation during the clinical presentation of diseases seems to alleviate the negative effects on reproductive biology. It is increasingly evident that animal health, not only at the time of breeding or pregnancy development but also in the period preceding breeding, is imperative for optimal reproduction in cattle and should always be considered in herd evaluations and managerial decisions.

Keywords: dairy cattle, embryo, fertility, inflammation.

Introduction

Optimization of reproductive efficiency is a necessity to maintain farms economically viable and sustainable (Ribeiro *et al.,* 2012; Rodgers *et al.,* 2012). Pregnancy loss in cattle, however, is substantial and impairs reproductive efficiency (Santos *et al.*, 2004). Although fertilization in cattle is estimated to be above 80, up to 50% of the potential zygotes fail to survive by the end of the fourth week of development (Ribeiro *et al.*, 2016a). Although less frequent, fetal mortality, after gestational day 42, is costly and reduce profitability significantly (De Vries, 2006).

Suboptimal uterine conditions and less competent embryos are ultimately the main reasons for reproductive failures, and these conditions are affected by many genetic and non-genetic factors in a complex series of interactions. Inflammatory diseases, however, have been identified as a major cause of reproductive failures in cattle. It is increasingly evident that animal health is imperative for optimal reproduction in cows. This review summarizes the current information evaluating the impact of inflammatory diseases on fertility in cattle, potential biological mechanisms involved and associated implications for health and reproductive management.

Incidence of clinical diseases

Clinical diseases caused by microbial infection and tissue injury are prevalent in postpartum dairy cows (Santos *et al.*, 2010; Ribeiro *et al.*, 2013, 2016a). Approximately one-third of dairy cows have at least one clinical disease in the first 3-weeks of lactation, and they represent 60 to 80% all clinical cases occurring in lactating cows. The most common clinical diseases observed in dairy herds are metritis, mastitis, digestive problems, lameness, and respiratory problems. The incidence of these diseases in the first 2-months of lactation of 8,268 cows in eight large dairy herds in USA was 21.3, 13.8, 6.4, 5.5, and 2.4%, respectively (Ribeiro, 2015). Combined, these diseases affected 40% of all cows.

The increased susceptibility to diseases in the early postpartum is mostly explained by reduced immunocompetence of dairy cows during this period. The nutritional status and associated metabolic scenario observed postpartum impair function of immune cells and increase the susceptibility to opportunistic microbial infections (Sordillo, 2016). In addition, the enlarged uterus postpartum contains placenta remnants and lochia that favor proliferation of microbes and development of uterine infections (Sheldon *et al.*, 2009). All diseases described above are also seen in beef cows. However, the epidemiology of diseases in postpartum beef cows is not well documented. In general, diseases are less prevalent in beef cattle compared with dairy cattle but could become a significant problem in cases of nutritional deficiencies or environmental stress.

Impact of clinical diseases on reproduction

Cows with clinical diseases have delayed resumption of estrous cyclicity postpartum (Santos *et al.*, 2010; Ribeiro *et al.,* 2013), which prolongs the interval between calving and first artificial insemination (AI) postpartum. In general, delayed first breeding causes reproductive inefficiency and economic losses (Ribeiro *et al.,* 2012). Timed AI programs can be used to assure

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proper time of first AI postpartum. However, the odds of being diagnosed pregnant 45 days after a timed AI is 30% smaller for cows that had postpartum disease compared with cows that did not have postpartum disease (Fig. 1). Further, the odds of pregnancy losses after day 45 of gestation are 2-times greater, and the odds of calving from first breeding postpartum are 42% smaller for cows that had postpartum diseases compared with cows that did not have disease (Fig. 1). Therefore, the impact of diseases is significant even when cows are subjected to timed AI programs. No differences in ovulation after synchronization of the estrous cycle or expression of estrus at timed AI were observed between cows that had or did not have postpartum diseases (Fig. 2). Therefore, the observed difference in pregnancy per breeding would be a result of reduced fertilization of oocytes and/or greater embryonic losses occurring before pregnancy diagnosis.

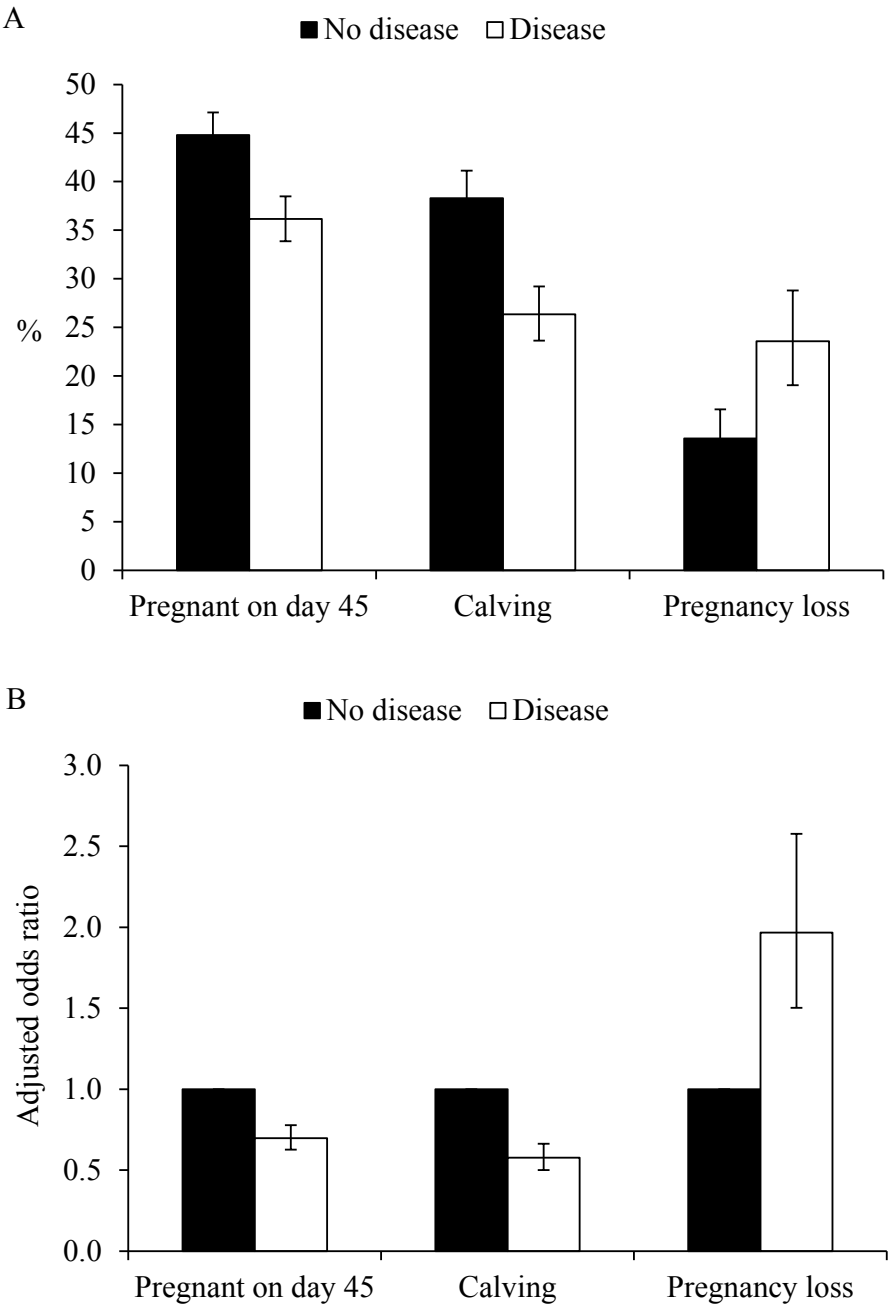

Figure 1. Adjusted means (Panel A) and adjusted odds ratio (Panel B) of the proportion of cows pregnant on day 45 after breeding, calving per breeding, and pregnancy loss after day 45 of gestation according incidence of clinical diseases before breeding. All outcomes were significantly ($P < 0.01$) affected by disease. Cows that did not have disease before breeding were used as reference for comparison (adjusted odds ratio = 1). Error bars represent the 95% confidence limits. Pregnancy on day 45 refers to data of 6,525 cows receiving their first breeding postpartum (Ribeiro, 2015). Calving per breeding and pregnancy loss after day 45 refer to data of 4,476 cows that were followed from first breeding postpartum until end of gestation (Ribeiro, 2015).

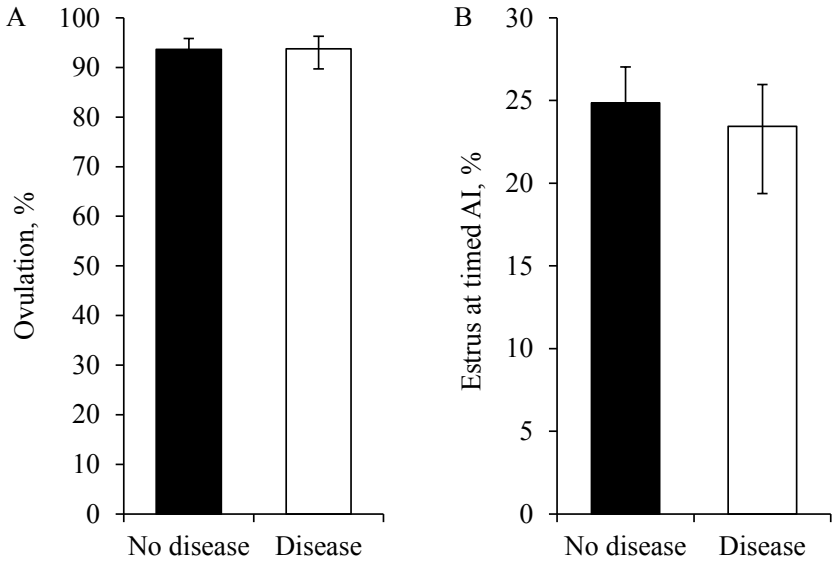

Figure 2. Adjusted means for the proportion of cows ovulating after synchronization of the estrous cycle (Panel A) and proportion of cows detected in estrus on the day of timed AI (Panel B) according to incidence of clinical diseases before AI. Disease before AI did not affect ovulation (P = 0.97) or estrous detection (P = 0.61). Error bars represent the 95% confidence limits. Data in Panel A refer to 746 cows receiving their first breeding postpartum whose ovulation was determined by ultrasonography examination of the ovaries 48 h after AI (Ribeiro, 2015). Data in Panel B refer to 1,265 cows receiving their first breeding postpartum whose detection of estrus was characterized by removal of tail chalk by the time of AI (Ribeiro, 2015).

To evaluate the impact of diseases on fertilization of oocytes, early embryo development and survival to morula stage, health information of 597 lactating cows was collected from parturition until first AI postpartum, and uterine flushing for recovery of ova-embryos was performed 5 or 6 days after AI. A total of 419 ova-embryos were recovered and evaluated for stage of development and quality. Cows with diseases before AI had reduced proportion of cleaved, live, and high-quality embryos relative to ova-embryos recovered (Ribeiro *et al.*, 2016a). Within cows with a recovered cleaved embryo, the odds of recovering a live embryo were reduced by 53.6% in cows with disease (Fig. 3). The reduction in cleaved embryos is likely caused by reduced fertilization of oocytes. Thus, the results indicate that postpartum disease reduces fertilization of oocytes and survival of zygotes in the first week of development.

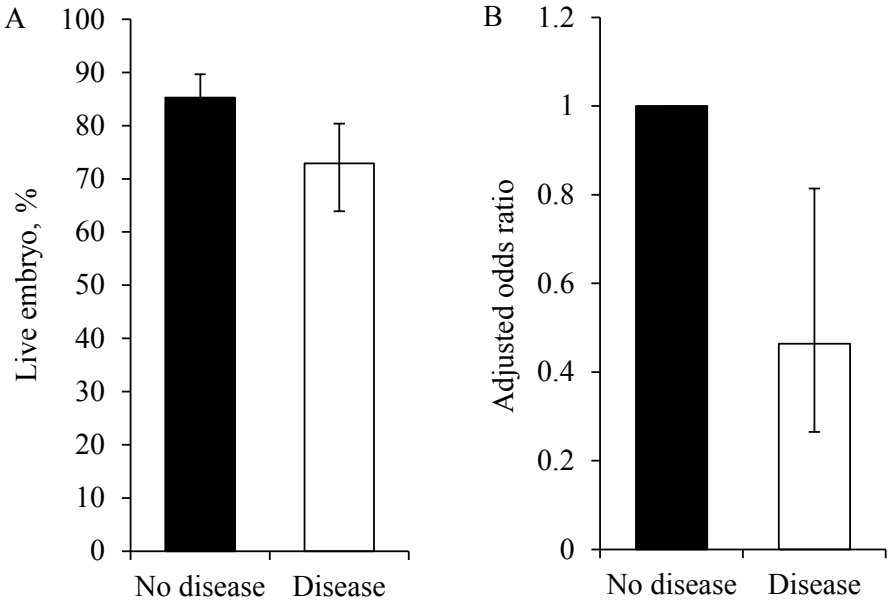

Figure 3. Adjusted means (Panel A) and adjusted odds ratio (Panel B) of the proportion of cows with a live embryo recovered from uterine flushes performed 5 or 6 days after AI according to incidence of diseases before AI. Cows that did not have disease before breeding were used as reference for comparison (adjusted odds ratio = 1). Error bars represent the 95% confidence limits. Data refer to 347 fertilized oocytes (cleaved embryos) recovered from 597 uterine flushes (Ribeiro *et al.*, 2016a).

To evaluate the impact of diseases on preimplantation conceptus elongation, health information of 148 lactating cows was collected from parturition until first AI postpartum, and uterine flushing for recovery of conceptuses was performed 15 or 16 days after AI. Cows with diseases had shorter conceptuses and reduced concentration of interferon (IFN)-τ in the uterine flush (Ribeiro *et al.*, 2016a). These results were supported by a second experiment that evaluated the transcript expression of IFN stimulated genes (ISGs) in peripheral blood leukocytes (PBL) on day 19 after AI (Ribeiro *et al.*, 2016a). Interferon-τ produced by the elongating conceptus in utero reaches maternal circulation and induces changes in gene expression in peripheral tissues including PBL (Oliveira *et al.*, 2008). Within cows that did not have disease before breeding, the expression of two ISGs (ISG15 and RTP4) was increased in cows later diagnosed as pregnant compared with those diagnosed not pregnant. However, this difference in gene expression of ISGs according to pregnancy status was not significant in cows that had diseases before AI, suggesting that production of IFN-τ by the elongating conceptuses in utero of cows that had postpartum diseases was reduced (Ribeiro *et al.*, 2016a).

Site of infection or tissue injury

In order to characterize the impact of diseases on reproductive biology of cattle, it is also important to understand how the impact is mediated, so that strategies to mitigate this negative association between diseases and reproduction might be developed. The site of infection or tissue injury is an important factor because the impact on reproduction and the mediator mechanism might change accordingly. Uterine diseases cause endometrial lesions that have detrimental effects on tissue integrity and physiology, hence suboptimal embryonic development and survival. Diseases that occur outside the uterus (i.e. mastitis, lameness, acidosis) have effects on reproductive biology that are mediated by a physiological response to infection or injury to tissues.

Ribeiro *et al.* (2016a) compared the effects of the uterine diseases (metritis) and non-uterine diseases (mastitis, lameness, digestive and respiratory problems) on reproduction of lactating dairy cows. Uterine and non-uterine diseases had similar impact on reproduction of dairy cows. Both type of disorder decreased pregnancy per breeding on day 45 after breeding, increased pregnancy loss after day 45 of gestation, and decreased calving per breeding (Fig. 4). Moreover, the two types of diseases have additive negative effects on reproductive outcomes. Cows that had both uterine and non-uterine diseases were 41% less likely to be pregnant on day 45 after breeding (adjusted odds ratio [AOR] = 0.59; CI = [0.47-0.75]), 3-times more likely to lose pregnancy after day 45 of gestation (AOR = 3.06; CI = [1.67-5.60]), and 60% less likely to calved from first breeding postpartum (AOR = 0.40; CI = [0.28-0.58]) compared with cows that did not have disease before breeding (Fig. 4). The effects of diseases on the development to morula and conceptus elongation were also similar between uterine and non-uterine diseases (Ribeiro *et al.*, 2016a).

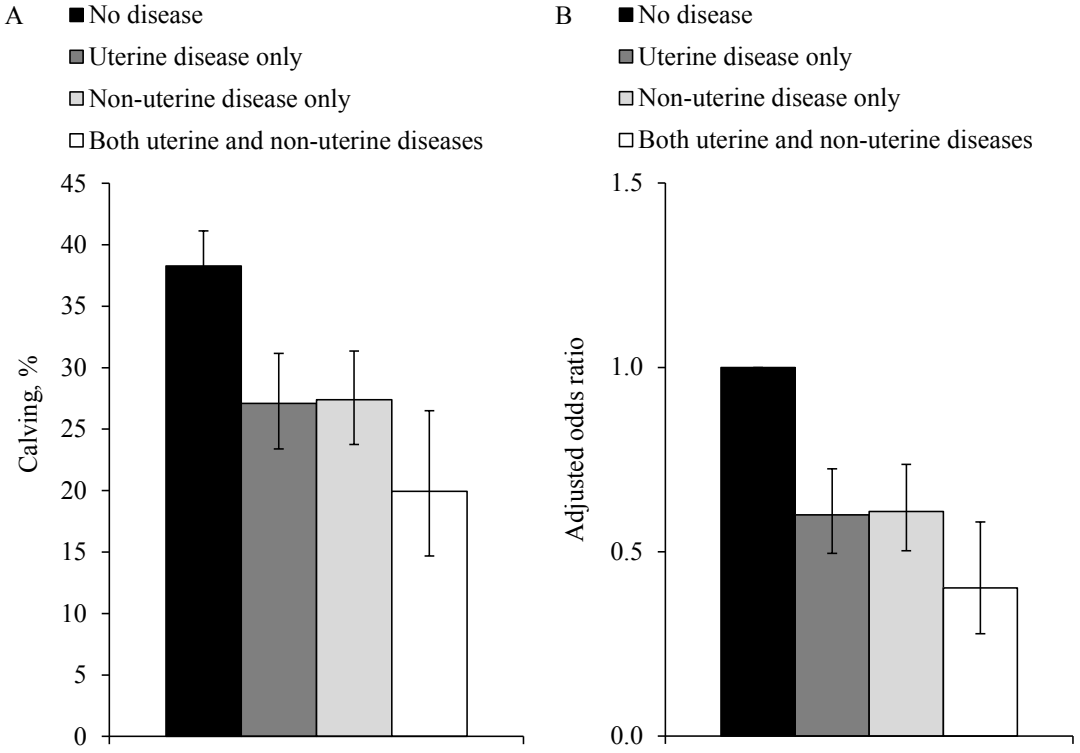

Figure 4. Adjusted means (Panel A) and adjusted odds ratio (Panel B) of the proportion of cows calving from first breeding postpartum according to the incidence of uterine and non-uterine diseases before breeding. Both uterine and non-uterine diseases significantly ($P < 0.01$) affected calving per breeding. The group of cows that did not have disease before breeding (no disease) were used as reference for comparison (adjusted odds ratio = 1). Error bars represent the 95% confidence limits. Information refers to data of 4,476 cows that were followed from first breeding postpartum until end of gestation (Ribeiro, 2015).

Physiological responses to microbial infections and tissue injury

The similar impact of uterine and non-uterine diseases on reproduction has led us to the hypothesis that physiological responses to microbe infections and tissue injury, the common factor between the two types of diseases, is the major mediator of the consequences on reproduction. Microbial infections and tissue injury cause a series of physiological adaptations not only locally in the insulted tissue, but also systemically in the individual (Colditz, 2002). One of the first responses of the affected tissue to an insult is inflammation, which is mediated by cells of the innate immune system that secrete inflammatory molecules such as cytokines, chemokines, eicosanoids and vasoactive amines. These inflammatory mediators recruit more immune cells to the site of injury and induce production of plasma proteins with the purpose of containing the infection or tissue injury (Medzhitov, 2008). Nonetheless, inflammation has consequences for energy metabolism (Colditz, 2002), and, when exacerbated, may generate excessive oxidative stress and further tissue damage and dysfunction (Medzhitov, 2008).

In general, cattle affected by diseases have reduced appetite, increased body weight loss, and altered partition of nutrients (Gifford *et al.*, 2012). The reduced appetite after establishment of disease is caused by the communication between immune and the central nervous systems (Dantzer and Kelley, 2007). Inflammation also increases energy expenditure to mount a response against infection and, therefore, partitions resources away from production and reproduction (Romanyukha *et al.*, 2006; Kvidera *et al.*, 2017). Collectively, reduced nutrient intake, increased nutrient and energy expenditure and altered nutrient partition caused by inflammation worsen the nutrient balance of postpartum dairy cows.

Indirect effects of disease on reproduction: anovulation and low body condition

The impact of inflammation on intake, expenditure, and partition of energy and nutrients cause compensative mobilization of body reserves, loss of body condition, and delayed resumption of estrous cyclicity postpartum (Ribeiro *et al.*, 2016a). Low body condition score (BCS) and anovulation at the onset of synchronization have been implicated individually with reduced fertility in dairy cows (Santos *et al.*, 2009). Therefore, it would be reasonable to speculate that the impact of diseases on reproduction is mediated indirectly by lower BCS at breeding and greater proportion of anovular cows at the onset of synchronization of estrous cycle.

To evaluate the hypothesis above, we collected information regarding health postpartum, estrous cyclicity before estrous cycle synchronization, BCS at AI, and conception risk at first AI postpartum in 2,190 cows (Ribeiro *et al.*, 2016a). As expected, cows with clinical diseases postpartum had greater proportion of anovular cows at the onset of synchronization (18.9 *vs*. 26.6%) and greater proportion of cows with low BCS at first AI postpartum (49.3 *vs*. 58.5%). Health status (disease or no disease), estrous cyclicity (anovular or cyclic), categorized BCS (low or adequate) and their interaction were then used concomitantly to predict conception risk of cows. All three predictors significantly reduced the probability of conception individually and did not interact with each other. The impact of disease on conception risk, therefore, was observed independently of estrous cyclicity and BCS of the cows. Thus, the results indicate that reduced BCS and increased incidence of anovulation caused by inflammatory diseases explain only a small portion of the impact of diseases on reproduction.

Time of infection or tissue injury relative to time of breeding

Inflammatory mediators are produced by immune cells in the insulted tissue and, through systemic circulation, reach other organs including brain, ovaries and uterus. These molecules have the potential to disrupt GnRH and LH secretion, oocyte developmental competence, and embryonic survival in cattle (Hansen *et al.*, 2004). Studies infusing lipopolysaccharide in the uterus, mammary gland, or intravenously, resulted in reduced secretion of GnRH and LH (Peter *et al.*, 1989; Lavon *et al.*, 2008). Incubation of granulosa cells with lipopolysaccharide or tumour necrosis factor (TNF) α resulted in smaller production of estradiol (Williams *et al.*, 2008). Incubation of immature oocytes in maturation media with TNFα reduced the development to blastocysts after fertilization *in vitro* (Soto *et al.*, 2003). Moreover, incubation of bovine embryos with TNFα five days after fertilization *in vitro* increased apoptosis of blastomeres (Soto *et al.*, 2003). These studies exemplify multiple consequences of inflammation on reproductive tissues.

It is important, however, to put in prospective the time of disease occurrence relative to the time of breeding and embryo development. Approximately 75% of the cases of clinical inflammatory diseases occurred in the first three weeks postpartum. Because the typical voluntary waiting period in most farms is 50 to 60 days, the first breeding postpartum in most cows with diseases occurs six to eight weeks after clinical resolution of disease. Conventional inflammatory mediators are unlikely to be at high concentrations in systemic circulation by the time breeding (Qu *et al.*, 2014) and perhaps are not directly important for pregnancy establishment and maintenance.

To evaluate whether the time of disease occurrence relative to breeding was important to determine the consequences on fertility, we compared the impact of non-uterine diseases occurring before breeding with those occurring after breeding (up to day 42 of gestation) on calving per breeding. Uterine disease was included as covariate in the statistical model. Calving per breeding was similarly affected by disease before breeding and by disease after breeding. The odds of calving from first breeding postpartum was reduced 47% (AOR = 0.53; CI = [0.34 – 0.84]) and 41% (AOR = 0.59; CI = [0.38 – 0.93]) by diseases occurring before breeding and after breeding, respectively. The two

variables did not interact, and cows that had both types, diseases before and also after breeding, had a reduction of 68% in the odds of calving from first breeding postpartum (Fig. 5). Thus, disease affects fertility of dairy cows not only at the time of breeding or early pregnancy development but also in the period preceding breeding, which confirms long-lasting effects of disease on fertility of cattle.

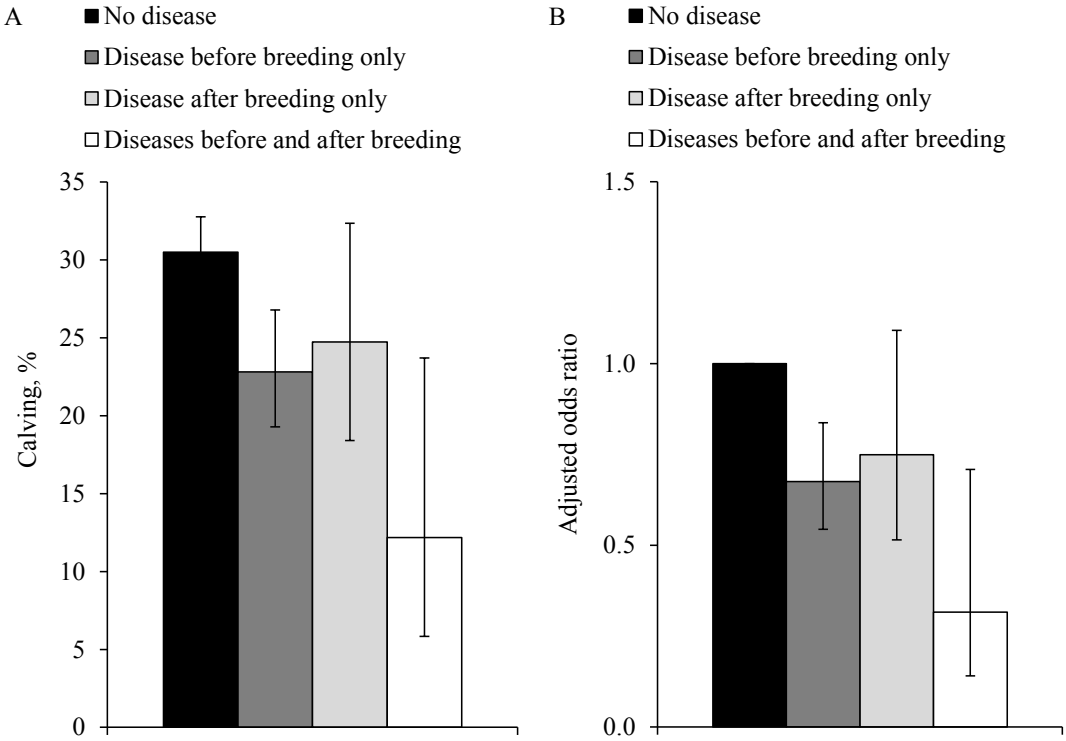

Figure 5. Adjusted means (Panel A) and adjusted odds ratio (Panel B) of the proportion of cows calving from first breeding postpartum according to the incidence of diseases before breeding and the incidence of diseases after breeding. Calving per breeding was significantly affected by disease before breeding ($P < 0.01$) and disease after breeding ($P = 0.02$), and these two variables did not interact ($P = 0.31$). The group of cows that did not have disease neither before nor after breeding (no disease) were used as reference for comparison (adjusted odds ratio = 1). Error bars represent the 95% confidence limits. Data from Ribeiro *et al.* (2016a).

Oocyte developmental competence *vs.* uterine environment

The interval from the activation of primordial follicles to the formation of preovulatory follicle is estimated to last 180 days (Fair, 2003), in which the majority of time would be spent in the pre-antral stages (138 days), and less time in the antral stages (42 days; Lussier *et al.*, 1987). During folliculogenesis, disease could potentially disturb the follicular environment and oocyte developmental competence without apparent effects on growth and ovulation (Bromfield *et al.*, 2015). Thus, a potential impact of postpartum disease on preantral or antral follicles is a plausible mechanism mediating the long-lasting effects of disease on reproduction.

If reduced oocyte developmental competence was the sole explanation for the long-lasting effects of postpartum diseases on reproduction, then fertility of cows in an embryo transfer (ET) program would not be affected by the occurrence of postpartum diseases. On the other hand, if diseases had an impact on fertility of cows receiving a viable embryo on day 7 of the cycle, then uterine environment should mediate at least part of the effects of disease on fertility of cattle. To test these hypotheses, information on the incidence of postpartum diseases, pregnancy and calving per breeding, and late pregnancy losses were collected in a large dairy farm using both AI and ET as part of the reproductive management for lactating cows (Ribeiro *et al.*, 2016a). Disease affected all reproductive outcomes, and the interaction with breeding technique was not significant (Tab. 1). Similar results were obtained when only uterine disease or only non-uterine diseases were considered, thereby suggesting that both types of disease have long-lasting effects on the uterine environment that impairs the ability to support pregnancy to term.

Furthermore, ET increased the proportion of cows calving from first breeding compared with AI (Tab. 1). The difference, however, was significant only in cows that had disease before breeding. The improvement in calving per breeding observed in cows that had disease when receiving ET suggests that oocyte quality and/or oviduct environment is also affected by disease. Supporting evidence for this interpretation is the slightly smaller change in adjusted odds ratios attributable to disease in cows receiving ET compared with those receiving AI (Tab. 1). Thus, reduced oocyte competence is a likely component in the carryover effects of disease in fertility of cows receiving AI, and impaired uterine environment is a reason for carryover effects of diseases in fertility of cows receiving AI and cows receiving ET.

Table 1. Reproductive outcomes of first breeding postpartum in dairy cows according to incidence of disease before breeding and breeding technique.

	Adjusted mean ± SEM [1]				P[2]			AOR (CI) [3]	
Item	No disease-AI	Disease-AI	No disease-ET	Disease-ET	DIS	BT	DIS*BT	Within AI	Within ET
Pregnant day 45	38.8 ± 1.8	31.0 ± 2.1	40.7 ± 1.7	35.9 ± 2.4	< 0.01	0.12	0.37	0.71 (0.58-0.87)	0.82 (0.65-1.02)
Calving	32.9 ± 1.7	22.2 ± 1.9	35.9 ± 1.7	28.2 ± 2.2	< 0.01	0.03	0.27	0.58 (0.46-0.73)	0.70 (0.55-0.90)
Pregnancy loss	12.4 ± 1.5	21.3 ± 3.1	11.1 ± 1.5	22.4 ± 3.4	< 0.01	0.87	0.59	1.92 (1.24-2.98)	2.30 (1.41-3.76)

[1]Adjusted mean and standard error of the mean for cows that had or not disease before breeding and were bred by artificial insemination (AI) or embryo transfer (ET). [2]Probability values: DIS = disease; BT = breeding technique; DIS*BT = interaction between disease and breeding technique. [3]Adjusted odds ratio (confidence interval) for the effect of disease within cows bred by AI and within cows bred by ET. Data from Ribeiro *et al.* (2016a).

Long-lasting effects of disease on uterine function

Conceptus cells sense changes in uterine environment and respond accordingly. Therefore, studying the transcriptome of conceptus cells could contribute to the discovery of a mechanism mediating long-lasting effect of inflammatory diseases on uterine biology. Ribeiro *et al.* (2016a) compared the transcriptome of conceptuses on day 16 of development from cows that had or did not have non-uterine diseases before AI. Five conceptuses recovered from cows that had non-uterine diseases before breeding were matched according to size with five conceptuses of cows that did not have disease before breeding and used for transcriptome analyses. Only a small number (n = 35) of transcripts were differently expressed between the two groups. Nonetheless, functional analysis of these transcripts revealed that changes in the transcriptome of conceptus cells recovered from cows that had diseases before breeding resemble an inflammatory response. Three proinflammatory molecules, lipopolysaccharide, IFN-γ and tumor necrosis factor were predicted to be potential upstream regulators of the changes in transcriptome. Moreover, the potential downstream consequences of these changes would include cell activation, particularly immune cells, and possibly problems with tissue rejection by immune system. These effects could result in rejection of the conceptus tissue by the maternal immune system and pregnancy loss. Nonetheless, it is not clear what specific alteration of the uterus would cause these inflammation-like changes in conceptus cells of cows that had postpartum diseases.

Lipids are important for elongation of the preimplantation conceptus (Ribeiro *et al.*, 2016b, c). They are used by trophectoderm cells for oxidation and generation of ATP, biosynthesis of membrane phospholipids and prostaglandins, cell signaling, and coordination of gene expression. Lipids accumulated in epithelial cells of the endometrium during diestrus are likely the most important source of fatty acids for utilization by the conceptus at the onset of elongation. Moreover, peroxisome proliferator-activated receptor (PPAR)-γ, a nuclear receptor activated by binding of fatty acids, seems to sense lipid composition in the histotroph and coordinate most of the lipid metabolism in trophectoderm cells (Ribeiro *et al.*, 2016b, c). The binding affinity of lipids to the ligand binding domain of PPARγ varies according to the biochemistry of the lipid molecule and modulates the activity of the transcription factor (McIntyre *et al.*, 2003; Itoh *et al.*, 2008). Thus, changing the composition of lipids in the endometrium can influence PPARγ activity and the biology of conceptus cells. Lipid composition of the endometrium at the time of breeding could be altered by long-lasting effects of postpartum diseases on energy metabolism of the lactating cow. Diseases worsen the negative energy balance of postpartum cows and increase the mobilization of fatty acids from adipose tissue (Ribeiro *et al.,* 2013), and excessive lipolysis postpartum may be sustained up to the time of breeding (Rukkwamsuk *et al.*, 1999; Contreras *et al.*, 2017). Adipose tissue derived-fatty acids, which are mostly saturated and monounsaturated fatty acids, are incorporated by multiple tissues (i.e. endometrium) and affect tissue physiology (Contreras *et al.*, 2017). Altered lipid composition of the endometrium could not only alter PPARγ activity in the conceptus but could also disturb the modulation of maternal immune cells in the endometrium at the time of conceptus elongation and early implantation. However, the contribution of altered composition of endometrial lipids to subfertility of cows with postpartum diseases still needs to be confirmed.

Transgenerational effects of postpartum inflammatory diseases

Since inflammatory diseases have substantial impact on pregnancy survival in cattle, Carvalho *et al.* (2017) investigated whether the lasting effects of inflammatory diseases would be extended into postnatal life in pregnancies that survive until term. Incidence of inflammatory diseases in 5,085 cows was recorded from calving until first breeding postpartum, and cows that became pregnant to first breeding were followed until calving. Female calves were followed up to 305 days in milk of their first lactation, and data related to morbidity, mortality, culling, reproduction and milk production were recorded. A total of 1,211 cows calved from the first breeding. Out of those, 872 cows did not have any diseases postpartum in the previous lactation (H-DAM) and 339 cows had at least one disease postpartum in the previous lactation (D-DAM). Out of the 339 D-DAM, 300 had a single disease (SD-DAM) and 39 had multiple diseases (MD-DAM). The proportion of female calves born did not differ among groups and averaged 51.9%. Incidence of dystocia was greater in D-DAM compared with H-DAM (39.8 *vs.* 30.2%). Rate of morbidity, mortality, and culling before and after first calving, age at first AI, pregnancy after first AI, age at first calving, and milk production in the first lactation did not differ between heifers born from H-DAM and those born from D-DAM. Nonetheless, the incidence of diseases before first calving was smaller for MD-DAM heifers compared with SD-DAM and H-DAM heifers (26.3 *vs.* 62.2 *vs.* 57.4%). The rate of morbidity also was less for MD-DAM compared with H-DAM (hazard ratio = 0.35) and S-DAM (hazard ratio = 0.34) heifers. These results indicate that transgenerational effects of postpartum inflammatory diseases exist but only when multiple diseases occurred before breeding and the effects were limited to distinct susceptibility of daughters to diseases at young age.

Strategies to alleviate the impact of postpartum diseases on reproduction

Prevention of postpartum inflammatory diseases is unquestionably the best approach to reduce the impact of diseases on fertility of cattle, and strategies to minimize the incidence of postpartum diseases were reviewed by others (LeBlanc *et al.*, 2006; Santos and Ribeiro, 2014). Nonetheless, understanding the mechanism mediating the impact of disease on

reproductive biology of cattle could lead to new strategies for mitigation of the negative consequences of diseases. Assuming that inflammation is the major mediator of subfertility in cows with postpartum diseases, control of inflammation during the clinical presentation of the disease could potentially mitigate the effects of inflammation on reproduction. McDougall *et al.* (2016) performed a randomized clinical trial testing the hypothesis that addition of a nonsteroidal anti-inflammatory drug (meloxicam) to antimicrobial treatment of clinical mastitis would improve subsequent fertility of dairy cows. Cows treated with meloxicam had greater conception risk in their first insemination postpartum and greater proportion of cows pregnant by day 120 after calving compared with the control group (Fig. 6). The results indicate that controlling inflammation during clinical presentation of an inflammatory disease might improve subsequent reproductive performance in dairy cows. A nutraceutical alternative for control of inflammation is reducing ratio of omega-6 to omega-3 fatty acids in the diet of postpartum cows (Greco *et al.*, 2015), which could also minimize the effects of inflammatory diseases on reproduction.

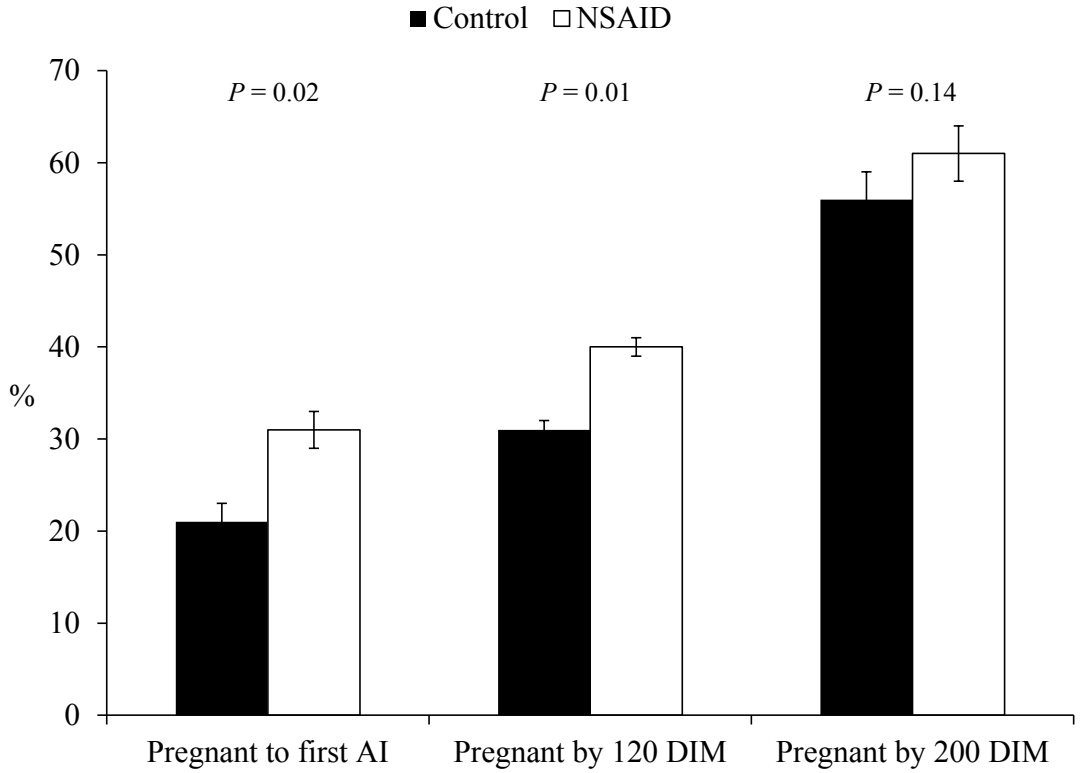

Figure 6. Proportion of dairy cows 1) pregnant to first AI, 2) pregnant by 120 days in milk (DIM) and 3) pregnant by 200 DIM according to treatment of clinical mastitis in a randomized clinical trial (McDougall *et al.*, 2016). Cows in the control group received only antimicrobial therapy and a placebo injection. Cows in the nonsteroidal anti-inflammatory drug (NSAID) group receive the same antimicrobial therapy of the control group plus a subcutaneous injection of 0.5 mg of meloxicam per kg of body weight. Error bars represent the standard errors of the means. Data from McDougall *et al.* (2016).

Conclusions

Inflammatory diseases occurring before breeding are very prevalent in dairy cows and have long-lasting effects on subsequent pregnancy establishment and survival. The effect of diseases on fertility does not seem to be mediated by a single mechanism, rather a combination of multiple mechanisms that have additive effects, which include reduced BCS at the time of breeding, reduced developmental competence of oocytes, and altered uterine environment (Fig. 7). In pregnancies that survive to term, the long-lasting effects of diseases on developmental biology seem to be extended to the postnatal life of heifers and their susceptibility to diseases in early life. In addition to prevention of diseases, control of inflammation during clinical presentation of the disease mitigates their impact on reproductive biology of cattle. Because of the high incidence and the high impact on reproduction, inflammatory diseases postpartum represent an important problem in reproductive management of cattle and should always be considered when evaluating reproductive efficiency of herds and recommending management changes.

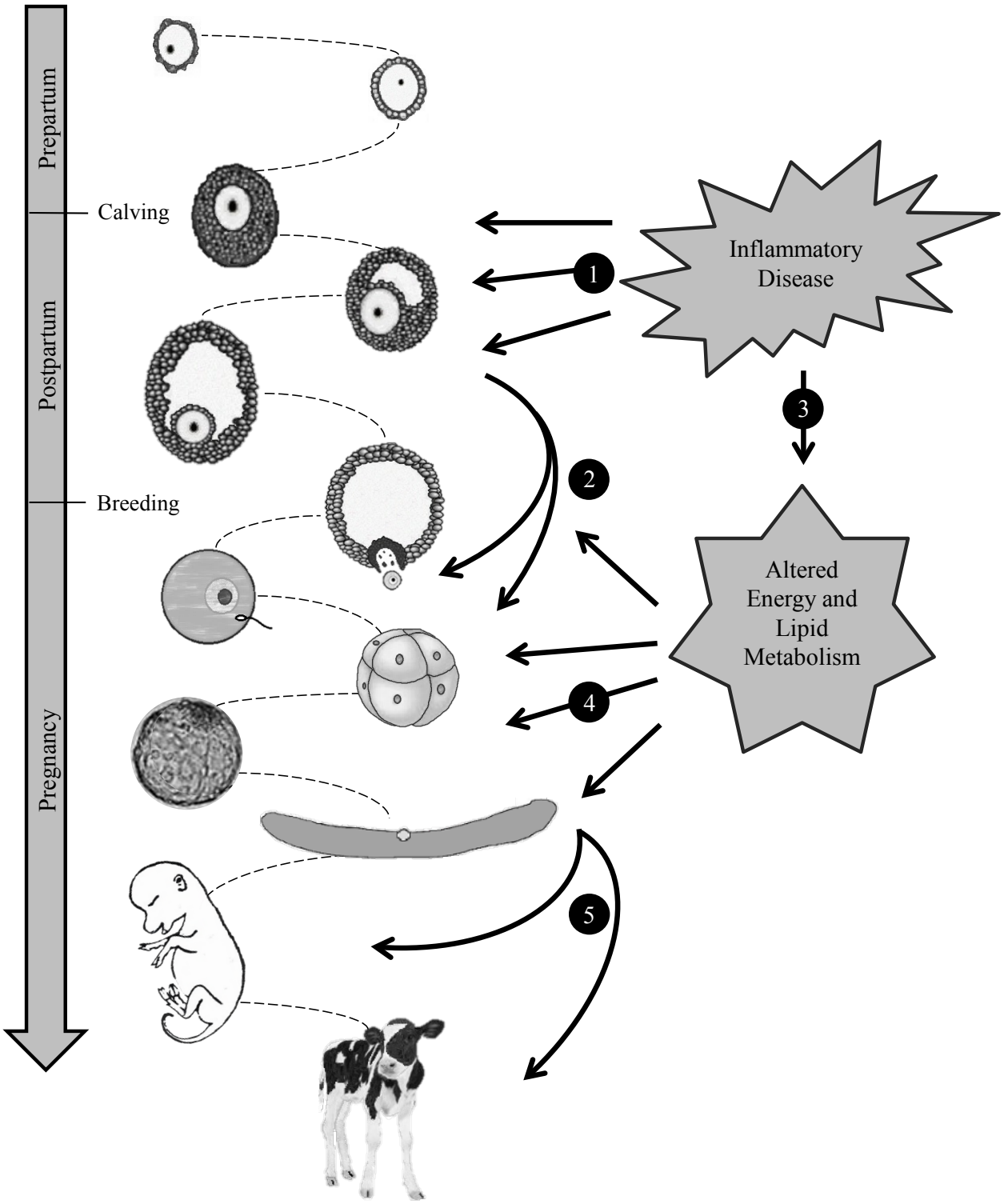

Figure 7. Schematic representation of putative long-lasting effects of postpartum inflammatory diseases on developmental biology and fertility of cows. Most clinical diseases occur in the first 3 weeks postpartum and cause inflammation. Inflammatory molecules secreted by innate immune cells in the insulted tissue reach the ovary through systemic circulation and alter ovarian follicle biology (1). Effects of inflammatory molecules on preantral and antral follicles reduce the future developmental competence of enclosed oocytes (2). Except for cows with chronical inflammation, concentration of inflammatory molecules in systemic circulation are not altered at the time of breeding, and the effects of postpartum diseases on uterine environment occur indirectly through lasting effects on energy and lipid metabolism of the cow (3). Altered metabolism of the cow during the time of embryonic development causes changes in uterine histotroph composition, impairs conceptus development and results in increased incidence of early pregnancy losses (4). Pregnancies that survive the embryonic period have altered programming of the developing fetus and placenta, which leads to increased incidence of late pregnancy losses and perhaps postnatal consequences in those that survive to term (5).

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