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Technical Series

Issue 17

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0800 4 DairyNZ (0800 4 324 7969) dairynz.co.nz

Fertility research released

Fertility of the dairy cow is rightly viewed as a key factor in the success of New Zealand seasonal dairy farming.

Internationally, fertility of the dairy cow has declined drastically in the last few decades but, fortunately, the decline has been much less severe in New Zealand. Previous DairyNZ research has indicated this is a feature of the genetics of the New Zealand Holstein-Friesian, with North American genetics being inferior for fertility.

Despite this clear New Zealand advantage, complacency is not acceptable given many New Zealand dairy farms do not achieve fertility targets.

InCalf, the DairyNZ tool, provides farmers, veterinarians and other advisors with the means to understand fertility issues and manage them better. However, it cannot deal with what is not known.

A six-year programme of research, involving key scientific skills in partner organisations, is now coming to an end. It has involved the University of Auckland, AgResearch, University of Victoria and DairyNZ, co-funded by the Ministry of Innovation, Business and Employment and New Zealand dairy farmers through DairyNZ.

This edition of the *Technical Series* reports part of the work and contains advice for dairy farmers on one important aspect of uterine health that significantly reduces fertility in the dairy cow.

The end of this project does not mean the end of work on fertility, it will remain an important research area until all on-farm targets can be met.

Subclinical endometritis in New Zealand dairy cows

Susanne Meier, DairyNZ Scientist – Animal Science; Nicola Priest, DairyNZ Post-graduate Student – Animal Science

Summary

- Research has found that the prevalence of subclinical endometritis in New Zealand cows is lower than reported internationally. On average, 35% of New Zealand study cows had subclinical endometritis two to three weeks post-calving.
- The number of cows with subclinical endometritis decreases with time after calving i.e. many cows recover with time. On average, 10% of study cows had subclinical endometritis five to six weeks post-calving.
- Cows with subclinical endometritis at four to six weeks after calving can have a 15-20% lower six-week in-calf rate than unaffected cows.
- Cows with lower body condition score at calving were more at risk of subclinical endometritis than cows with higher body condition scores.
- Disease prevalence differs between study herds.

This project was funded by the Ministry of Business, Innovation and Employment, and New Zealand dairy farmers through DairyNZ. The support of Fonterra and their staff is acknowledged for contributing to this research.

International research has identified subclinical endometritis as a disease that reduces cow and herd level reproductive performance[1-3].

If highly prevalent in New Zealand herds, this disease could contribute to lower than expected reproductive performance and have significant economic impact.

Evaluating the risk this disease poses to the New Zealand dairy industry has formed one part of a research programme undertaken by DairyNZ, with industry collaborators. The research has determined the prevalence of subclinical endometritis in study herds and the mechanisms through which it affects reproductive performance.

The key targets have been to:

- measure the prevalence of subclinical endometritis and the effect on herd-level reproductive performance
- understand how subclinical endometritis affects reproduction, at the level of the ovary and the reproductive tract
- evaluate ways of identifying cows that may be at risk of subclinical endometritis.

What is subclinical endometritis?

Subclinical endometritis is a disease of the uterus that occurs in cows post-calving when the uterus undergoes involution, a natural recovery process to its pre-calving status in the first four weeks after calving[4].

During this period most, if not all, cows will have some level of inflammation, and possibly bacterial contamination, in the uterus[5]. This is normal and many cows recover from this during the involution process.

If the inflammation or infection persists, then this is uterine disease and can result in long-term problems, including poor

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reproductive performance[3, 6]. For a description of the three main uterine diseases, see the research snippet from Dr Scott McDougall and Melvin de Boer (pg 12).

Subclinical endometritis is a 'silent' disease (no obvious signs to aid diagnosis). However, it can be diagnosed in research studies using uterine cytology (a method that allows the number of white blood cells in the uterus to be counted)[1]. The cells of interest are a specialised group of white blood cells, which are immune cells that fight infection and produce inflammation. These are called polymorphonuclear (PMN) cells[1, 2]. When an infection in the uterus develops, more PMN cells are recruited from the blood to fight the infection, so an increased concentration of these cells in the uterus is indicative of uterine disease.

To determine the PMN count in the uterus, the cyto-brush technique is used. A small brush is guided through the cervix to take a swab sample from the body of the uterus.

Uterine cytology, measuring PMN cells, was found to be a better predictor of reproductive performance when compared with gross vaginal inflammation scores (measured using Metricheck) or with culturing bacteria present in the uterus[7]. This made the uterine cytology technique the most accurate way to determine the prevalence of subclinical endometritis.

Prevalence of subclinical endometritis

This research found that, on average, 35% of cows tested positive at 14-21 days after calving, reducing to 10% by 40-45 days after calving (Figure 1).

These data come from research farms in Taranaki and the Waikato. This prevalence of subclinical endometritis is less than that reported in international studies, where 20-50% of cows have subclinical endometritis 40-50 days after calving[2, 8, 9].

Subclinical endometritis is diagnosed when PMN cells exceed a certain level. However, this 'cut-off' level changes over time, as PMN cells naturally decrease in number. The cut-off is set high in the first two to three weeks after calving (more than 18% of cells collected are PMN cells) and declines progressively with time (more than 9% are PMN cells at four to five weeks, and more than 7% beyond six weeks after calving).

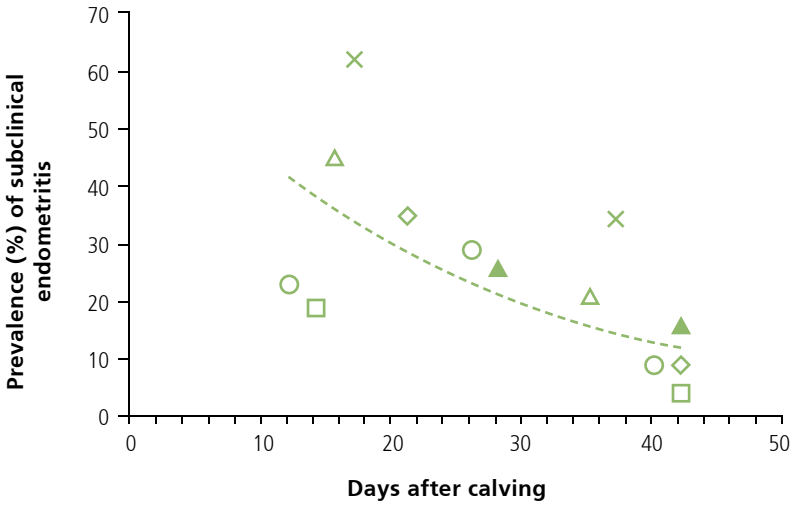
A good way to determine the cut-off point is to identify at which level it affects an outcome, such as reproductive performance[1]. For example, cows with more than 8% PMN cells have lower conception rates to first service, making this is a valid cut-off point.

This is compared with a cut-off of less than 3% PMN cells, where there is no reduction in first service conception rate (see Figure 1, pg 6).

Figure 1. The prevalence of subclinical endometritis (cut-off ≥18% PMN cells between 12-21 days; ≥9% PMN 26-28 days, and ≥7% PMN between 37-42 days after calving) for 1172 cows enrolled during the programme.

Symbols represent data from different studies:

- ▲ (n=228)[7];
- △ (n=40 to 66)[7];
- ○ (n=56)(Roche et al 2013 unpublished);
- ◇ (n=46)[10];
- □ (n=224)[11];
- × (n=552)(Meier et al unpublished)

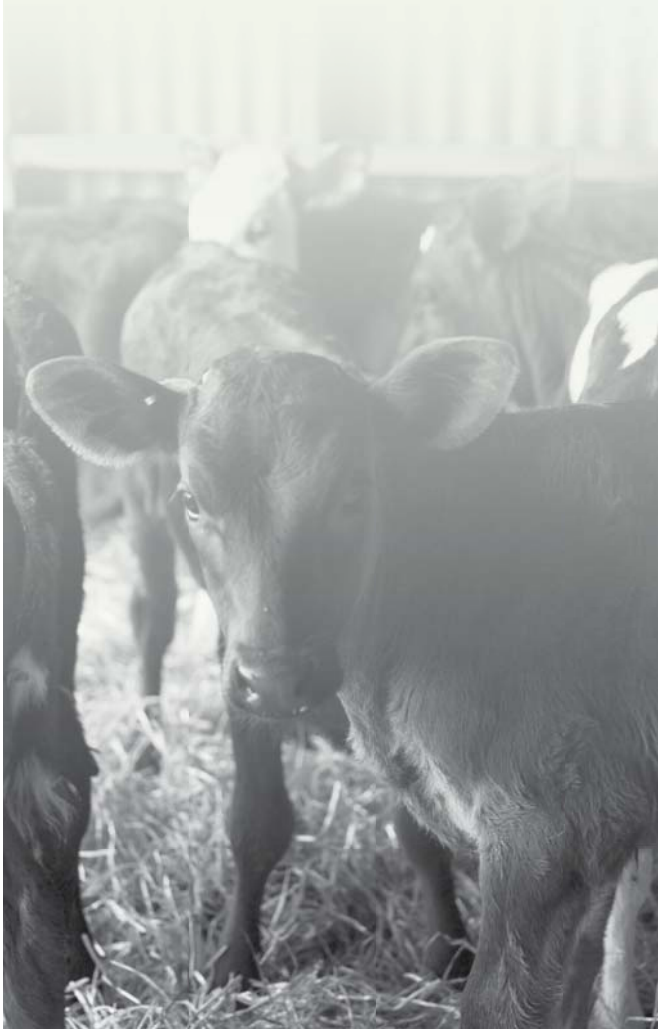


Subclinical endometritis causes poor repro performance

Susanne Meier, DairyNZ Scientist – Animal Science; Nicola Priest, DairyNZ Post-graduate Student – Animal Science

Summary

- Cows with subclinical endometritis are less likely to fail to ovulate by 63-70 days after calving.
- Cows with subclinical endometritis at 42 days after calving have lower conception rates.
- Prolonged subclinical endometritis reduces the six-week in-calf rate and increases empty rates.



Many cows with subclinical endometritis were still not showing heats (were anoestrus) nine to ten weeks after calving[7, 12], took longer to conceive, and had lower conception and pregnancy rates.

This is consistent with international data reporting that cows with subclinical endometritis have poor reproductive performance, low first service conception and pregnancy rates, require more services per conception and take longer to get pregnant[1, 2, 8].

In this study, the impact of subclinical endometritis on reproductive performance was further examined by grouping cows into four groups based on the length and severity of subclinical endometritis.

The groups were:

1. cows with a low PMN count (healthy cows) throughout
2. cows that had subclinical endometritis at four weeks but had recovered by six weeks after calving
3. cows that were healthy at four weeks but had subclinical endometritis at six weeks
4. cows that had subclinical endometritis both at four and six weeks after calving (prolonged subclinical endometritis).

Those cows with prolonged subclinical endometritis (i.e. group 4) had the greatest reduction in reproductive performance (see Figure 2, pg 6). This group made up less than 10% of the herd.

Cows in group 4:

- conceived 14-24 days later than the healthy group (56 days compared with 32-42 days after the start of mating)[7]
- had a lower first service conception rate (Figure 1, pg 6)
- were less likely to be pregnant at the end of mating (Figure 2, pg 6).

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How subclinical endometritis affects reproduction

How much harm is caused by the inflammation and infection of endometritis will determine how long a cow remains anoestrous and how likely she is to conceive and maintain her pregnancy[7, 12, 13].

Research conducted by project partners from AgResearch and Victoria University of Wellington identified that subclinical endometritis might affect the quality of the eggs and, therefore, interfere with ovulation and conception.

At Victoria University, the research is continuing to investigate the importance of egg quality, using the latest techniques and markers to identify healthy eggs. For more details, see the research snippet by Dr Janet Pitman and Prof Ken McNatty (pg 13).

Another part of the programme has identified that the uterine environment of cows with subclinical endometritis does not support good embryo development[14]. To understand more about how subclinical endometritis alters the uterine environment, changes in gene expression in response to uterine diseases are being investigated.

This is important, as research evidence identified that a poor uterine environment has the potential to increase early embryo death, and this is reflected by low conception and pregnancy rates.

An outline of this work is provided by Dr Caroline Walker and Prof Murray Mitchell, pg 13.

Figure 1. The reduction in first service conception rate in cows with increased severity of subclinical endometritis (severity determined by the percentage of PMN cells in the uterus)[13].

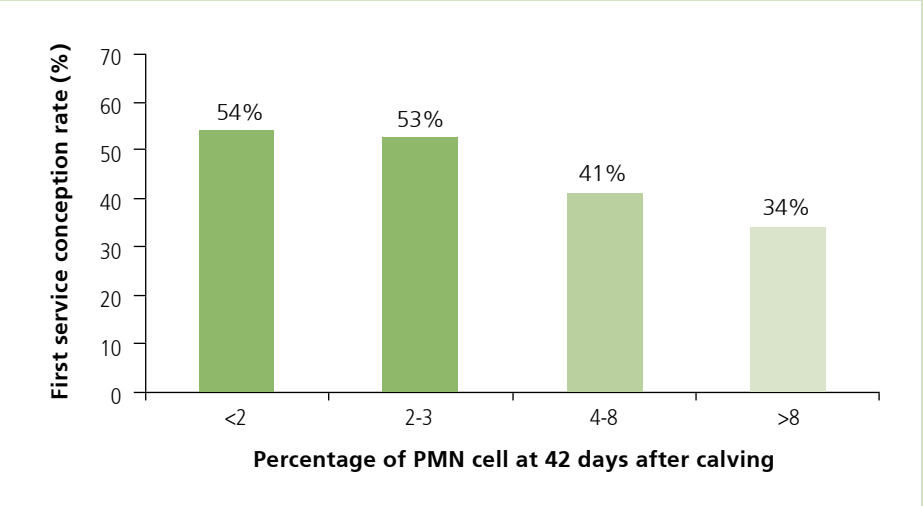
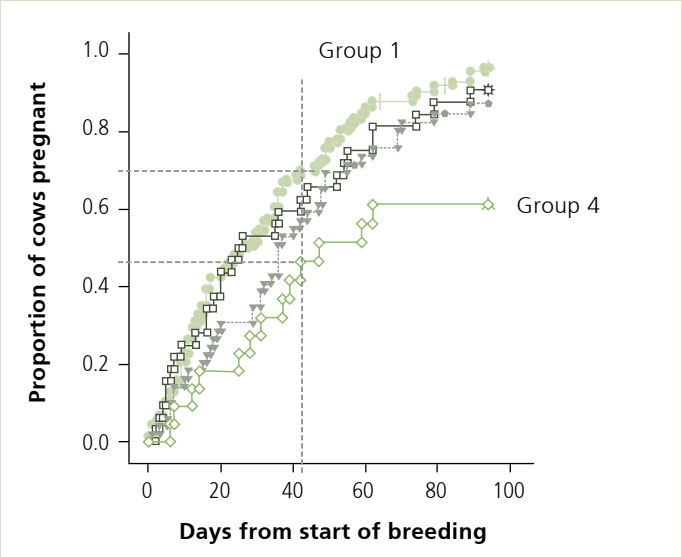


Figure 2. Percentage of cows pregnant after the start of breeding. This includes cows that had a low PMN count throughout (healthy cows) (Group 1 in green circles); and cows with prolonged subclinical endometritis both at four and six weeks after calving (Group 4 in green diamonds).

The remaining two groups were: Group 2 – cows that had subclinical endometritis at four weeks but recovered by six weeks after calving (grey triangles) and Group 3 – cows that were healhty at four weeks but had subclinical endometritis at six weeks (black squares)[7]. Grey dashed lines represent the approximate proportion of cows pregnant 42 days after the start of breeding.



Counting the cost and treatment

Susanne Meier, DairyNZ Scientist – Animal Science; Nicola Priest, DairyNZ Post-graduate Student – Animal Science

Summary

- Where more than 10% of cows are affected by prolonged subclinical endometritis, significant financial loss occurs.
- For a 393 cow herd and a $5.50 milk price:
  - if 5% of cows are affected, the economic loss equates to approximately $9,400
  - if 30% of cows are affected, the economic loss equates to approximately $46,000.
- Treatment options are currently limited and, in this case, prevention is better than cure.
- The risk can be reduced by calving cows in good body condition, and monitoring and reducing other diseases around calving.

Using data produced from the programme, the potential economic losses due to subclinical endometritis were estimated.

Cows with subclinical endometritis at four and six weeks post-calving had a six-week in-calf rate of 48% and the proportion of cows not pregnant (empty rate) after 10 weeks of mating was 35%[7, 12].

In comparison, cows without subclinical endometritis had a six-week in-calf rate of 70% and empty rate of 5% after 10 weeks of mating (see Figure 2, pg 6).

Given such a negative impact on reproductive performance at the cow level, subclinical endometritis at the herd level is likely to have a significant impact on overall farm reproductive and financial performance.

The flow-on economic impact if 0, 5, 10, 20 or 30% of cows in a herd have subclinical endometritis has been evaluated using the Economics of Reproductive Performance Tool (InCalf, DairyNZ).

Economics of repro performance tool

This tool is a gap calculator, comparing overall reproductive performance with industry targets and it estimates the potential increase in operating profit, with improved reproductive performance (find it at **dairynz.co.nz/incalftools**).

It uses two key targets to work out the economic gap, the six-week in-calf rate and the empty rate.

To calculate the economic impact of increasing prevalence of subclinical endometritis (Table 1), the assumptions used were that:

- healthy cows had a 70% six-week in-calf rate and 5% empty rate after a 10-week mating
- cows with subclinical endometritis had a 22% reduction in six-week in-calf rate (from 70% to 48%)
- cows with prolonged subclinical endometritis had a 30% increase in empty rate after a 10-week mating period (from 5% increased to 35%)
- herd size was 393 cows (2011/12 national average; *New Zealand Dairy Statistics,* **dairynz.co.nz/dairystats**).

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The economic loss associated with subclinical endometritis relates to the prevalence of the disease in the herd. The more cows with subclinical endometritis, the greater the economic losses, as shown in Table 1 and Table 2.

As the prevalence of subclinical endometritis increased, the greater economic loss was associated with the increased empty rate, accounting for around 75% of the total loss. When a 30% prevalence of prolonged subclinical endometritis was used, the total economic loss was calculated to be more than $46,000 (Table 1 (C) and Table 2 (F)).

Table 1. Reduced six-week in-calf rate

The economic loss due to a reduced six-week in-calf rate from prolonged subclinical endometritis (SCE), with prevalences ranging from 0-30%. (A) was calculated by: (healthy % of herd x healthy six-week in-calf rate) + (SCE % of herd x SCE six-week in-calf rate).

Uterine status of herd (% of herd)		Six-week in-calf rate		
Subclinical*	**Healthy***	**Herd in-calf rate (A)**	**Gap (B**-A)**	**Economic gap (C)*****
0%	100%	70%	0%	$0
5%	95%	69%	1%	$1,572
10%	90%	68%	2%	$3,144
20%	80%	66%	4%	$6,288
30%	70%	63%	7%	$11,004

*Six-week in-calf rate for cows with subclinical endometritis = 48%, for healthy cows = 70%

**(B) Six-week in-calf rate (target) = 70%: actual performance of healthy cows in this herd

***The economic gap associated with reductions in the six-week in-calf rate is calculated using the formula:
Gap (B-A) ……. X $4^ X ……. cows in herd = $ ……. (C) per year

^This economic multiplier was estimated through modelling assuming a $5.50 per kg MS milk price.

Table 2. Increased empty rate

The economic loss due to increased empty rates associated with prolonged subclinical endometritis (SCE), with prevalences ranging from 0-30%. (E) was calculated by: (healthy % of herd x healthy empty rate) + (SCE % of herd x SCE empty rate).

Uterine status of herd (% of herd)		Cows not pregnant after 10 weeks of mating		
Subclinical*	**Healthy***	**Herd empty rate (D)**	**Gap (D-E**)**	**Economic gap (F)*****
0%	100%	5%	0%	$0
5%	95%	7%	2%	$7,860
10%	90%	8%	3%	$11,790
20%	80%	11%	6%	$23,580
30%	70%	14%	9%	$35,370

*Empty rate after 10 weeks of mating for cows with subclinical endometritis = 35%, for healthy cows = 5%

**(E) Empty rate (target) = 5%: actual performance of healthy cows in this herd

***The economic gap associated with increased empty rate is calculated using the formula:
Gap (D-E) ……. % X $10^ X ……. cows in herd = $ ……. (F) per year

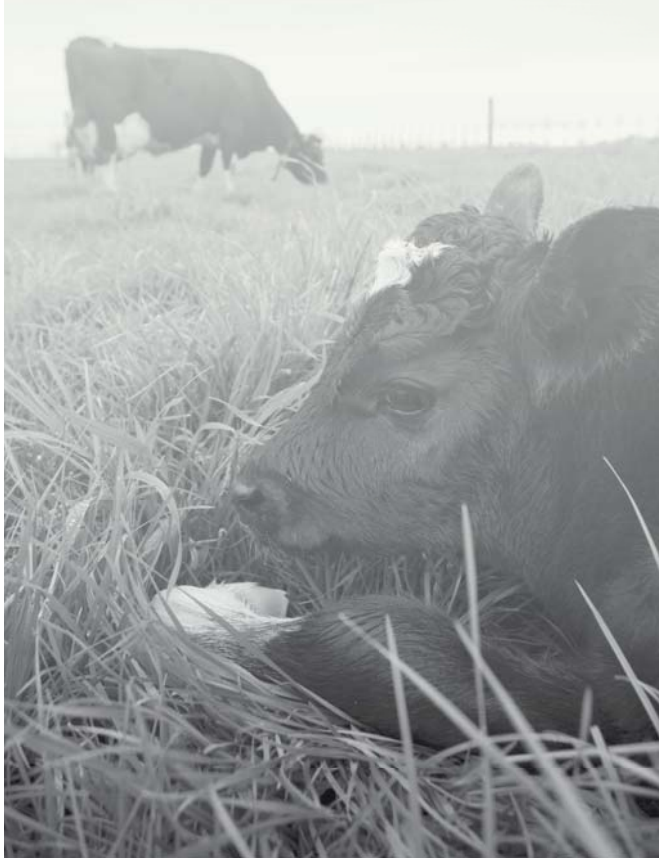
^This economic multiplier assumes a $1000 value differential between an empty and a pregnant cow.

Identifying subclinical endometritis on the farm

Susanne Meier, DairyNZ Scientist – Animal Science; Nicola Priest, DairyNZ Post-graduate Student – Animal Science

Summary

- Currently, all cows in a herd would need to be tested to identify cows with subclinical endometritis six weeks after calving.
- Low body condition at calving puts cows at risk of subclinical endometritis.
- Disease and illnesses during the transition period (three weeks pre- and post-calving) may increase the risk of uterine disease.



Identifying cows with subclinical endometritis on-farm is difficult. The technique used for research (uterine cytology) is time-consuming and costly.

Developing an easier method to identify at-risk herds or cows has benefits. With a simple method, cows could be easily identified and treated as an individual case.

AgResearch are developing a simplified test that uses 'markers'. In this case, the marker is a protein that is found in the fluid collected from either the reproductive tract or vaginal mucus. More details are in the research snippet by Dr Rita Lee, pg 14.

In 2012, cows calving at low (3.6), moderate (4.6) and high (5.4) body condition score (BCS) were examined to determine the effect of BCS on subclinical endometritis. Cows calving in low body condition had poor uterine health compared with those calving at a moderate or high body condition.

Figure 1 (pg 10) demonstrates this, with cows calving at a high body condition having the lowest uterine PMN counts and very low levels by six weeks after calving. Cows calving at a low body condition had higher levels at four weeks after calving and still moderately high levels at six weeks (Roche et al 2013, unpublished).

These data are consistent with results from other DairyNZ studies, linking subclinical endometritis with low body condition at calving[7, 11].

Cows that develop any form of illness after calving are at greater risk of uterine disease and subsequent reduction in reproductive performance.

Any sickness (clinical or subclinical) during the three weeks before and after calving slows down post-calving recovery, including involution of the uterus. This means there is more time for uterine disease to develop[15].

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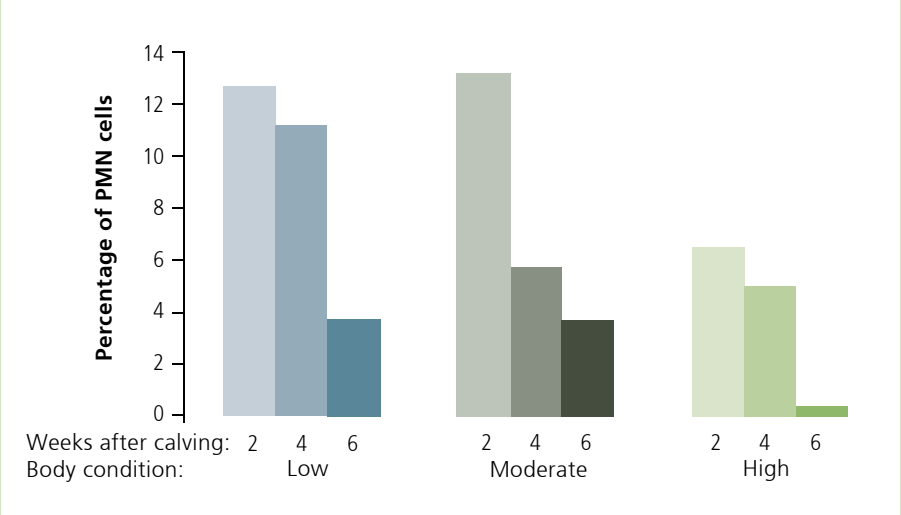
Figure 1. Average uterine PMN cells at two, four and six weeks after calving for cows calving at low (3.6), moderate (4.6) and high (5.4) body condition scores.



The key research findings are that:

- Cows with a clinical disease (e.g. milk fever, ketosis or retained placenta) have delayed uterine involution[4] and are at a higher risk of uterine disease (e.g. endometritis)[16, 17].
- Cows with any disease have a greater chance of not getting pregnant[18].
- Cows with a subclinical disease (e.g. subclinical milk fever or ketosis) have a 10% lower pregnancy rate[18].
- Poor energy status decreases immune function, including the activity of the PMN cells in the uterus, thereby increasing the chances that an infection will persist[15].

In addition, pre-calving immune status and liver function were also found to differ for cows that contracted subclinical endometritis, when compared with cows that were healthy[12].

This means that some cows may be pre-disposed to contract subclinical endometritis because of events occurring before calving.

Preventing subclinical endometritis

Uterine disease is complex and subclinical endometritis is not easily identifiable on-farm. So, the primary focus must be to ensure calving cows are in good body condition and that the risk of cows contracting other diseases post-calving, such as metabolic diseases and mastitis, is reduced as much as possible.

For information on avoiding metabolic disease around calving, refer to the June 2012 DairyNZ *Technical Series*, pg 13-18.

Treating affected cows

Subclinical endometritis can have a significant negative impact on reproduction and can reduce farm profit. However, even if a diagnostic tool for this disease was available on-farm, there is limited information available on how to treat this disease[9, 19].

International research reports the use of both antibiotics and prostaglandins as treatments for subclinical endometritis:

- **Antibiotics**
  This targets bacterial infections and aims to reduce bacterial activity to a level where the cow's natural defences can gain dominance, allowing the cow to clear the infection and reduce uterine inflammation[20].
- **Prostaglandins**
  These are administered to regress the corpus luteum (the part of the ovary that develops after ovulation and produces progesterone), stimulating cows to ovulate. This treatment is used for two reasons: 1) to induce ovulation and stimulate uterine contractions that help clear pus from the uterus; 2) to reduce progesterone concentrations, as progesterone supresses the cow's immune system, hindering the ability to self-cure[21, 22], induce a high oestrogen environment to stimulate uterine activity and remodelling.

However, results from these studies have demonstrated that neither of these treatments is consistently effective for subclinical endometritis, with results on both disease cure rate and reproductive performance being variable[9, 19, 23].

Such inconsistencies may result because not all cows with subclinical endometritis have bacteria in their uterus to respond to antibiotic treatment (inflammation only) or have no corpus luteum to regress.

Another option that DairyNZ has investigated is the use of a non-steroidal anti-inflammatory drug (NSAID) to improve the reproductive performance of cows with subclinical endometritis. This work is outlined in the research snippet by Nicola Priest, pg 15.

Treatment limitations

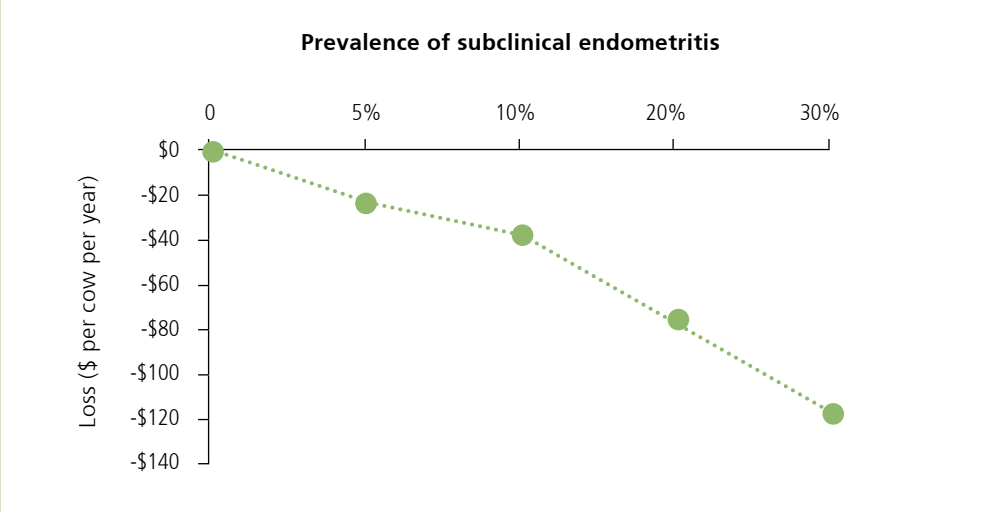
Currently, there is no useful on-farm diagnostic tool and no obviously effective treatment available for subclinical endometritis.

Looking forward, any effective treatment has to be considered for use based on the costs and benefits of diagnosis and treatment, or treatment only if blanket therapy is possible.

Where the disease is common, the economic benefit of blanket treatment will be greater. For instance, using the gap calculator, a low prevalence of 5% of cows left untreated results in a loss of $9,400, while a high prevalence at 30% generates a loss of $46,000.

Spread across the herd, this loss equates to between $24 and $118 per cow (Figure 2). Therefore, the cost of treatment will govern the degree of uptake by the industry, with more severely affected herds likely to achieve the greatest benefit from an effective treatment.

Figure 2. The economic impact (loss of income per cow per herd per year) of subclinical endometritis (see Tables 1 and 2, pg 8.



Research snippets

Research highlights are presented from scientists working in specialist areas of the programme, including the University of Auckland, AgResearch, Victoria University of Wellington, Cognosco Anexa Animal Health and DairyNZ.

Diagnosis and treatment of uterine disease

Scott McDougall, Cognosco Anexa Animal Health Veterinarian (Morrinsville); Melvin de Boer, Cognosco Anexa Animal Health Post-graduate Student

There are three common forms of uterine disease.

Clinical endometritis

Endometritis occurs more than three weeks after calving. Endometritis can be divided into clinical endometritis, where inflammation is visually evident in the vagina, and subclinical endometritis, where laboratory tests (e.g. cytology) are required to find evidence of inflammation.

Cows with endometritis are not systemically ill (e.g. are still eating and do not have a high temperature).

Cows with clinical endometritis have purulent (pus-like) material in the uterus (and commonly the vagina) which is identified by the Metricheck device, vaginoscopy, insertion of a gloved hand into the vagina or by ultrasonography. The proportion of affected cows varies amongst herds, however, most herds have 5-20% of cows affected.

Clinical endometritis is commonly treated by infusion of antibiotics into the uterus. In those cows with a high Metricheck score, systemic antibiotics may be useful. Where there is a corpus luteum present (a part of the ovary that develops after ovulation and produces progesterone), prostaglandins may be used either alone or preceding antibiotic treatment.

Metritis

Metritis occurs in the first three weeks after calving and affected cows have a foul smelling, bloody discharge and are often seriously ill. Normally 1-2% of cows in a herd have metritis.

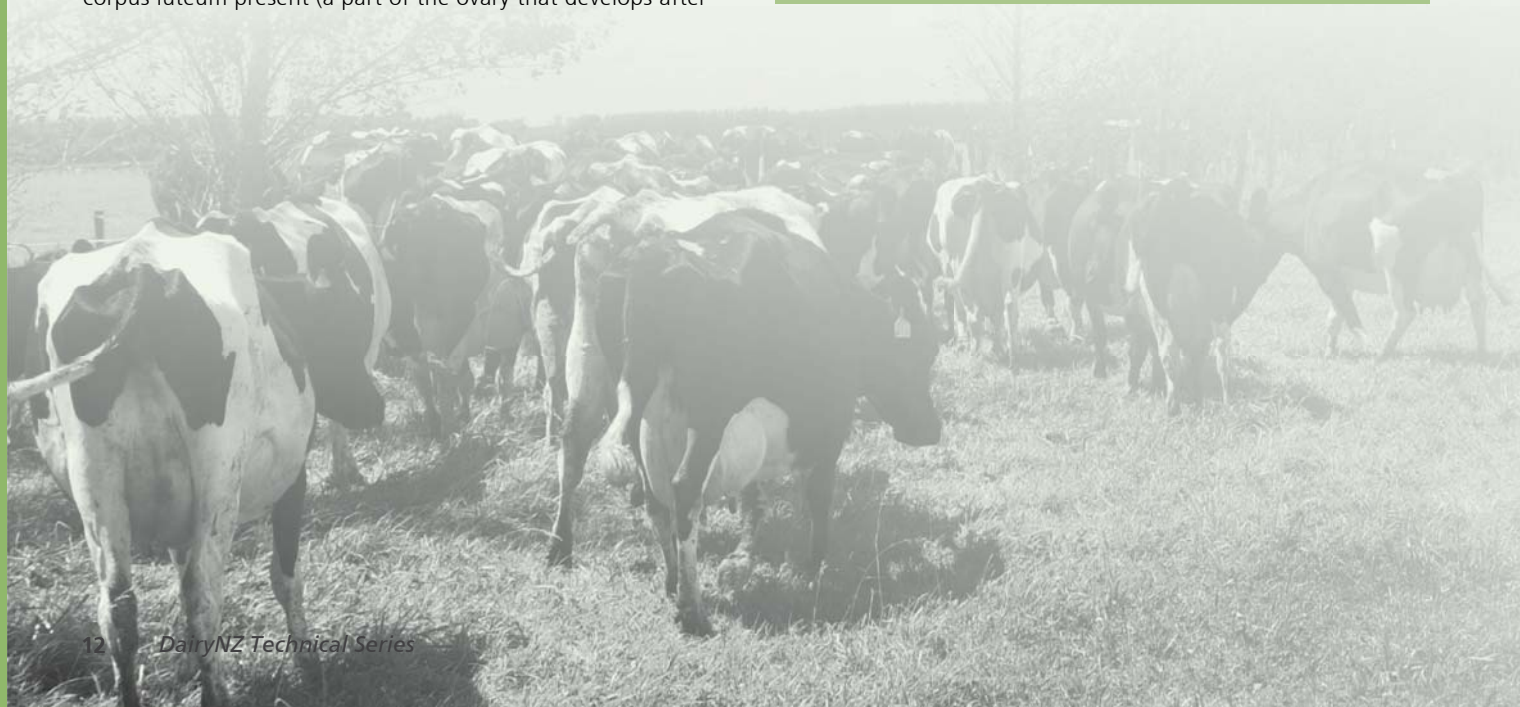
Cows with metritis require systemic antibiotics and non-steroidal anti-inflammatories. In some cases, they require fluid therapy due to dehydration.

Pyometra

Pyometra is where there is gross pus in the uterus, the cervix is closed and there is a corpus luteum present. Pyometra is relatively rare, with generally fewer than 1% of cows affected.

Pyometra is treated with prostaglandins.

Reproductive performance of cows with uterine disease (even when treated) is lower than in unaffected herd mates. Hence, it is important to record cows with uterine disease and the associated risk factors (e.g. difficult calvings, dead calves, twins etc), assess the total number of affected cows and optimise management to minimise the number of cows affected.



Nutrition and its effects on egg quality

Janet Pitman, Victoria University of Wellington, Senior Lecturer; Ken McNatty, Victoria University of Wellington Professor

In New Zealand dairy cows, the re-calving rate has declined progressively[24]. Recent reports indicate that poor oestrus, low conception rate and early embryo loss are commonly caused by poor egg quality[25].

In one study, egg quality was indirectly linked to its nutritional and energy status[25]. As eggs mature, they increasingly require amino acids to make protein, glucose and fatty acids for energy. Therefore, the nutrient and energy composition in the fluid surrounding the egg (called follicular fluid) is critical to its health.

This collaborative project measured the important nutrients (amino acids, non-esterified fatty acids and glucose) in blood and the follicular fluid[26, 27].

The amino acid composition in follicular fluid mirrored what was in the blood. The composition in New Zealand cows was very different to that reported in UK and USA cows. New Zealand cows function with fewer (25-75%) nutrients in the blood and follicular fluid.

This research was conducted through the collection of ovaries from slaughtered cows to extract the eggs, making sure the closely associated support cells (called cumulus cells) around each egg were still intact. Eggs receive many of their nutrients from the cumulus cells through tiny channels called gap junctions, which connect these cells to one another and the egg[28].

To assess the ability of nutrients to transfer from cumulus cells to the egg, eggs were incubated in media that simulated the composition in follicular fluid and monitored by examining the transfer of a fluorescent dye from cumulus cells into the egg.

At amino acid compositions of 50-100% of that in a UK cow, there was no effect on dye transfer. However, when amino acid composition was lowered to levels similar to those in a New Zealand dairy cow, dye transfer was significantly compromised and was further negatively influenced by high concentrations of both cholesterol and fatty acids (e.g. oleic acid).

The focus now is to better understand the nutritional thresholds that compromise egg quality and how this affects re-calving rates.

The nutritional and immune status of cows will affect the quality of the egg and, therefore, the quality of the embryo. A greater understanding of how the eggs and follicles are altered will provide tools to allow reproduction to be enhanced.

How the uterus responds to inflammation

Caroline Walker, DairyNZ Post-doctoral Scientist; Murray Mitchell, University of Queensland and University of Auckland Professor

After calving, the uterus of all cows can be contaminated with bacteria and is often inflamed.

The uterus responds by attracting polymorphonuclear cells (PMN), specialised white blood cells, to help support uterine recovery and clean up any bacteria. The uterus also responds by altering what it secretes, to discourage bacterial growth.

In many cases the cow can take care of these uterine problems in a timely manner, yet some cows are unable to resolve the contamination, resulting in uterine inflammation for a longer period (six weeks after calving). As a result, these cows have low conception and pregnancy rates.

To understand why these cows fail to resolve postpartum contamination, genes in the cells of the uterus were studied to identify if there was a difference in cows with and without subclinical endometritis.

Cows diagnosed as having subclinical endometritis at 30 days after calving had more than 1000 genes active in different amounts, compared with cows with a low PMN count.

Of these genes, most were involved in the immune response, and in particular, the genes of the innate immune response (the body's first line of defence, including PMN cells and inflammation).

The innate immune response is a rapid but non-specific response to hold off an infection, while the more specialised part of the immune system (the adaptive immune response) develops antibodies specific for the invading bacteria.

Inflammation is an essential part of the innate immune response. However, if prolonged, it can cause tissue damage and affect fertility.

Subclinical endometritis alters the uterine environment in such a way that normal early pregnancy is affected. This research provides a greater understanding of how the uterine environment is altered, which may help develop tools to support a more normal pregnancy.

(cont'd pg 14)

Uterine fluid proteins as markers for endometritis

Rita Lee, AgResearch Senior Scientist; Anita Ledgard, AgResearch Research Associate; Mark Green, AgResearch Scientist (now a University of Melbourne lecturer)

Endometritis can be difficult to diagnose due to lack of systemic illness. This is especially so for subclinical cases.

Currently, the most reliable method for detecting subclinical endometritis is by uterine cytology to measure prevalence of specific white blood cells in the uterus called polymorphonuclear (PMN) cells. However, it is not practical for on-farm detection, being costly and time-consuming.

Because identifying cows with subclinical endometritis on-farm is difficult, developing an easier method to identify at-risk herds or cows has benefits, as cows can be targeted for managing and treating the disease.

Ideally, such a test could be completed on-farm in minutes and would be easy and inexpensive. It also needs to identify cows with subclinical endometritis accurately (few or no false positives) to ensure the right cows are treated.

This is the focus of this research and findings to date indicate the potential for such a diagnostic tool.

Certain proteins in the uterine fluid have been found to be associated with the presence of immune cells (PMN cells). Proteins secreted by the PMN cells could be used as biomarkers for endometritis.

Interestingly, the vaginal mucus contained many of these same proteins, meaning potential tests could be less invasive (sampling vaginal mucus rather than uterine fluid).

Although tests on vaginal mucus may not distinguish between different forms of inflammation (endometritis, vaginitis and cervicitis), the presence of these biomarkers would indicate infections that may impair cow fertility within the reproductive tract.

That way, farmers can concentrate on treating only the cows with uterine disease. Early detection and treatment may be crucial in successfully reversing the negative effects of endometritis on reproduction.

It is also possible that markers of a 'healthy' uterus could emerge from the research. These can be used, in conjunction with markers for endometritis, to give farmers a quick uterine health assessment of the cow, allowing for easy identification of cows with subclinical endometritis.

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Treating subclinical endometritis

Nicola Priest, DairyNZ Post-graduate Student – Animal Science

Cows with subclinical endometritis may have low level signs of systemic inflammation[12] as well as inflammation in the uterus.

It follows that reducing inflammation should lead to fewer immune cells in the uterus and an improved uterine environment supportive of maintaining pregnancy.

To test this, 27 cows diagnosed with subclinical endometritis between 12 and 16 days postpartum, were treated with a non-steroidal anti-inflammatory drug (NSAID) between 21 and 31 days postpartum to reduce both systemic and uterine inflammation.

Reproductive performance was compared with cows diagnosed with subclinical endometritis that received no treatment (n=27).

The results demonstrated that the NSAID treatment reduced the levels of systemic inflammation in all the cows treated, but surprisingly did not reduce uterine inflammation.

However, reduction of systemic inflammation alone may have improved the uterine environment, such that the six-week in-calf rate for cows with subclinical endometritis was improved by 23% (Table 1).

This improvement in pregnancy rate when cows with subclinical endometritis were treated with a NSAID indicates that the inflammation associated with the disease is a significant part of the mechanism which reduces fertility.

It's important to note that, while the difference in pregnancy rate at four weeks after the planned start of mating was significant, at eight and ten weeks the difference was not as great. Because of the low number of animals, a larger study is needed to confirm the positive effect of NSAID on pregnancy rates for cows diagnosed with subclinical endometritis.

Table 1: Difference in pregnancy rate between cows with subclinical endometritis that were treated with a non-steroidal anti-inflammatory drug (NSAID) or those left untreated[*,10].

Weeks after start of mating	Pregnancy rate (%) Untreated	NSAID
4	42	69
6	51	74
8	54	78
10	64	84

*The herd had a 10-week mating period. The difference between treated and untreated cows was only significant ($P < 0.05$) at four weeks after mating, but there was a trend ($P = 0.06$-0.09) for the differences to be significant at eight and 10.

Compared with the treatments available for clinical endometritis, there are currently no effective treatments for subclinical endmetritis. Although early results from this research look promising, there is not yet sufficient confidence that NSAID is a cost-effective treatment for subclinical endometritis.

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Improving herd fertility

Caroline Walker, DairyNZ Post-doctoral Scientist; Chris Burke, DairyNZ Reproduction Team Leader; Susanne Meier, DairyNZ Scientist; Murray Mitchell, University of Queensland, Centre for Clinical Research Director

Summary

- Majority of pregnancy losses occur in the first three weeks after conception.
- Poor uterine environment is a likely cause of pregnancy loss.
- This can be due to:
  - failure of the cow to respond to signals received from the embryo
  - inadequate amounts of pregnancy supportive genes produced by the uterus
  - genetic differences which influence how much of a gene is produced
  - epigenetics, where the level of gene expression is altered by direct modification of the DNA itself.
- Selecting cows based on genetics associated with greater fertility and manipulation of epigenetics can improve reproductive outcomes.

Ninety percent of cows conceive to an insemination performed at the right time, but only about half of the fertilised embryos survive the next three weeks[2].

During this perilous first three weeks, the developing embryo is growing rapidly and needs to signal its presence to the cow. In turn, the cow needs to recognise the presence of the embryo and provide a receptive uterine environment for it to thrive.

Why does the uterus not always support the embryo?

A fertilised embryo may fail within the first three weeks because it was formed from a poor-quality egg or has genetic defects, but early embryonic losses can also be attributed to a non-receptive uterine environment.

The role of the uterus in supporting embryonic development has received a lot of research attention. However, the underlying processes that prevent the cow from cycling again, and her own immune system from attacking the embryo, still remain poorly understood.

A receptive uterine environment will:

- Provide nutrients for embryo growth and development.
- Maintain high levels of progesterone by not allowing the cow to cycle. It does this by preventing luteolysis or 'killing of the corpus luteum'. The corpus luteum is the part of the ovary that develops after ovulation and produces progesterone.
- Adjust the immune system to tolerate the "foreign" embryo.

Regulation of the uterine response to pregnancy

The requirements for a receptive uterine environment are coordinated by regulating gene expression or quantity of specific gene products within the uterus (see Figure 1).

The quantity of these gene products is regulated by pregnancy hormones, such as progesterone, as well as biochemical signals received from the embryo. Certain genes need to be active

at the right time to ensure the correct balance of proteins is produced to nurture the newly-established pregnancy.

A genetic basis for improving uterine receptivity

The DairyNZ 'Strain Trial'[6] investigated if the uterine environment differed between fertile New Zealand strain and sub-fertile North American strain cows, during the time of greatest pregnancy loss.

Holstein-Friesian cows with more than 85% North American ancestry were classified as 'sub-fertile' compared with the more 'fertile' New Zealand strain. The fertile strain was derived predominantly through decades of selection with the New Zealand seasonal, pasture-based system where a greater genetic selection pressure is placed on fertility traits.

Sub-fertile North American cows are characterised as having inferior reproductive performance compared to fertile New Zealand cows.

North American cows have:

- poorer egg and embryo quality
- lower conception rate to first and second service
- lower six-week in-calf rate
- lower overall pregnancy rate.

DairyNZ research has demonstrated that the more 'fertile' New Zealand strain of cows respond better to the presence of the embryo than sub-fertile North American cows. Genes regulating uterine receptivity were expressed in greater quantities in fertile dairy cows compared with sub-fertile dairy cows.

A similar pattern was evident for genes involved in preventing luteolysis and promoting embryo growth and development[13, 14]. The uterus of New Zealand cows was more receptive.

In addition, the uterus of the fertile New Zealand cows supplied greater quantities of nutrients (fatty acids and amino acids) to nourish the growing embryo, compared with the uterus of sub-fertile North American cows[7, 8].

The more fertile New Zealand cows also had a dampened immune response to the embryo compared with sub-fertile North American cows. This indicates that their greater fertility is partly due to a greater immune tolerance of the developing embryo. It is one feature that could explain variances in the ability of some cows to get back in-calf quicker than others, even within the relatively fertile New Zealand population of cows.

To understand why sub-fertile dairy cows do not respond sufficiently to the embryo, the genetic and epigenetic regulation of gene expression are being studied.

Genetics

Several studies worldwide have identified areas of the genome (i.e. the cow's complete DNA makeup) where genetic variation or "spelling mistakes" in the DNA (see Figure 2) are associated with fertility traits[1, 3, 4, 9, 10].

Data from LIC and DairyNZ were used to identify areas of the genome that contain variants that may explain differences in fertility within the New Zealand dairy cow population. A particular area of the genome was identified that had variants associated with fertility. This area was also the location of an immune-suppressing gene that was expressed in greater quantities in fertile New Zealand cows relative to sub-fertile North American cows, indicating that it plays a key role in early pregnancy.

Preliminary analysis of this gene has not identified the specific spelling mistake that causes less of it to be produced. Ultimately,

Figure 1. Gene expression. An animal's full complement of DNA contains thousands of genes. These genes are used as a recipe to make RNA, which is then used as a template to make proteins that the body needs to survive. The amount of protein produced is limited to the amount of RNA template produced. In this example, the animal receives a signal from the embryo (E+) that activates the production of RNA for a particular gene that is used as a template to make a protein important for pregnancy maintenance. The production of RNA is known commonly as gene expression.

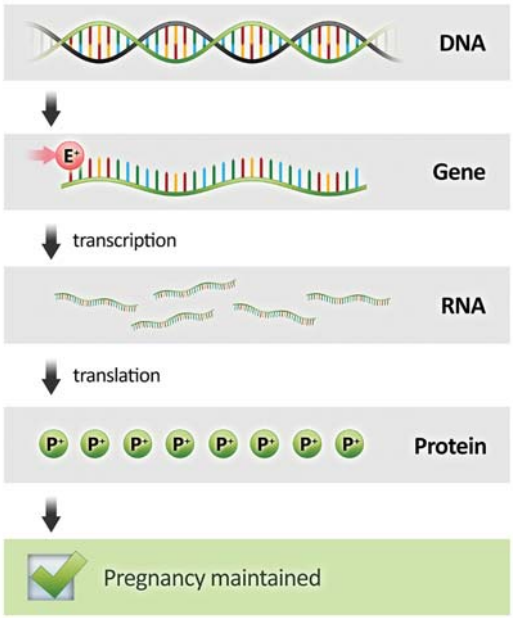

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Figure 2. Genetic variation and DNA methylation regulate gene expression. Normally, embryo signals can bind to a specific piece of DNA to activate production of a gene. However, if that piece of DNA contains spelling mistakes, then the embryo's signal will not be able to bind to the DNA and signal production of the gene. Additionally, if the DNA is chemically modified in a process known as DNA methylation, it will not be able to bind to the gene and, again, RNA and then protein required for pregnancy maintenance will not be made.

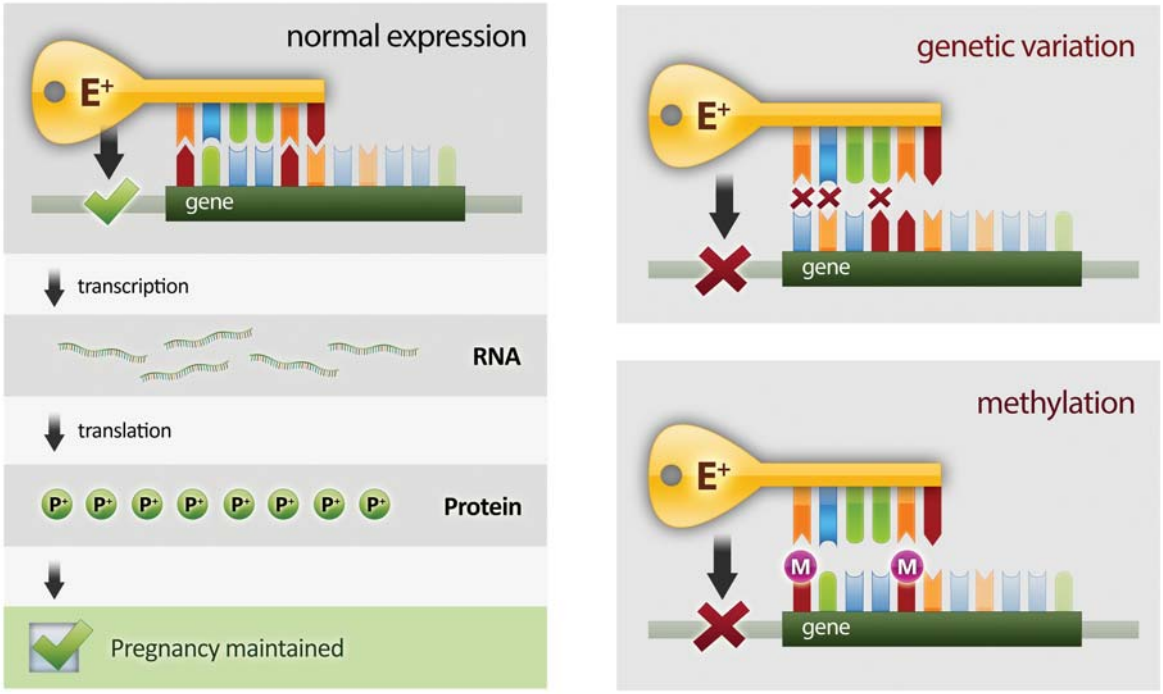
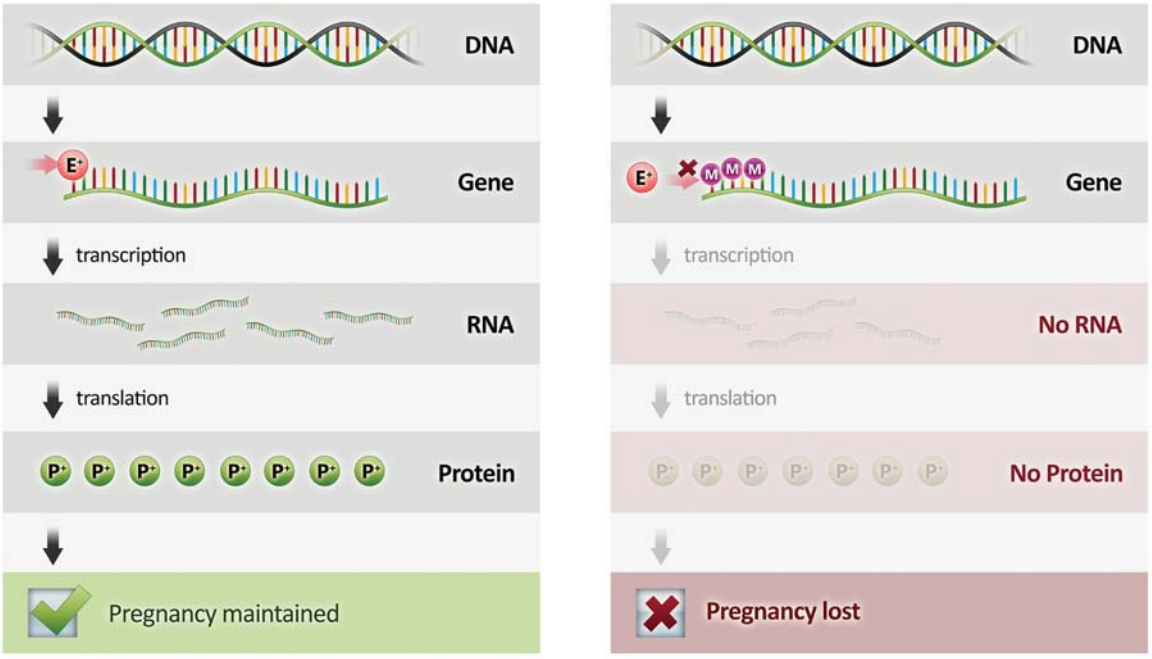


Figure 3. Inappropriate DNA methylation can lead to pregnancy failure if it prevents the cow from responding to signals received from the embryo.



identifying spelling mistakes in cows' DNA associated with a better uterine environment will enable integration into breeding programmes to select the most suitable fertile cows for a particular production system[11].

Epigenetics

Epigenetics is where the level of gene expression is altered by direct modification of the DNA itself. This is commonly achieved by methylation, a chemical modification to the DNA, which suppresses gene expression (see Figure 2).

The DairyNZ-led research has demonstrated that DNA methylation regulates the expression of several important genes involved in the early-response pathways for pregnancy[12]. Inappropriate DNA methylation may, therefore, explain why important genes are not being expressed in sufficient quantities in sub-fertile cows.

This research has identified a specific gene, normally activated and produced in response to signals received from the embryo, that is not active when it is methylated. This gene is a master regulator of the response to pregnancy and this may explain why sub-fertile dairy cows are not responding sufficiently to the embryo (see Figure 3) and lose more pregnancies.

It is not fully understood how reproductive outcomes can be influenced by manipulating DNA methylation. Several substances, such as folic acid, choline, vitamin B6 and vitamin B12, can influence DNA methylation.

Supplementing cows with a B-vitamin complex can improve their reproductive performance[5]. Therefore, the use of these substances to improve reproductive outcome and their influence on DNA methylation and gene expression in the uterus should be investigated.

Conclusion

The combination of selecting cows based on genetics associated with better fertility and manipulation of DNA methylation provides exciting avenues for future research to improve the reproductive performance of New Zealand dairy cows.

Greater understanding of which genetic variations or spelling mistakes can result in the best gene expression profile, to create an optimal uterine environment, will enable the selection of cows based on their ability to maintain pregnancy. Integration of this information into breeding programmes has the potential to improve the reproductive efficiency of dairy herds in New Zealand.

Manipulation of DNA methylation profiles through supplementation provides another mechanism to improve reproductive outcomes.

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Focus on international research

The following is a brief summary of some key science papers recently published.

Cassida and others (2012) Available soil phosphorus affects herbage yield and stand persistence in forage chicory

Agronomy Journal 104: 807-816

An experiment evaluated the effect of phosphorus (P) fertiliser on the herbage yield and persistence of three chicory cultivars in the United States (US). Across two sites, 95% of maximum dry matter (DM) yield was achieved when soil P was 30 mg kg^{-1} Bray-P. Dry matter yield declined from year one to year two for each of the cultivars: Puna (45%), Forage Feast (69%) and Lacerta (89%). Stand density increased 12% when available soil P increased from low to medium levels. There was an indication of lower persistence at high levels of P fertilisation, as well as increased bolting or reproductive stem development. A stand density of 50 plants/m^2 was indicative of poor DM yield in the second year after planting. Overall, P fertilisation improved chicory DM yield but the improvement in stand density was not sufficient to overcome the large reduction in DM yield in the second year.

DairyNZ comment: Although 'Choice' and 'Puna II' have replaced 'Puna' as dominant New Zealand cultivars, this paper is relevant as it discusses DM yield and persistence responses which are agronomic variables being evaluated in DairyNZ programmes. In contrast to the US study, DairyNZ has found that a minimum of 25-30 plants/m^2 is required to achieve a satisfactory yield in the second year after planting. Further work is required to determine the economic viability of taking chicory swards through a second year. Furthermore, the relationship between Olsen P and chicory DM yield has not been established for New Zealand soils. Currently, DairyNZ recommends fertiliser applications for chicory follow the guidelines for perennial ryegrass. This study confirms that best practice fertiliser guidelines for specialist forages like chicory need to be defined.

Hamilton and others (2013) Stubble height management changes the productivity of perennial ryegrass and tall fescue pastures

Agronomy Journal 105: 557-562

This two-year American study evaluated the herbage accumulation, nutritive value and tiller density of tall fescue *(Lolium arundincea)* and perennial ryegrass *(Lolium perenne)* swards when repeatedly mowed to stubble heights between 2.5 and 15 cm. In year one, tall fescue mowed to 2.5 cm recorded the highest DM yield. In year two, tall fescue mowed to 2.5, 5.0 or 7.5 cm recorded similar yields, and approximately 15% greater than recorded for perennial ryegrass mowed to the same heights. Nutritive value was greater for both species when harvested at stubble heights ≥ 10 cm than at heights of ≤ 7.5 cm. Stand persistence of perennial ryegrass, measured by change in tiller density, was 60% less at stubble heights ≤ 7.5 cm vs. stubble heights ≥10 cm. Stubble height did not affect tiller density across the two years for tall fescue. The authors concluded that, when considering all three variables, DM yield, nutritive value and persistence, the optimum stubble height for tall fescue was 7.5 cm or less. A less clear pattern emerged for perennial ryegrass. Tiller density was higher when stubble height was 10 cm or more but herbage production was highest at stubble heights of 7.5 cm or less.

DairyNZ comment: New Zealand and Australian trials have shown tall fescue offers superior summer-autumn growth over perennial ryegrass, particularly in summer dry areas. DairyNZ guidelines for optimal growth and nutritive value is to graze perennial ryegrass and tall fescue at between the two and three-leaf stages of regrowth, and to a residual of approximately 4-5 cm during spring, summer and autumn, in general agreement with the conclusions in the study above. Research data from DairyNZ have not shown any increase in perennial ryegrass persistence when mown or grazed to stubble heights of greater than 10 cm. Further, the data in the above study suggest that stubble height did not decrease tall fescue persistence but New Zealand data have shown that tall fescue can be sensitive to over-grazing during summer and should be managed accordingly.