J. Dairy Sci. 97:3472–3487
http://dx.doi.org/10.3168/jds.2013-7404


# Risk factors that affect reproductive target achievement in fertile dairy cows

**S. P. M. Aungier,* J. F. Roche,* M. G. Diskin,† and M. A. Crowe*‡[1]**
*School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
†Animal and Grassland Research and Innovation Centre, Teagasc, Mellows Campus, Athenry, Co. Galway, Ireland
‡Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland

## ABSTRACT

The aims of the present study were to investigate (1) the risk factors that influence the achievement of reproductive targets postpartum (pp) and (2) the key factors that influence pregnancy rate following first artificial insemination (AI) in dairy cows. Ninety-eight Holstein-Friesian pp cows were blood sampled from wk 1 to 4 pp for hematology and biochemistry. Reproductive tract health was assessed weekly by ultrasonography and vaginal mucus scoring. Body condition score (BCS), lameness score, and milk yield were assessed every 2 wk. Milk samples for progesterone assay were collected twice weekly and on d 4, 5, and 7 after AI. Risk factors associated with achieving reproductive targets depended on (1) increased metabolic activity of the liver (increased glutamate dehydrogenase at calving and increased γ-glutamyl transpeptidase in wk 4), (2) a competent immune system (increased neutrophils in wk 1; decreased $\alpha_1$-acid glycoprotein in wk 1, 2, and 3), (3) an endocrine system that was capable of responding by producing sufficient triiodothyronine in wk 2 and increased insulin-like growth factor I in wk 3 and 4, (4) a lower negative energy balance status (decreased nonesterified fatty acid concentration in wk 1; decreased β-hydroxybutyrate concentration in wk 2; BCS loss between calving and d 28 pp <0.5), (5) good reproductive tract health [normal uterine scan at d 45 pp; clear vaginal mucus discharge at first ovulation and at d 45 pp; resumed ovarian cyclicity by the end of the voluntary waiting period (≥d 35 pp)], and (6) adequate diet (to ensure increased glutathione peroxidase in wk 2 and 3 and increased magnesium in wk 4). Risk factors that increased the odds of a successful first AI were previous ovulation(s) (odds ratio = 3.17 per ovulation), BCS >2.5 at AI (odds ratio = 3.01), and clear vaginal mucus (score = 0) compared with purulent mucus (score >0) 4 d after first AI (odds ratio = 2.99). In conclusion, this study identified key risk factors in the early pp period that give a higher probability of cows achieving their reproductive targets and of having a first-AI pregnancy.
**Key words:** fertility, insulin-like growth factor I, negative energy balance, thyroid function

## INTRODUCTION

The reproductive efficiency of high-yielding dairy cows is influenced by their postpartum (**pp**) intervals to uterine involution, first ovulation, first AI, and conception. Thatcher et al. (2006) suggested that complete physical involution should occur in a short period of time pp before first ovulation. It has been suggested that early resumption (<21 d) of ovulation pp was associated with improved reproductive performance in those cows that continue to have normal estrous cycles (Opsomer et al., 2000; Galvão et al., 2010). However, dairy cows enter a period of negative energy balance (**NEB**), which induces decreased circulating IGF-I concentration during the time pp when the uterus must undergo extensive repair after calving. Wathes et al. (2011) found that cows with a severe NEB had alterations to the IGF- and insulin-signaling pathways in the pp endometrium. This may affect the rate of tissue repair, with a possible negative effect on subsequent fertility. One of the key physiological roles of $PGF_{2\alpha}$ in the first month pp is to induce uterine contractility and so promote uterine involution (LeBlanc, 2008). Circulating $PGF_{2\alpha}$ is converted rapidly to $PGF_{2\alpha}$ metabolite (**PGFM**) in the lungs. In normal cows, the longer the time that concentrations of PGFM remained elevated pp, the shorter the time to gross uterine involution (Lindell et al., 1982; Madej et al., 1984) and resumption of normal estrous cycles (Madej et al., 1984). In cows with purulent uterine discharge, PGFM concentrations were higher and remained elevated for longer than in normal cows, yet involution was delayed (Lindell et al., 1982; Del Vecchio et al., 1994). The inflamed uterus may provoke an increased magnitude and duration of $PGF_{2\alpha}$ release from the uterus (Lindell et al., 1982) and it may become refractory to this continuous release of $PGF_{2\alpha}$ (Rodriguez-Martinez et al., 1987). In healthy cows, the previously gravid uterine horn should achieve a diameter of 3 to 4 cm by 25 to 30 d pp and a cervical

Received August 22, 2013.
Accepted February 12, 2014.
[1]Corresponding author: mark.crowe@ucd.ie

diameter <5 cm by 40 d pp (Gier and Marion, 1968). However, involution of the uterus and cervix is not complete until approximately 40 to 50 d pp (Gier and Marion, 1968; Sheldon, 2004; Scully et al., 2013). Uterine involution can also be affected by age, breed, nutrition, and other factors, so delayed uterine involution is not a specific indicator of uterine disease (Fonseca et al., 1983).

A study by Dubuc et al. (2012) identified risk factors associated with early ovulation (<21 d pp), which included season, parity, decreased haptoglobinemia, and decreased serum NEFA concentration before and after parturition. Risk factors for prolonged anovulation (anovulatory >63 d pp) included cytological endometritis, increased haptoglobinemia, and greater serum NEFA concentrations before and after parturition. Negative energy balance inhibits LH pulse frequency, and low concentrations of blood glucose, insulin, and IGF-I result in reduced estradiol-17β production by the dominant follicles (Butler, 2000). Negative energy balance not only reduces insulin and IGF-I concentrations (Beam and Butler, 1999; Butler, 2000) but also increases growth hormone (**GH**) concentrations (Vicini et al., 1991), leading to delays in resumption of estrous cycles, impaired oocyte quality and corpus luteum (**CL**) function. The development of extended luteal phases after first ovulation pp has been reported to be higher in cows with pp endometritis and pyometra (Mateus et al., 2002). The extended luteal phase probably occurs because the infection switches the endometrial epithelial secretion of prostaglandins from the F to the E series by a phospholipase A2-mediated mechanism that disrupts luteolysis (Sheldon et al., 2008; Herath et al., 2009).

A decline in pregnancy rate to first AI has been associated with abnormal reproductive, metabolic, and endocrine factors (Diskin, 2008). Conception rates were found to be approximately 20% lower for cows with endometritis, with the calving to conception interval being 31 d longer (Borsberry and Dobson, 1989). Despite the multifactorial nature of bovine subfertility, the current literature is lacking in research where a multifactorial approach has been used to identify the key risk factors associated with cows that can achieve reproductive targets and become pregnant in a timely fashion. Therefore, the aims of the present paper were to determine (1) the risk factors that influence the achievement of reproductive targets pp and (2) the key factors that influence pregnancy rate at 30 d after first AI in dairy cows. To address these aims, the following hypotheses were tested: (1) specific metabolic, endocrine, mineral, reproductive tract health, liver function, immune status, BCS, and lameness score factors monitored in the early pp period are related to fertile cows achieving their reproductive targets (interval to ovulation, involution, first AI, and conception); and (2) at the time of the first AI, reproductive tract health, milk production, BCS and lameness score variables are related to the probability of a pregnancy following this AI in fertile cows.

## MATERIALS AND METHODS

### *Study Animals*

The study was conducted between January and August 2009 on a commercial all-year-round milk-producing dairy farm in County Kildare, Ireland. A total of 98 cows from the spring-calving cohort were selected on the basis of having had single-calf births and calving after January 19. The cows were divided into 3 groups representing first lactation (n = 31), second lactation (n = 27), and a third lactation group made up of lactations 3 to 8 (n = 40). The calving period was from January 19 to May 5, with a calving distribution of 26, 22, 35, 14, and 1 cow in the months January to May, respectively. From the day of drying-off up to 21 d before calving, dry cows and in-calf heifers were fed a far-off diet consisting of grass silage, straw, and a vitamin and mineral mix. The close-up diet, from 21 d before calving to expected day of calving, aimed to exceed the energy requirements. The diet supplied, on average, 117 MJ/animal per day, as the parities were not separated. The diet consisted of grass silage, straw, soybean meal, Dairy Blend (1 kg of 20% CP; Charles R. Wynne Foods Ltd., Kildare, Ireland), and vitamin and mineral mix [150 g/cow per day of the mix: vitamin A (600,000 IU/kg), vitamin $D_3$ (200,000 IU/kg), vitamin E (10,000 IU/kg), cobalt (100 mg/kg), iodine (500 mg/kg), selenium (42 mg/kg), manganese (3,800 mg/kg), copper (3,000 mg/kg), zinc (8,000 mg/kg), phosphorus (6%), and magnesium (26.5%)].From calving, cows were fed grass silage, straw, maize silage, proprietary coarse blend (8 kg of 20% CP) and brewers grain. This was topped off with 1 kg of a proprietary compounded concentrate (15% CP) in the parlor at both a.m. and p.m. milking. From late March, cows that had calved were let out to graze grass by day and the level of grass and maize silage was reduced accordingly. From mid April, cows were outdoors full time, but were fed a TMR buffer diet, with reduced grass and maize silage consumption, after each milking, and were fed the proprietary compounded concentrate during each milking. The cows in the study group had a mean ± standard error of the mean 305-d milk yield of 8,800 ± 154 kg with a mean peak yield of 43.9 kg/d for 2009 to 2010. The automatic heat activity monitor Heatime (SCR Engineers Ltd., Netanya, Israel).was used to de-