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BRIEF COMMUNICATION: Differences in follicle dynamics and the importance of luteal support in young and old cohorts of genetically identical dairy cows

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INTRODUCTION

There has been a documented decrease in fertility in the modern dairy cow selected for milk yield (Pryce *et al.,* 2004). This emphasises the need for farmers in a seasonal grass-based system such as that in New Zealand to correctly manage herd genetics in conjunction with nutrition to optimise cow reproductive performance. The question remains however as to the relative influence and interdependence of these two factors in establishing a pregnancy, as an optimal size for the ovulatory follicle is directly related to successful conception and pregnancy (Perry *et al.*, 2005).

Decreased fertility is also reported as a consequence of maternal aging, with older animals having a decreased ovarian reserve of follicles (Erickson *et al*., 1976), an altered hormone secretion pattern (Malhi *et al.*, 2005) and a diminished ability of their oocytes to support early embryo development (Webb *et al.*, 2004). However the mechanisms controlling follicular growth are not fully understood.

Previous studies have used mother-daughter pairs to examine the effect of maternal aging on fertility. The aim of this preliminary study was to use genetically identical cloned cows to investigate whether ovarian follicle dynamics are genetically determined and influenced by maternal age when environmental factors, such as nutrition and management are controlled.

MATERIALS AND METHODS

This study was approved by the Ruakura Animal Ethics Committee (AEC11817) and conducted from July to October 2009. Follicle dynamics were monitored over four successive oestrous cycles in two cohorts of pasture-fed non-lactating genetically identical dairy cows aged 3 years (n = 7) and 8 years (n = 5). All these cloned females were generated as described (Wells *et al.,* 1999) and managed as one group grazed on ryegrass (*Lolium perenne L.*) – white clover (*Trifolium repens L.*) pasture sufficient for maintenance at approximately 10 kg DM/cow/d.

Follicular diameters were measured by ultrasonography using a 7.5 mHz transvaginal sector probe (PieMed 200S, Pie Medical Inc., Maastricht, The Netherlands). Prior to ultrasound scanning, cows received epidural anaesthesia (4 mL 2% Lidocaine; Bomacaine, Bomac Laboratories Ltd., Auckland, New Zealand). Follicle populations were mapped and the diameters of individual follicles measured using the internal callipers of the ultrasound system, every third day after oestrus (Day 0).

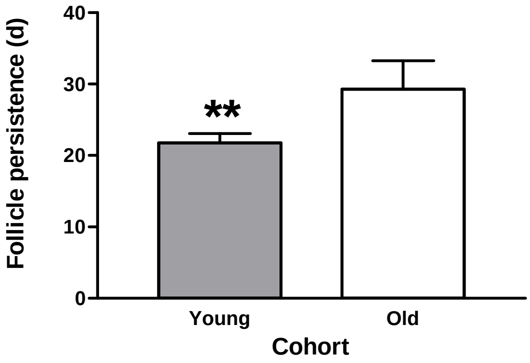
The importance of luteal support for follicular growth was also investigated over two oestrous cycles in five 5-year-old genetically identical non-lactating cows, through the removal of luteal tissue via administration of prostaglandin (2 mL Estroplan; Cloprostenol, Parnell Labs, Auckland, New Zealand) on day 10. On day 10 and 12 post-oestrus follicles greater than 4 mm were aspirated using 19Gx1.5”BD Precision-Glide needles (Becton Dickinson, Auckland, New Zealand) with 25 mm Hg vacuum using an aspiration pump (Karl Storz GmbH, Tuttlingen, Germany). The subsequent growth of new follicles in a progesterone free environment was then measured daily via ultrasonography until a dominant follicle with a diameter of 16 mm was attained.

Maximum follicle size was determined for both the dominant non-ovulatory and ovulatory follicle of each cycle. Daily growth rate (mm) was calculated for the ovulatory follicles from first measure (4 mm) to peak divided by the number of days. Morphological persistence of the non-ovulatory follicles was calculated from the time of first measure (4 mm) to regression (<6 mm or diameter at the end of the trial if follicles failed to regress to <6 mm). Follicle size, daily growth rate and morphological persistence were analysed using two-way ANOVA in SAS version 9.1 (SAS, 1997) with maternal age and cycle incorporated in the model. Results are presented as the mean ± standard error of the mean unless otherwise stated.

RESULTS

All young and old females demonstrated two follicular waves per cycle, with no difference ($P > 0.1$) in the maximal size of either the non-ovulatory; from the first wave (old 16.2 ± 0.6 mm,

FIGURE 1: Morphological persistence (days) of the dominant non-ovulatory follicle of seven young and five old, cloned dairy cows. Mean ± standard error; ** (P = 0.007).



young 16.0 ± 0.5 mm), or ovulatory; from the second wave, follicle (old 18.0 ± 0.6 mm, young 17.7 ± 0.4 mm). There was no difference (P >0.1) in the average ovulatory follicle growth rate over the four cycles (old 1.44 ± 0.08 mm/d, young 1.43 ± 0.07 mm/d). However, seasonal effects were identified with ovulatory follicles from the 3rd and 4th cycles during September through October, having a higher (P = 0.05) mean daily growth rate irrespective of age (Cycles 1 to 2 1.30 ± 0.07 mm/d, Cycles 3 to 4 1.57 ± 0.07 mm/d). Interestingly, morphological persistence of the non-ovulatory follicle was extended (P = 0.007) in older cows (old 29.3 ± 4.0 days, young 21.8 ± 1.3 days) (Figure 1).

In the second study, removal of luteal support resulted in behavioural oestrus in all cattle on average, three days after treatment on Day 15 of the original cycle, once a 6 mm follicle was present. Despite displaying behavioural oestrus, follicles failed to ovulate and had limited growth beyond 6 mm.

DISCUSSION

The results of the current study indicate that genetic control of follicle dynamics is limited, with large variation in follicular growth rate and morphological persistency between genetically identical individuals, both within and between ages, although dynamics appeared highly repeatable within individuals. This study supports the low estimated heritability of fertility, specifically 0.16 for follicle size (MacNeil *et al*., 2006). Thus, follicle dynamics are more likely influenced by the numerous environmental factors that impact upon local ovarian mechanisms (Webb *et al.*, 2004).

Maternal age was shown to affect follicular turnover as the older cows showed a greater morphological persistence of first-wave dominant follicles of approximately eight days, indicating slower follicular turnover. It should be noted that only morphological, rather than functional persistence which is the ability of the dominant follicle to suppress a new follicle wave, was determined in the current study. Prolonged follicle turnover is evident in sub-fertile cows, whose delay in the timing of ovulation and slower luteinisation of the subsequent corpus luteum (CL) resulted in a delay in the early progesterone (P4) rise (Mann & Lamming, 2001). Lower circulating P4 concentrations and CL weight are found in old cows (Malhi *et al*., 2005), as well as younger sub-fertile cows with reduced embryo development (Green *et al.*, 2005). This delayed rise in P4 is critical, as it is associated with slower growing embryos that are less likely to maintain a pregnancy due to mis-timing of interferon-tau production, the maternal pregnancy recognition signal, and thus reduced pregnancy rates (Mann & Lamming, 2001). This is why endocrine measures, such as commencement of P4 production are being investigated for inclusion in fertility indices.

The results of the second study clearly demonstrate that luteal support is obligatory for both the maintenance of healthy follicle growth and ovulation in the cow. Changes in concentrations and ratio of P4 and oestrogen are important in triggering behavioural oestrus (Boer *et al.*, 2010). This explains why in the current study in the absence of any P4 even the small concentration of oestradiol produced by a 6 mm follicle, which potentially lacks functional LH receptors, compared to that produced by a pre-ovulatory follicle (>14 mm), may have the ability to elicit a behavioural oestrus response but fail to ovulate.

Collectively, the current findings illustrate how important exposure to, and maintenance of, P4 is to ovulation of the correct sized follicle, since this is indirectly associated with a successful conception. Therefore, farmers should monitor post-partum P4 profiles in order to improve the chances of their cows conceiving within the limited breeding period.

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