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## Preovulatory follicle characteristics and oocyte competence in repeat breeder dairy cows

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### ABSTRACT

The varied and elusive etiology of repeat breeding (RB) in dairy cows necessitates evaluation of oocytes and follicles, which have not previously been assessed together. Accordingly, we evaluated characteristics of preovulatory follicles and the competence of oocytes in control (CTL) and RB cows. The estrous cycles of 35 cows (18 CTL and 17 RB) were synchronized using PGF<sub>2α</sub> and estrus detection. Cows with a corpus luteum were treated with PGF<sub>2α</sub> and, 14 to 15 d after a visible behavioral estrus, they were administered a second PGF<sub>2α</sub>, followed 48 h later by follicular fluid (FF) aspiration of the preovulatory follicles. Estradiol (E<sub>2</sub>)-active preovulatory follicles did not differ in diameter between the 2 groups of cows. However, FF of RB cows had higher E<sub>2</sub> concentrations than that of CTL cows: 1,854.9 and 1,073.6 ng/mL, respectively, but similar androstenedione and progesterone concentrations. In the second part of the study, 14 consecutive ovum pick-ups (OPU) were performed in 5 CTL and 5 RB cows, at 3- to 4-d intervals. The RB and CTL cows did not differ in average numbers of follicles available per cow per session (7.1 and 7.3, respectively), oocyte recovery rates (42.2 and 44.1%, respectively), or cleavage rates (57.6 and 63.4%, respectively), but blastocyst production was markedly less in RB than in CTL cows (12.5 and 29.2%, respectively). We conclude that part of the RB cows' etiology occurs at an earlier phase of folliculogenesis, thereby impairing oocyte competence, and subsequently reducing the probability of normal fertilization, which diminish embryo vitality and development.

**Key words:** repeat breeder dairy cow, preovulatory follicle, oocyte competence

### INTRODUCTION

The incidence of repeat breeding (RB) in some dairy cattle herds is as high as 24% (Yusuf et al., 2010); in Israel, the frequency of RB in multiparous dairy cows in recent years has been ~30% (Israeli Dairy Herdbooks; Israel Cattle Breeders' Association, 2014, 2015). Among other costs, the RB phenomenon results in loss of genetic gain and increased culling rates (Bartlett et al., 1986). Reproduction in cows depends on many factors and involves tight coordination of numerous events that occur at various points between the higher brain centers and the ovarian compartment. Fertilization failure (Graden et al., 1968) and embryo mortality (Gustafsson, 1985) are the inherent mechanisms that restrict reproduction under various adverse conditions, of which RB is a pertinent example. There are several documented reasons for subnormal fertility in RB cows; some investigations found endocrine and ovulatory disturbances to be the preponderant reasons (Båge et al., 2002; Saumande and Humbolt, 2005; Bloch et al., 2006; Sood et al., 2015). However, these can be diagnosed from clinical manifestations (e.g., prolonged estrus) or endocrinological assessments, and treated accordingly (López-Gatiús et al., 2005). Nevertheless, other groups of RB cows had normal endocrine and ovulation status, normal genital organs (Sood et al., 2015), and a uterine environment that was favorable for pregnancy (Tanabe et al., 1985). In such cases, determining the exact reason for RB remains a serious challenge, and the etiology remains obscure in 37.8% of the RB cows (Perez-Marin and Espana, 2007). Thus, the causes of RB lie beyond endocrine or ovulatory perturbations, and we assumed that RB might be determined much earlier, during the course of oocyte development. In a previous study, RB cows yielded substantially increased numbers of morphologically deviated and degenerated 7-d-old embryos, which were collected nonsurgically from their uterus (Gustafsson, 1985). Other studies revealed excess inferior-quality oocytes in RB cows (Gustafsson and Emanuelson, 2002; Båge et al., 2003; Kurykin et

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al., 2011). Ferreira et al. (2011) found no differences in quality or cleavage rate between oocytes retrieved from RB cows and those from cows in peak lactation, but they observed reduced blastocyst production in the RB cows; however, Ferreira et al. (2011) performed their study after involvement of exogenous hormones in the synchronization protocol, which might influence oocyte quality or competence (Blondin et al., 2012). The oocyte shares a close and dynamic relationship with the antral follicular fluid (FF), which constitutes a specialized microenvironment specifically suited to the needs of the developing oocyte. Composition of the FF, especially the steroids, can influence follicle health and oocyte quality (Wehrman et al., 1993; Revelli et al., 2009). Aardema et al. (2013) examined the association, in superstimulated healthy heifers, between steroid concentrations in preovulatory follicles and oocyte developmental competence: they found differences in the criteria for selection of cumulus oocyte complexes between superstimulated heifers and nonstimulated cows, and this finding highlights the need to examine the RB phenomenon in nonstimulated cows.

Zachut et al. (2010) investigated the FF steroidal environment and oocyte competence in cows subjected to dietary changes, and Roth et al. (2008) did so for those in various stages of lactation, but neither group addressed RB cows. Therefore, the aim of the present study was to delineate the RB mechanisms, per se, in unstimulated RB cows.

## MATERIALS AND METHODS

### Cows and Diet

All procedures used in the present study were approved by the Volcani Center Animal Care Committee. The animals used were primiparous and multiparous lactating Israeli Holstein cows, housed and maintained at the Volcani Center Experimental Farm (Rishon Lezion, Israel). All the selected cows were normal cyclic, with normal estrus duration, and before the start of

the study had no history of dystocia, retained placenta, or metritis. Also, we ruled out occurrence of abnormal genital discharge and of pathological abnormalities of the reproductive tract, including cystic ovaries, by performing 2 successive examinations separated by 10 to 11 d, before the study commenced; using an Aquila 5-MHz linear array transducer (Pie Medical, Maastricht, the Netherlands). The other exclusion criteria were severe mastitis, lameness, or severe digestive disorders during the current lactation.

The cows were grouped into 2 categories: control (CTL) and RB. The CTL cows were >60 d in lactation, with a range of 62 to 133 d, cycling, and not inseminated. A cow was considered RB if it exhibited no clinically detected abnormality and did not become pregnant after at least 4 successive AI during spontaneous estrus, in the current lactation, with normal intervals between inseminations. Duration of estrus was recorded by means of H-tag collar-mounted tags for neck movements (SCR Engineers, Hadarim, Netanya, Israel) and pedometers (AfiFarm System, SAE Afikim, Israel).

Forty cows were investigated during 2 successive years. In the first year, the study involved 25 cows, 13 CTL and 12 RB; we assigned 20 cows (10 from each group) to preovulatory follicular aspiration and 10 cows (5 from each group) to ovum pick-up (OPU). Five cows (2 CTL and 3 RB) were subjected to both procedures, and 5 cows (3 CTL and 2 RB) were subjected only to OPU. In the second year, 15 cows (8 CTL and 7 RB) were assigned only to preovulatory FF aspiration; see Table 1 for distribution of cows among both procedures. We conducted the experiment during the same season in each year (late winter to early spring) to avoid heat stress effects.

The cows were fed according to NRC (2001) recommendations and housed together in a covered loose pen with an adjacent outside yard. The animals were milked 3 times/d, and milk yields and BW were automatically recorded daily with an automatic AfiFarm System (SAE Afikim). A single technician determined the BCS

**Table 1.** Distribution of cows for preovulatory follicle aspiration and ovum pick-up (OPU) procedures in both years<sup>1</sup>

Procedure	First year		Second year		Total cows
	CTL	RB	CTL	RB	
Preovulatory follicle aspiration only	8	7	8	7	30
Preovulatory follicle aspiration + OPU	2	3	0	0	5
OPU only	3	2	0	0	5
Total preovulatory follicle aspiration	10	10	8	7	35
Total OPU	5	5	0	0	10
Total cows	13	12	8	7	40

<sup>1</sup>CTL = control cows; RB = repeat breeder cows.

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