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Surgical translocation and ultrasound bio-microscopy of the ovaries in rabbits

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ABSTRACT

Experiments were designed to validate the use of ultrasound bio-microscopy (UBM) as a method for assessing ovarian structures in rabbits. In Experiment 1, female New Zealand White (NZW) rabbits ($n=4$) were given an ovulation-inducing treatment and the ovaries were examined *ex situ* by UBM using a 25 MHz oscillating sector transducer before being processed for histology. Pairwise correlations revealed strong relationships between UBM and histology in the number (Mean ± SEM) of follicles ≥0.6 mm (17.3 ± 2.3 compared with19.0 ± 1.6, respectively; $r=0.96$; $P=0.040$), CL (8.5 ± 2.9 compared with 8.8 ± 3.0; $r=0.99$; $P=0.003$), the diameter of follicles (1.1 ± 0.05 compared with 1.1 ± 0.03 mm; $r=0.96$; $P=0.035$) and CL (2.1 ± 0.7 compared with 1.8 ± 0.6 mm, $r=0.99$; $P<0.001$). In Experiment 2, the ovaries of NZW rabbits ($n=12$) were surgically translocated to a subcutaneous position in the flank region to permit serial examination of ovarian structures *in vivo* by UBM. Beginning 2 weeks after surgery, the ovaries were examined by UBM daily for at least 18 days, and again 2 months after surgery. Post-operative complications were minor, and both ovaries of each rabbit were identified consistently. The number and diameter of follicles ≥0.6 mm were readily visualized during each examination. Multiple corpora lutea were detected in two rabbits, and serial follicular and luteal dynamics in these two rabbits were used to document the consistency of UBM and the retention of ovarian function after surgery. It is concluded that UBM is a valid tool for instant assessment of rabbit ovarian structures (follicles, corpora lutea, and cumulus-oocyte complexes) *ex situ*, and for serial assessment *in vivo* using a transcutaneous approach. Surgical translocation had no apparent untoward effect on ovarian function.

1. Introduction

Rabbits have been used as a research model for humans and other mammals in a variety of areas (*e.g.*, physiology, toxicology, biomedical research, placentation) (Kaplan and Timmons, 1979, Foote and Carney, 2000, reviewed by Brewer, 2006, Bõsze and Houdebine, 2006; Carter, 2007) because they are of medium size, readily accessible, easy to handle, and easy to maintain (Manning et al., 1994). Study of ovarian function in the rabbit has been based on findings at post-mortem examination and histology (Deanesly, 1930; Armstrong et al., 1978; Kranzfelder et al., 1984; Agrawal and Jose, 2011), laparotomy (Marshall and Verney, 1936; Kauffman et al., 1998; Agrawal and Jose, 2011), laparoscopy (Blasco et al., 1994; Argente et al., 2010), and endocrinology (Miller and Keyes, 1978; Labib et al., 1978; Caillol et al., 1983; Mills and Gerardot, 1984). These terminal or invasive methods, however, provide

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little or no opportunity for serial examination and restrict progress in our understanding of follicle development and ovulation in rabbits.

The advent of real-time B-mode ultrasonography resulted in rapid advancement of the understanding of ovarian follicular and luteal dynamics in every species in which it has been used, including cattle (Pierson and Ginther, 1984; Adams and Pierson, 1995), horse (Ginther and Pierson, 1984), camelids (Adams et al., 1989), sheep (Ravindra et al., 1994) and humans (Queenan et al., 1980; Pierson et al., 1990). Conventional ultrasonography, using frequencies of 5–7.5 MHz, has been used widely for ovarian studies in large species, but resolution is inadequate for detailed study of small structures ≤2 mm; *i.e.*, for visualization of ovarian structures in small laboratory species (Pallares and Gonzalez-Bulnes, 2008). Based on a report in which conventional ultrasonography (using a 7.5 MHz transducer) was described as a method of evaluating follicular dynamics in rabbits (Marongiu and Gulinati, 2008), a preliminary study was initiated to examine the effects of ovulation-inducing treatments in rabbits *in vivo* using conventional transabdominal ultrasonography with a 12 MHz transducer. The images obtained, however, were inadequate for resolving dynamic changes in follicular development or for evaluating ovarian response to ovulation-inducing treatments in the rabbits.

High frequency ultrasound systems (ultrasound biomicroscopy) have provided the technological basis for imaging tissue at the cellular level (Czarnota et al., 1997). Ultrasound bio-microscopy (UBM), using frequencies of 20 to 55 MHz, has enabled the *in vivo* study of embryogenesis in mice (Turnbull and Foster, 2002), as well as ovarian follicular dynamics of the theca/granulosa cell layers, and visualization of cumulus-oocyte complex in mice (Singh *et al.*, 2003) and in humans ovaries *in vitro* (Baerwald et al., 2009). Recent studies documented the feasibility of UBM for imaging ovarian structures in mice (Pallares and Gonzalez-Bulnes, 2008; Mircea et al., 2009). In one study, serial *in vivo* UBM was used to characterize ovarian dynamics in mice using a transcutaneous approach; however, a similar approach was not successful in rats (Jaiswal et al., 2009). To date, studies of ovarian function in rabbits using UBM have not been reported.

The objectives of the present study were to validate the use of ultrasound bio-microscopy as a method for assessing ovarian structures in rabbits, and to develop a method that permits serial noninvasive evaluation of ovarian structures *in vivo* in rabbits.

2. Materials and methods

New Zealand White rabbits were housed under controlled environmental conditions (20.2 ± 2 °C, 14:10-h light:dark cycle) and were allowed *ad libitum* access to food and water. The experimental protocol was approved by the University of Saskatchewan's Animal Research Ethics Board, in accordance with guidelines of the Canadian Council on Animal Care.

2.1. Experiment 1

The experiment was conducted to validate the use of ultrasound bio-microscopy (UBM) as a method for assessing follicles and corpora lutea in rabbit ovaries. Four female rabbits (5–5.5 months old) were given 20 μg of GnRH (Fertagyl; Intervet Canada Ltd., Whitby, Ontario, Canada) intramuscularly as an ovulation-inducing treatment. The day of treatment was designated Day 0. Rabbits were euthanized on Day 8 by an overdose of sodium pentobarbital, and the ovaries were removed for evaluation *ex situ*. Immediately following removal, each ovary was imaged in a saline bath using a high-resolution ultrasound bio-microscope (UBM; Vevo 660 Imaging System™, Visual Sonics Inc., Toronto, Ontario, Canada) equipped with a 25 MHz oscillating sector transducer (RMV 704; Fig. 1). The measured lateral resolution of the transducer was 75 μm, the axial resolution was 40 μm, and the depth range of imaging was 1.5 to 10 mm with a focal length of 6 mm (http://www.visualsonics.com). The saline bath was placed on a manually operated mechanical stage and moved through two horizontal axes (forward-to-back and side-to-side). The transducer was placed in a stationary holder and the plane of the beam was oriented transverse to the long axis of the ovary. The stage was moved until the ovary was visualized from one end to the other, and images were recorded in cine-loops (300 frames in 10 s). Following UBM imaging, the ovaries were cleaned of adhering connective tissue and fat, fixed in 10% formaldehyde, and stored at room temperature until histological processing.

Individual follicles ≥0.6 mm and corpora lutea were identified, counted and measured by scrolling through the digital UBM cine-loops frame-by-frame. Two orthogonal diameter measurements were taken from images that contained the greatest cross-sectional area of the structure in question. The overall diameter (mm) of the structure was taken as the average of the two measurements.

Standard histological procedures were used to prepare ovarian sections. In brief, the ovaries were dehydrated, embedded in paraffin wax, serially sectioned at 10 μm thicknesses, dewaxed, hydrated, and stained with Masson's trichrome. Stained slides were examined with conventional light microscopy, and digital photomicrographs of every third section (*i.e.*, at 30 μm intervals) of each ovary were taken. Photomicrographs were sequentially loaded into Image J NIH Software System (National Institutes of Health, Bethesda, Maryland, USA), and individual follicles ≥0.6 mm and corpora lutea were identified, counted and measured by scrolling through the serial digital images. The procedure to measure the structures of interest was similar that described for assessing UBM images. All UBM and histological measurements and counts were performed by a single observer.

2.2. Experiment 2

Attempts were made in Experiment 1 to assess ovarian structures *in vivo* by trans-abdominal UBM, but the acoustic impedance of the body wall prevented acquisition of high-quality ovarian images. Therefore, a novel approach