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In vitro culture of oocytes and granulosa cells collected from normal, obese, emaciated and metabolically stressed ewes

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ABSTRACT

The present study was undertaken to investigate the oocyte morphology, its fertilizing capacity and granulosa cell functions in ewes (obese, normal, metabolic stressed and emaciated). Ewes (*Ovis aries*) of approximately 3 years of age (Bellary breed) from a local village were screened, chosen and categorized into a) normal b) obese but not metabolically stressed, c) Emaciated but not metabolically stressed d) Metabolically stressed based on body condition scoring and blood markers. Oocytes and granulosa cells were collected from ovaries of the ewes of all categories after slaughter and were classified into good (oocytes with more than three layers of cumulus cells and homogenous ooplasm), fair (oocytes one or two layers of cumulus cells and homogenous ooplasm) and poor (denuded oocytes or with dark ooplasm). The good and fair quality oocytes were *in vitro* matured and cultured with fresh semen present and the fertilization, cleavage and blastocyst development were observed. The granulosa cells were cultured for evaluation of metabolic activity by use of the MTT assay, and cell viability, cell number as well as estrogen and progesterone production were assessed. It was observed that the good and fair quality oocytes had greater metabolic activity when collected from normal and obese ewes compared with those from emaciated and metabolically stressed ewes. No significant difference was observed in oocyte quality and maturation amongst the oocytes collected from normal and obese ewes. The cleavage and blastocyst production rates were different for the various body condition classifications and when ranked were: normal > obese > metabolically stressed > emaciated. Lesser metabolic activity was observed in granulosa cells obtained from ovaries of emaciated ewes. However, no changes were observed in viability and cell number of granulosa cells obtained from ewes with the different body condition categories. Estrogen and progesterone production from cultured granulosa cells were not different in normal and obese ewes. Estrogen and progesterone secretions were less from granulosa cells recovered from metabolically stressed and emaciated ewes. The results suggested that oocyte morphology, fertilizing capacity and granulosa cell growth were dependent on body condition and feeding status of the animals.

1. Introduction

Poor reproductive performance has been associated with metabolic stress, such as poor feeding practices that

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influence body composition. These factors can influence fertility by effecting ovarian function, yield of oocytes and quality of embryos (Kubovicova et al., 2013). Dietary status is a foremost feature influencing an animal's capacity to reproduce (O'Callaghan et al., 2000). Nutritional status has been associated with embryo viability and is a key aspect influencing competence in assisted reproductive technologies (Webb et al., 2004). Nutrition and subsequently metabolic status of the female can affect the capacity of females to reproduce (Bridges et al., 2012). For assessing the nutritional status of animals, body condition scoring (BCS) is a useful practice (Hady et al., 1994). Body condition score (BCS) is the perceptible evaluation of body condition and sequential change in BCS is used to examine dietary and physical condition of animals used for meat and milk production (Berry et al., 2007). The BCS is associated with reproductive performance, both phenotypically and genetically (Kubovicova et al., 2013). During periods of meat and milk production, animals can enter a metabolic state where negative energy balance (NEB) occurs (Formigoni et al., 2003). Fertility is a complex variable and its decrease can be caused not only by BCS, but also by several other non-genetic effects such as season, heat stress, nutritional stress, breed, age and individual differences between animals (Kubovicova et al., 2013). Physiological condition of animals has a considerable effect on embryo quality (Walsh et al., 2011). Feeding diets with less energy than that required for body maintenance results in lipolysis and is characteristically associated with elevated non esterified fatty acid (NEFA) concentrations in association with lesser glucose concentrations in serum (Leroy et al., 2004). Endocrine signaling is altered as a result of reduced metabolism and a reduced quality of oocytes and/or embryos can result (van Knegsel et al., 2007). Oocyte quality can be influenced by metabolic status of animals throughout the several month maturation periods when there is sensitivity to negative influences such as nutritional deficiencies or obese conditioning (Farman et al., 2015). Short-duration changes in plane of nutrition do not influence ovarian follicular dynamics in ruminants by changes in circulating concentrations of gonadotropins (Webb et al., 2003; Fouladi-Nashta et al., 2007). Short-term changes in nutritional energy intake can result in enhanced oocyte morphology and developmental capacity (Adamiak et al., 2006; Fouladi-Nashta et al., 2007). The sum total of nutrients and energy available to the animal's tissues at a given time is termed the metabolic status of the animal and it depends on three factors: amount of food consumed, amount of body reserves and rate of expenditure of energy (Blache et al., 2006; Martin and Walkden-Brown, 1995). Metabolic status includes an integrative dimension that is illustrated by the number and range of physiological control processes that influence reproductive function (Blache and Martin 2009). Significantly, changes in any of these three components can influence the reproductive capacity in both males and females.

It was hypothesized in the present study that oocyte and granulosa cell function are dependent on body condition of an animal. The present study was undertaken to investigate the oocyte morphology and its fertilizing capacity in sheep (normal, obese, metabolically stressed and emaciated). Granulosa cell growth and secretory functions in normal, obese, metabolically stressed and emaciated ewes were also assessed.

2. Materials and methods

2.1. Animals

Mature, non-pregnant, estrous cycling, multiparous ewes (*Ovis aries*) of the Bellary breed that were 2.5 to 3 years of age were used in this study ($n = 306$; breed average for body weight is 32.6 kg). Ewes were from a local farm (Sira, Tumkur, Karnataka, India) and were screened for this study. Bellary sheep are a local breed in India. This breed is known for its strong physical characteristics medium sized and has body colour ranging from white through various combinations of white and black. The lambing percentage ranges 80% to 85% with a litter size of one/lambing. The animals used in the present study were under regular veterinary care and management throughout the study. The ambient temperature ranged from 26 to 28 °C during the experimental period. Ewes ($n = 200$) were chosen and categorized into (a) Normal ($n = 50$, Average body weight: 32.3 kg), (b) Obese but not metabolically stressed ($n = 50$, Average body weight: 44.6 kg), (c) Emaciated but not metabolically stressed ($n = 50$, Average body weight:15.4 kg) and (d) Metabolically stressed ($n = 50$, Average body weight: 30.2 kg) based on body condition scoring (Thompson and Meyer, 2002) and blood sampling. The metabolically stressed ewes were identified/selected by estimating serum ammonia, blood urea nitrogen (BUN), total non-esterified fatty acids and β-hydroxybutyric acid (Nandi et al., 2013a; Nandi et al., 2013b). The metabolically stressed ewes had more than 130 μM, 6.5 mM, 105 μM and 0.5 mM of serum ammonia, BUN, total non-esterified fatty acids and β-hydroxybutyric acid, respectively. The experiments were conducted under good laboratory conditions and animal experimentation was done under guidance from the Institute Ethical Clearance Committee. The diet consisted of wheat bran, groundnut cake, maize grain, ragi straw, mineral mixture. The animals had *ad libitum* access to good quality drinking water. The total duration from screening to slaughter of animals was 2 weeks. The average ambient temperature during the study was 23.4 °C (Maximum temperature: 30.8 °C, Minimum temperature: 18.5 °C).

2.2. Collection of sheep oocytes and semen

Selected ewes were estrous-synchronized (Lutalyse 15 mg) using two injections of dinoprost tromethamine 9 days apart with multiple ovulations being induced by mid-estrous cycle treatments with Equine Chorionic Gonadotropin [eCG, 1000 IU one dose] to maximize the sample size (Nandi et al., 2015). After 3 days subsequent to cessation of treatments, ewes were slaughtered and ovaries were collected. The ovaries were transported to the laboratory within 2 h of slaughter. Oocytes were collected from all ovaries by aspiration of all visible follicles and the aspiration medium consisted of TCM–199 + phosphate buffered saline (PBS) + Bovine serum