Animal Reproduction Science 172 (2016) 114–122

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Oestrus synchronisation and superovulation alter the production and biochemical constituents of ovine cervicovaginal mucus

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ARTICLE INFO

Article history:
Received 25 January 2016
Received in revised form 30 June 2016
Accepted 14 July 2016
Available online 22 July 2016

Keywords:
Progesterone
Prostaglandin
Oestrogen
Oestrus
Hormone
Cervicovaginal

ABSTRACT

Controlled breeding programmes utilising exogenous hormones are common in the Australian sheep industry, however the effects of such programmes on cervicovaginal mucus properties are lacking. As such, the aim of this study was to investigate cervicovaginal (CV) mucus from naturally cycling (NAT), progesterone synchronised (P_4), prostaglandin synchronised ($PGF_2\alpha$), and superovulated (SOV) Merino ewes. Experiment 1; volume, colour, spinnbarkeit, chemical profile and protein concentration of mucus (NAT, P_4, $PGF_2\alpha$ and SOV; n = 5 ewes/treatment) during the follicular (5 d) and luteal phases (8 d) was investigated. Experiment 2; *in vivo* mucus pH and *in vitro* mucus penetration by frozen-thawed spermatozoa (NAT, P_4 and SOV; n = 11 ewes/treatment) was investigated over oestrus (2 d) and the mid-luteal phase (pH only, 2 d). Oestrus mucus was more abundant, clearer in colour and less proteinaceous than luteal phase mucus ($p < 0.05$). SOV increased mucus production and protein concentration ($p < 0.05$) while $PGF_2\alpha$ reduced mucus volume ($p < 0.05$). Mucus pH (oestrus 6.2–6.5), chemical profile and mucus penetration by sperm were unchanged ($p > 0.05$). Results indicate that exogenous hormones used for controlled breeding affect cervicovaginal mucus production, but few other tested characteristics. Further research is required to explain fertility differences between synchronised and naturally cycling animals following cervical AI.

1. Introduction

The use of exogenous hormones for oestrus synchronisation and superovulation is commonplace in the Australian sheep industry, allowing farmers a high degree of control over timing of flock mating and insemination, increased reproductive efficiency and widespread dissemination of genetics. However, several studies have reported reduced fertility rates (Armstrong and Evans, 1983) and reduced numbers of spermatozoa in the reproductive tract of the ewe compared to naturally cycling animals, suggesting a failure in maintenance of cervical reservoirs as a possible cause (Quinlivan, 1963; Quinlivan and Robinson,

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http://dx.doi.org/10.1016/j.anireprosci.2016.07.008
0378-4320/

1969; Croker and Shelton, 1974; Hawk and Cooper, 1977; Salamon and Maxwell, 1995). Progesterone synchronisation has been shown to have a variable effect with reports of increased production (Croker and Shelton, 1974; Rexroad and Barb, 1977), decreased production (Smith and Allison, 1971) and no change to mucus production (Allison, 1971) in the ewe during oestrus. Altered mucus production could result in a less compatible tract for sperm migration and reduce the effectiveness of foreign body clearance from the tract by mucus. These results, while somewhat contradictory, do serve to indicate the marked effect progesterone synchronisation can have on fluids within the female tract relevant to successful reproduction. In humans, it is known that exogenous hormones impact mucus characteristics such as viscosity and protein content (Chappell et al., 2014) and that these changes negatively impact on sperm penetration in mucus (Lewis et al., 2010) as these are the principal mechanisms of action for contraceptives in women. The use of Protaglandin-$F_2\alpha$ for synchronisation of oestrus in cattle has also been linked with altered mucus protein content (Yildiz and Aydin, 2005), but similar recent studies on the cervical or cervicovaginal mucus of sheep have not been undertaken. Superovulation treatment has also been linked to reduced fertility (Armstrong and Evans, 1983) and lower sperm numbers in the female tract when compared to naturally cycling ewes (Evans and Armstrong, 1984). This effect may be caused by reduced sperm transport through the tract possibly due to larger volumes of cervical mucus present as a result of amplified levels of circulating oestrogen (Evans and Armstrong, 1983), but this remains unclear. Treatment with exogenous oestrogen, a model for superovulation treatments, has been shown to increase mucus wet weight (Adams and Tang, 1979), but its addition in conjunction with exogenous progestagens has resulted in decreased mucus production (Croker and Shelton, 1974). In addition to the effects of exogenous hormones, the natural changes in mucus production and composition that result due to circulating endogenous hormones have not been fully defined in the ewe.

While it is clear that controlled breeding practices may impact mucus production within the female tract and even fertility, the means by which these phenomena occur have yet to be fully established. The effect of exogenous hormones on mucus characteristics such as pH, spinnbarkeit, chemical composition and protein concentration, and any correlation these changes have on sperm transport both *in vivo* and *in vitro* remains unknown. As such, the aim of this study was to (1) investigate the properties (volume, colour, spinnbarkeit, protein concentration, pH) and chemical profile (sodium, calcium, potassium, magnesium, chloride) of ovine cervicovaginal mucus in naturally cycling, progesterone synchronised, superovulated and prostaglandin-$F_2\alpha$ synchronised ewes across the oestrous cycle, and (2) examine the influence of these hormonal treatments on mucus properties, chemical profile and *in vitro* penetration by spermatozoa.

2. Materials and methods

2.1. Experimental design

Procedures herein were approved by the University of Sydney Animal Ethics Committee (protocol number 2013/5999). In experiment 1, 20 mature Merino ewes (housed at the University of Sydney, Camden campus, Australia) were randomised into four treatment groups: naturally cycling ewes (NAT, n = 5), progesterone sponge synchronised ewes (P_4, n = 5), superovulated ewes (SOV, n = 5) and prostaglandin-$F_2\alpha$ synchronised ewes ($PGF_2\alpha$, n = 5). Mucus was collected from each ewe every 6 h for 4 days over the follicular phase (with oestrus occurring in last two days of the follicular phase for all ewes), then daily for 8 days over the luteal phase. Circulating progesterone levels and androgenised wethers were used to ascertain precise onset of oestrus and timing of follicular and luteal phases (data not shown). Initial assessments of volume, spinnbarkeit and colour were made on samples at time of collection whilst chemical profile and protein concentration were determined at a later date.

In experiment 2, 32 mature Merino ewes were randomised into three treatment groups: naturally cycling ewes (NAT, n = 10), progesterone sponge synchronised ewes (P_4, n = 11) and superovulated ewes (SOV, n = 11). Treatments were applied so oestrus occurred at approximately the same point for all treatment groups. Measurement of circulating progesterone and oestrogen as well as marking by androgenised wethers was used to ascertain precise onset of oestrus and timing of follicular and luteal phases (data not shown). Mucus was collected daily for 2 days over the follicular phase (oestrus) then for 2 days during the mid-luteal phase. The experiment was replicated twice in the breeding season (March and June 2014), using the same animals. pH measurements were taken *in vivo* prior to mucus sample collection and sperm migration tests carried out following sample collection.

2.2. Hormone administration

Hormone schedules were the same for both experiments, with treatments applied so that expected onset of oestrus occurred at the same time for all treatment groups (although this was also confirmed by circulating hormone levels and markings by androgenised wethers). To ensure all ewes in the NAT treatment group cycled at approximately the same time, they were treated in the cycle prior to the start of sample collection with intra vaginal progesterone sponges (30 mg Flugestone acetate; Vetoquinol, Lure cedex, France) for 14 days and injected with equine chorionic gonadotropin (400 IU; Pregnocol, Vetoquinol) at sponge removal (Evans and Maxwell, 1987). Mucus was collected from the second oestrous cycle following synchronisation, at which time mucus was determined not to be directly affected by hormonal administration. P_4 ewes were treated with intra vaginal progesterone sponges (30 mg Flugestone acetate; Vetoquinol) for 14 days and injected with equine chorionic gonadotropin (400 IU, Pregnocol; Vetoquinol) at sponge removal (Evans and Maxwell 1987). SOV ewes were treated