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Influence of seminal plasma on leucocyte migration and amount of COX-2 protein in the jenny endometrium after insemination with frozen–thawed semen

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ABSTRACT

After mating, seminal plasma has an immuno-modulatory effect on the endometrium in some mammals. In jennies, achieving conception via artificial insemination (AI) with frozen–thawed semen is generally much more difficult than in mares. The endometrial inflammatory response is hypothesized to be a contributing factor to the lesser fertility. Following a cross-over experimental design, the uterine inflammatory response of six jennies was evaluated at 6 h after AI with frozen–thawed semen (deposited in the uterine body) in the presence or absence of autologous seminal plasma (+SP or −SP). The endometrial cytology and histology of the animals were examined by uterine lavage, uterine swabbing and biopsy. The amount of cyclooxygenase-2 (COX-2) protein in endometrial cells was also evaluated. As a control (C), the same examinations were made before any AI procedure (i.e., when the jennies were in oestrus). Large numbers of polymorphonuclear neutrophils (PMN) were observed in the −SP and +SP cytology and biopsy samples; more than in the C samples. The −SP samples also had intense COX-2 labelling; less labelling was detected in the +SP and C samples (no significant difference between these latter two types). Thus, while the presence of SP does not change the post-AI number of PMNs with regard to that detected in its absence, it does reduce COX-2 protein. Further research into the complex mix of molecules in SP and its effects during AI might help increase the pregnancy rates achieved in jennies.

1. Introduction

The spermatozoa and seminal fluid deposited in mammalian females during natural mating and artificial insemination (AI) can induce an acute inflammatory response in the endometrium (Troedsson et al., 2001; Robertson, 2007). This reaction is species-specific and varies with the type of inseminate (cryo-preserved, cooled or fresh semen), semen quality, and deposition site (Kotilainen et al., 1994; Katila, 2005, 2012; Fiala et al., 2007). When the cervical and epithelial endometrial cells come into contact with semen, proinflammatory cytokines such as prostaglandins are produced, mainly through the action of the COX-2 enzyme in the arachidonic acid synthesis cascade. This is thought to lead to

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increase intrauterine fluid production as well as the chemoattraction of polymorphonuclear neutrophils (PMN) to the uterine lumen. Macrophages, monocytes and lymphocytes are also attracted to the sub-epithelial endometrium via the activation of complement (Troedsson et al., 2001, 2005; Robertson, 2007) in a process that peaks after 4–6 h. In mares, this leads to the cleansing of the excess semen by 24–48 h after insemination (Katila, 2001).

Seminal plasma (SP) is a mixture of molecules that functions as a transport medium for sperm. It also has species-specific inhibitory and stimulatory properties (Robertson, 2005). Although SP is not necessary for fertilization to occur, it facilitates interactions between the most competent male gametes and the uterus, thus maximizing the probability of conception and embryo viability (Töpfer-Petersen et al., 2005; Robertson, 2007). In gilts and mares with a previously inflamed uterine environment, even short SP contact times can promote pregnancy (Troedsson et al., 2001; Rozeboom et al., 2001; Fiala et al., 2002), perhaps by reducing this inflammation. The SP is also reported to have favourable effects on sperm motility and on the survival of sperm cells in the uterus by helping to avoid oxidative damage and impairing their binding to PMN and other phagocytes (Alghamdi et al., 2004; Rota et al., 2008; Aloé et al., 2012). It may also facilitate the elimination of non-viable spermatozoa via selective phagocytosis (Tomlinson et al., 1992; Troedsson et al., 2005). In addition, SP may promote the production and inhibition of different cytokines during the post-breeding inflammatory process, helping to elicit the transient immune tolerance essential for implantation and embryo development (O'Leary et al., 2004; Robertson, 2005; Jiwakanon et al., 2011; Sharkey et al., 2012). The removal of SP is, however, a necessary step when trying to cryo-preserve semen.

In mares, fertility outcomes with frozen–thawed semen have improved over the years (Sieme et al., 2004; Vidament, 2005), but fertility outcomes remain disappointing in jennies (Oliveira et al., 2006; Vidament et al., 2009). This is true even if the cryo-preserved donkey semen is of excellent quality and successful at inducing pregnancy in mares (Jepsen et al., 2010; Canisso et al., 2011). It was thought that the problem was an irritational effect of the glycerol cryoprotectant (Trimeche et al., 1998; Vidament et al., 2009). In a recent study of Rota et al. (2012), no differences were observed between semen frozen with glycerol or ethilene glycol as cryo-protectors, in post-thaw motility or fertility. Both cryo-protectors had a similar irritant effect on the jennies' endometrium post AI.

Vidament et al. (2009) using cryo-preserved donkey semen in INRA-82 and glycerol as cryo-protector obtained greater pregnancy rates in mares (36%) than in jennies (11%). Rota et al. (2012) inseminating Amiata jennies with Amiata jackass semen frozen in INRA-96 and glycerol, however, found there was only a 20% pregnancy rate and this rate was only 23.1% when cryo-preserved semen went through the post-thaw process and was re-extended with INRA-96.

Pregnancy results after AI with frozen semen in donkeys are very poor. When using frozen–thawed semen, healthy jennies could be more likely than healthy mares to develop endometritis. This might be a consequence of the specific physiology differences between the two because of the anatomical conformation of the reproductive tract (with its long and tortuous cervix), lesser uterine drainage, and presence of eosinophils in non-inflamed jenny endometrial tissue (Vendramini et al., 1998; Renner-Martin et al., 2009; Miró et al., 2011; Climent et al., 2012; Vilés et al., 2013).

Studies on the role of SP in mares have focused on sperm-induced endometritis and its effect on fertility (Troedsson et al., 2002; Alghamdi et al., 2004; Schuberth et al., 2008). In jennies, in vitro studies have shown a favourable effect of SP on sperm motility and reduced PMN-binding (Rota et al., 2008; Miró and Vilés, 2012). An in vivo study by means the post-thaw jack semen dilution in seminal plasma showed a trend towards the improvement of fertility (Rota et al., 2012). The mechanism though which SP could improve fertility is its action on the uterine immune response. The aim of the present work was to determine the effect of autologous SP on the endometrial inflammatory response in jennies after AI with frozen–thawed semen, following the changes that occur in uterine histology (via the examination of cytology and biopsy samples) and COX-2 immunohistochemistry.

2. Material and methods

2.1. Animals and experimental design

This research was approved by the Ethics Committee on Animal and Human Experimentation (CEEAH) of the Autonomous University of Barcelona. The experimental animals were six healthy jennies (5–14 years old) in oestrus, all with negative uterine cultures, and one male donkey (6 years old). All were known to be fertile. These animals were fed 2 kg of concentrate per day and had free access to hay, straw and water.

In this cross-over design experiment, endometrial cytology and biopsy samples were taken from each female on the following occasions: (1) during oestrus before any AI procedure (C group), (2) following two subsequent periods of oestrus at 6 h after AI with frozen–thawed sperm (collected from the male donkey) in the absence of SP (−SP group), and (3) following two additional periods of oestrus at 6 h after AI with frozen–thawed sperm (collected from the male donkey) in the presence of SP (+SP group). All semen and SP was deposited directly into the uterus.

2.2. Detection of oestrus and insemination protocol

Oestrus was determined by symptomology, transrectal palpation and use of ultrasounic techniques using an Esaote® MyLab™30 5 MHz linear transducer (VET, Genoa, Italy) to check for uterine fluid accumulation, oedema and follicle growth (detection of a follicle >40 mm in diameter and the absence of a corpus luteum). In the–SP and +SP treatments, the jennies were inseminated 24 h after iv-administering 2000 IU of human chorionic gonadotrophin (hCG; Chorulon®) (Intervet, Vienna, Austria). The tail was covered with a disposable examination glove and the vulva and perineal region rinsed in warm water and then cleaned with dry paper. Each jenny was