A role for seminal plasma in modulating pregnancy outcomes in domestic species

John J Bromfield

D H Barron Reproductive and Perinatal Biology Research Program, Department of Animal Sciences, University of Florida, Gainesville, Florida, USA

Correspondence should be addressed to J J Bromfield; Email: jbromfield@ufl.edu

Abstract

Seminal plasma is a complex fluid produced by the accessory glands of the male reproductive tract. Seminal plasma acts primarily as a transport medium for sperm on its arduous journey through the male and then female reproductive tract following ejaculation. This spermatozoan expedition will hopefully result in the meeting of and resultant fertilization of an oocyte, perpetuating the genetic lineage of both sexes. Whereas seminal plasma has historically been perceived as only a transport medium providing a nutrient-rich fluid environment for sperm during this exchange of genetic material, new insights into a complex communication pathway between males and females has been unraveled in the past 30 years. This new research suggests seminal plasma as a method to promote early pregnancy success by modulating cellular and molecular adaptions of the maternal environment required to facilitate healthy, successful pregnancy outcomes. Whereas much work on this exciting new communication process has focused on mice and translation to human reproduction, here we review the current evidence in domestic species where artificial insemination in the absence of seminal plasma is routine. Improving artificial insemination in domestic species to optimize offspring health and productivity could have far-reaching impacts on agriculturally relevant species such as cattle, sheep, pigs and horses.
Reproduction (2016) **152** R223–R232

Introduction

Transport of sperm through the reproductive tract of males and then females is key to the functional role of seminal plasma (SP). The molecular makeup of SP is complex and species specific. SP is comprised of energy substrates for sperm in the form of simple sugars, antioxidant agents to prevent potential sperm DNA damage, minerals and salts, and a large number of diverse protein moieties of unknown function. It is now postulated that a role for some of these SP proteins is direct communication with the female reproductive tract to alter uterine function and drive physiological changes to the maternal milieu that increase pregnancy success and promote healthy outcomes for potential offspring (summarized in Fig. 1). Whereas much of this groundbreaking work has been performed elegantly in rodents for reasons pertaining to model accessibility and translation to human health, the question remains whether the communication pathway between males and females via SP exist in livestock species. It has been demonstrated that such communication exists between males and females of the insect world where postmating SP exposure in crickets, mosquitos and flies can elicit behavioral and physiological changes in females pertaining to reproductive outcomes (reviewed in Avila *et al.* (2011)). However, in domestic species where artificial insemination (AI) in the absence (or significantly reduced presence) of SP is sometimes more prevalent than natural conception, we do not know the full extent of SP's actions on the female reproductive tract or pregnancy outcomes. It is also important to note the anatomical and physiological differences in reproduction among domestic species, such as ruminants where embryo implantation occurs much later and placentation is less invasive than the mouse or human where these processes share greater anatomical and temporal similarities. This review will focus on the newer paradigm of SP acting beyond a transport medium for sperm and as an agent that may optimize pregnancy outcomes in mammals.

What is the role of SP in modulating reproduction in the mouse and human?

Much of the cutting-edge investigations into the role SP plays in modulating the maternal environment have been performed in rodents and humans. These studies have broadened our basic understanding of biology and have been approached to impact human reproductive health. However, when interpreting
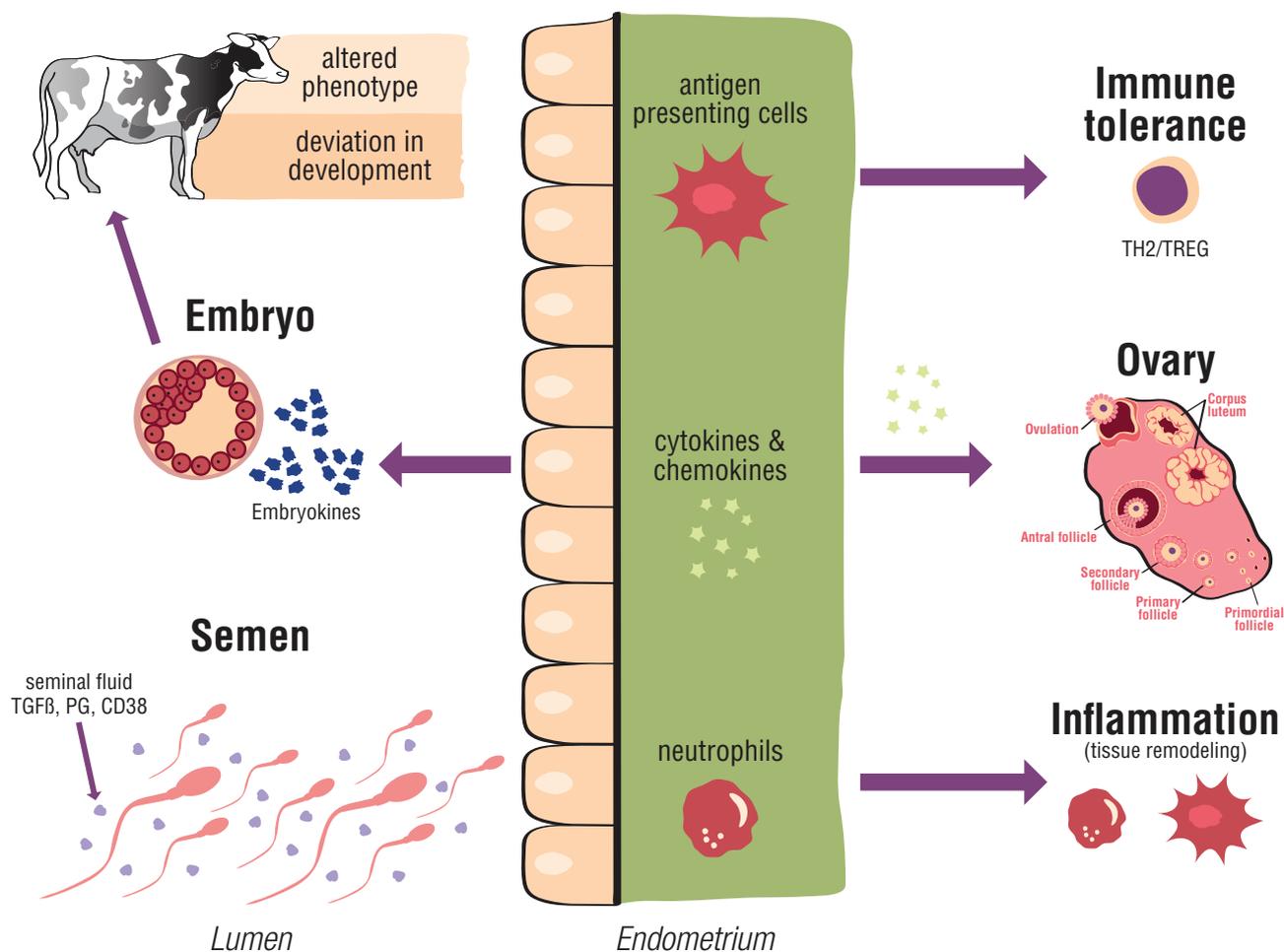
Figure 1 Schematic representation of the proposed impact of seminal plasma on the maternal environment. This figure represents the all-encompassing impact of seminal plasma on endometrial inflammation, production of embryokines and modulation of the maternal immune response to pregnancy and the ovary. We propose that these modulations in combination can effect embryo growth, pregnancy success and potentially the health and productivity of offspring. Inflammation; factors in seminal plasma induce expression of endometrial cytokines and chemokines in turn recruiting inflammatory cells and driving tissue remodeling. Embryo; the embryo is positively affected by the secretion of embryokines in the oviduct and uterus in response to seminal plasma exposure. Immune tolerance; insemination increases hypertrophy in the draining lymph nodes of the uterus, seminal plasma in turn increases proliferation of T-regulatory cells reactive toward paternal antigens expressed in semen, while a systemic skewing of the maternal immune system is directed to a beneficial Th2 profile. Ovary; seminal plasma exposure increases inflammation in the ovary following insemination and alters corpus luteum growth and progesterone production. All of these effects in combination culminate by improving pregnancy outcomes and optimizing the health and efficiency of offspring. Illustration by Tracy Bryant, UF/IFAS.

studies performed in the mouse, while undoubtedly valuable, caution must be applied when extrapolating to humans or domestic species. Research has demonstrated that murine models of inflammation are considerably divergent between mice and humans (Seok *et al*. 2013). Moreover, the reproductive physiology of ruminants is considerably different from mice with very different forms of placentation and temporal embryonic development and implantation. Many domestic species have noninvasive, or semi-invasive placental types where a priming of the maternal immune system with paternal antigen may not be as beneficial as in hemochorial placental mammals.

SP-induced inflammation and immune modulation in maternal tissues

Following insemination in both rodents and humans an acute inflammatory reaction is observed in the endometrium and cervix (Robertson *et al*. 1996, Sharkey *et al*. 2012b). It is believed that this acute inflammatory response which results in the recruitment of leukocytes including neutrophils, macrophages and dendritic cells to the endometrium and cervix is to facilitate clearance of debris and pathogens introduced during coitus, sperm selection and most intriguingly induction of immune tolerance to paternal antigens potentially expressed on the resultant semi-allogeneic conceptus. Post-insemination inflammation is not

evident in mice mated to males replete of SP following surgical removal of the seminal vesicle glands or in women following condom-protected intercourse, suggesting that the initiating factor(s) for the observed inflammation is contained within SP (Robertson *et al.* 1996, Sharkey *et al.* 2012*b*). Indeed, *in vitro* studies using both rodent and human semen have been able to demonstrate that the cytokine transforming growth factor beta (TGFβ) is the key modulating factor in SP to result in post-insemination maternal inflammation (Tremellen *et al.* 1998, Gutsche *et al.* 2003, Sharkey *et al.* 2012*a*). A major role of SP-derived TGFβ is to induce the expression of proinflammatory cytokines by the endometrium or cervix and initiate the post-insemination recruitment of inflammatory immune cells (reviewed in Robertson (2005)). New evidence suggests that the innate immune system is also involved with post-insemination inflammation. The importance of toll-like receptor (TLR) 4 signaling has been implicated in SP-induced proinflammatory cytokine expression. In a similar fashion to TLR4 involvement during cumulus oocyte-complex (COC) expansion where endogenous TLR4 ligands induce the expression of proinflammatory mediators important for COC expansion, such as interleukin (IL) 6 (Shimada *et al.* 2008), SP modulates TLR4-induced cytokines like colony-stimulating factor 2 (CSF2) (Shimada *et al.* 2008, Schjenken *et al.* 2015). The importance of SP exposure on embryonic development has also been demonstrated in mice and hamsters (Peitz & Olds-Clarke 1986, O *et al.* 1988). Interestingly, many of the proinflammatory cytokines induced by SP have a physiological role as embryokines in mouse. Cytokines including CSF2 (Sjoblom *et al.* 2005), leukemia inhibitory factor (LIF) (Mitchell *et al.* 2002), IL6 (Shen *et al.* 2009) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Riley *et al.* 2004) have all been demonstrated to be important in embryo quality in the mouse or human and are shown to be regulated by SP exposure in the oviduct of mice at a time when the developing embryo is present (Bromfield *et al.* 2014). Indeed, the absence of SP at insemination is detrimental to the developmental progression of embryos, resulting in fewer blastocysts with increased abnormalities and fewer blastomeres (Bromfield *et al.* 2014).

It is now postulated that the observed SP-derived immune changes during the post-insemination period may impact the maternal adaptive immune system in preparedness for the implanting embryo, facilitating recognition and tolerance of foreign paternal antigens expressed by the subsequent conceptus. Medwar proposed the immunological paradigm of viviparity in the 1950s and postulated that specific mechanisms must exist to allow the semi-allogenic conceptus to reside within the maternal tissues throughout gestation without the threat of immunological clearance (Medwar 1953). SP itself has the capacity to modulate the function of immune cells *in vitro* (Anderson & Tarter 1982, Saxena *et al.* 1985). However, it has become evident that SP has the capacity to also alter systemic immune function when delivered at coitus. Whereas research has demonstrated that lymphocyte populations become anergic to paternal antigens during pregnancy (Tafuri *et al.* 1995), an elegant study demonstrated that this hyporesponsiveness is achieved in a paternal-specific manner (Robertson *et al.* 1997). Robertson and colleagues demonstrated that tumor growth in female mice could be induced if mated to males with a matching MHC haplotype to that of the introduced tumor cell line. Tumor growth in virgin mice or those mated to a disparate MHC haplotype to the tumor, however, was inhibited (Robertson *et al.* 1997). This provides direct evidence that exposure to semen can induce systemic immune tolerance to potential paternal antigens. In 2004, a seminal paper demonstrated the importance of T-regulatory (Treg) cells in facilitating pregnancy success in mice. In the described mouse model of Treg cell depletion, no mice were observed to be pregnant in late gestation following allogeneic mating (Aluvihare *et al.* 2004). Whereas mating induces a transient hypertrophy of the uterine-draining lymph nodes resulting in expansion of a number of lymphocyte subpopulations, it has been demonstrated that SP or TGFβ induces antigen-specific Treg cell expansion in mice and humans (Clark *et al.* 2008, Guerin *et al.* 2011, Balandya *et al.* 2012, Shima *et al.* 2015). In addition, Kim and colleagues have identified SP-derived soluble CD38 as the active factor to induce the production of these important Treg cells and when depleted from the ejaculate of mice pregnancy failure ensued (Kim *et al.* 2015). Others have demonstrated the importance of SP-derived prostaglandins in expanding tolerogenic dendritic cells thought to be important in immune tolerance of pregnancy (Remes Lenicov *et al.* 2012). The critical role for SP-derived changes to the immune system stretch beyond what is presented here, but what does this imply for current assisted reproductive techniques and pathologies of pregnancy?

The impact of semen in pregnancy pathologies and optimizing pregnancy outcomes in humans and rodents

Current evidence is clear that SP is not required to achieve pregnancy in many species including human, rodent, domestic and wild species. *In vitro* fertilization (IVF) is the classic example of human conception in the absence of SP (or coitus). Several, large, well-controlled studies have demonstrated an increased risk of premature birth, very low birth weight, complications during delivery and serious birth defects in pregnancies derived from IVF (Perri *et al.* 2001, Hansen *et al.* 2002, Schieve *et al.* 2002, Wang *et al.* 2002, Ochsenkuhn *et al.* 2003. It is interesting to note a number of studies which have demonstrated a beneficial effect of SP exposure at or around the time of embryo transfer (ET)

to improve pregnancy rates in women (Marconi *et al.* 1989, Tremellen *et al.* 2000). In addition to these causal studies, epidemiological evidence suggest that semen exposure in a partner specific manner can help to prevent pregnancy pathologies such as preeclampsia where placentation is thought to be suboptimal (Klonoff-Cohen *et al.* 1989, Robillard *et al.* 1995). The use of SP containing pessaries has been reported to improve pregnancy outcomes in women suffering from recurrent spontaneous abortion (Coulam & Stern 1995). These studies and others pertaining to pregnancy pathologies suggest that the events induced by SP at insemination may 'program' the maternal environment and/ or immune system to accommodate the semi-allogeneic conceptus and optimize pregnancy outcomes. Whereas tumor studies described above indicate such treatments induces systemic changes, it is suggested that even ingestion of SP can improve reproductive outcomes in women by reducing the incidence of pre-eclampsia (Koelman *et al.* 2000). The authors surmise that oral exposure to paternal HLA antigens could induce maternal tolerance to paternal antigens expressed by the conceptus. Interpreting such studies is difficult, however, as the couples described likely have increased SP exposure due to increased frequency of intercourse and no mechanistic studies exist to make clear conclusions.

Our own studies in mice have demonstrated that a lack of SP exposure at conception alters the phenotype in subsequent offspring (Bromfield *et al.* 2014). These studies suggest that suboptimal embryonic development and placentation due to an absence of SP exposure results in offspring with increased fat deposition, increased blood pressure and altered metabolic profile. Moreover, when embryos developed for the first 48 h in a reproductive tract exposed to SP and then transferred to an oviduct replete of SP exposure, a similar phenotype was witnessed in female offspring, suggesting a window of susceptibility to programming due to an absence of SP exposure. These studies also described the developmental environment of the oviduct following SP exposure, suggesting that SP promoted the expression of embryokines, which may optimize developmental competence of the embryo. One of these key oviductal SP-induced embryokines is CSF2 (formerly GM-CSF). *In vitro* culture of human embryos has been reported to benefit from exogenous CSF2 resulting in increased development and live births (Sjoblom *et al.* 1999, Ziebe *et al.* 2013). A number of studies now suggest that the paternal contribution to pregnancy success is far greater than simple delivery of genetic material. Ng and colleagues demonstrated a nongenetic transmission of paternal phenotype in rats fed a high-fat diet (Ng *et al.* 2010). Male rats fed a high-fat diet produced female offspring with impaired glucose tolerance and β-cell dysfunction independent of fat deposition. An interesting paradigm of SP exposure has recently been described, which possibly pertains to the immune-modulating

ability of SP. Xenografting of human endometriosis lesions to nude mice has shown that exposure to SP promotes lesion growth (McGuane *et al.* 2015). These studies suggest that the immunomodulatory properties of SP may indeed be detrimental to certain pathological conditions such as endometriosis.

Collectively, the studies presented here (and others) are suggestive of a beneficial role of SP exposure to promote early pregnancy success in women and rodents by modulating the maternal immune response to the conceptus and promoting early embryonic development. Rodent studies elude to maternal SP exposure contributing to programing the health of subsequent offspring (Fig. 1).

Can the benefits of SP be extrapolated to domestic species?

The role of SP in altering the maternal environment is now well documented in mice and may extend to humans. However, agriculturally and economically important domestic species have yet to be investigated to the same extent.

Reproduction in many relevant domestic species including poultry, horses, sheep, cows and pigs is being managed very efficiently by commercial producers to optimize the genetic merit of offspring by using reproductive technologies such as AI. During the processing of semen to increase the number of applicable inseminations from a single ejaculate SP is either removed or considerably diluted, while the volume of semen used during AI is markedly reduced compared with that of natural insemination. All of these factors could potentially reduce the efficacy of any active moieties present in semen, and consequently reduce any positive effects on reproductive outcomes. For example, an average Holstein bull produces 3.75 mL of semen per ejaculate containing a total of 5000×10^6 sperm. If a single AI dose requires 5×10^6 sperm in 0.25 mL, the initial ejaculate would provide 1000 inseminations resulting in a dilution of SP in semen extender by a factor of 332 (numbers adapted from Den Daas *et al.* (1998) and Hering *et al.* (2014)).

SP signaling and bovine reproduction

Much of the modern bovine dairy industry relies heavily on AI and the use of high genetic merit sires, while the beef industry still uses a high proportion of live bull cover (natural insemination). To date, only a single study has evaluated the ability of semen to induce inflammation in the endometrium of cattle (Mahajan & Menge 1967), while the effect of SP is yet to be determined. However, studies have demonstrated that the presence of SP does alter sperm parameters including viability and motility (Baas *et al.* 1983). SP has also been demonstrated to

increase neutrophil binding to sperm in the bovine (Alghamdi *et al.* 2009). Conversely, the addition of SP at very low concentrations (0.2% v/v) to *in vitro* assays using peripheral blood leukocytes was shown to reduce net migration and reactive oxygen production while increasing cell membrane damage (Aloe *et al.* 2012), further suggesting a role of SP in modulating local inflammatory response.

In induced ovulatory species such as camelids (including the llama), SP contains specific ovulation-inducing factors like β-nerve growth factor (β-NGF) (Ratto *et al.* 2012). Whereas the bovine is not an inducible ovulatory, it is interesting to note that bovine SP also contains β-NGF (Tribulo *et al.* 2015). Bovine SP has the capacity to induce ovulation in the llama in addition to acting as a luteotrophic agent by promoting progesterone secretion when administered as an intramuscular injection in cattle (Tribulo *et al.* 2015). Proteomics has verified the presence of β-NGF in bovine SP in addition to 98 other identified proteins including BSP-PDC109 (BSP-A1/A2), BSP-A3 and BSP-30 kDa, which comprise approximately half of all the proteins in SP (Manjunath & Sairam 1987, Desnoyers *et al.* 1994, Kelly *et al.* 2006). Whereas the chemokine monocyte chemoattractant protein 1 (MCP1, known as CCL2) was identified in bovine SP, these papers were unable to identify other immune-modulating factors including TGFβ, which is known to exist in SP of other species. Using ELISA we have been able to quantify the presence of total TGFβ isoforms 1 and 2 in SP of 33 bulls (7.11 ± 1.55 and 6.07 ± 1.16 ng/mL respectively).

The ability of SP to increase pregnancy outcomes in cow is of great interest. In 1968, the importance of the seminal vesicle glands in the bull were tested by surgical removal (resulting in increase in sperm concentration due to a halving in ejaculate volume) and subsequent breeding of bulls to heifers (Faulkner *et al.* 1968). Minimal impact on sperm motility and viability was observed in bulls following seminal vesicle removal. Surprisingly, when seminal vesicle-deficient bulls were bred to heifers a 60% conception rate was still observed (Table 1). These findings may be due to the fact that the seminal vesicles only contribute half of the total volume of SP to the ejaculate, while the Cowper's and prostate glands may be significantly more important in the bovine than in rodents where 90% of SP is seminal vesicle derived. Importantly, these studies did not use intact control bulls when assessing fertility, which does not allow the interpretation that the seminal vesicles are not essential to fertility in the bull as concluded by the authors.

Odhiambo and colleagues (2009) demonstrated that exposure to SP around the time of AI numerically increased pregnancy rates at day 35–40 by 13.9% in dairy cows (this was not statistically significant). An interesting observation from the same study reported that when reproductive performance was optimal,

SP (or the known active moiety in the rodent, TGFβ) had no effect on pregnancy rates. However when reproductive performance was suboptimal (which readily occurs) administration of TGFβ improved conception rates by 28.1%.

SP signaling and porcine reproduction

Natural insemination in pig occurs intracervically resulting in up to 500 mL of ejaculate being deposited in the uterus of the sow. This process is considerably different to all other species discussed here where insemination normally results in semen being deposited in the vagina near the external os of the cervix. The fact that semen is deposited into the pig's uterus lends a unique opportunity for SP to directly interact with the endometrium. Indeed, prototypic inflammation in the endometrium is observed 34 h after the infusion of SP in pig denoted by increased CD45+, MHCII+ and HB142+ (macrophages) cells (O'Leary *et al.* 2004). These studies show that SP-induced inflammation is greatest 5 days after treatment and in some cases still evident up to 9 days following SP infusion. Conversely, it has been shown that sperm alone can increase luminal leukocytes and when sperm is administered in conjunction with SP a reduction in luminal leukocytes is observed within 36 h of treatment (Rozeboom *et al.* 1999).

Sperm survival and motility are improved when stored in SP compared with samples washed and stored in commercial extenders (Chutia *et al.* 2014). A subsequent bonus of improved sperm quality may be the observed increase in early embryos when AI is followed by exposure to SP. An increase in the average number of total and viable embryos collected from pigs up to day nine following AI was observed when SP is infused at insemination (O'Leary *et al.* 2004). In this regard, it is important to also consider the observed effects of SP on the ovary, which may contribute to increased embryonic viability in pig (or cow as discussed above). Intrauterine SP infusion significantly increases plasma progesterone, corpus luteum weight and infiltration of leukocytes into ovarian tissue, all of which may change the developmental competency of ovulated oocytes and early embryo survival (O'Leary *et al.* 2006).

Notwithstanding the documented actions of SP on the maternal tissues and early pregnancy discussed here, it is interesting to note that of the species studied in regard to SPs action, pig has the least invasive placenta and likely requires the least immune modulation of the species discussed.

SP signaling and equine reproduction

Similar to the species discussed already, commercial horse reproduction (with the exception of thoroughbreds) relies heavily on AI using washed, SP-free sperm. A number of studies have demonstrated the potential of SP

Table 1 Effect of seminal vesicle removal on bull fertility.

Bull ID		Volume (mL)	Concentration score	Heifers bred	Conception rate (%)
2172	Before	2.7	3.2		
	After	1.7*	4.1*	7	71
2240	Before	3.2	2.9		
	After	1.3*	3.9*	8	75
3018	Before	6.2	4.1		
	After	3.5	4.5*	16	75
3024	Before	4.9	4.0		
	After	3.0*	4.9*	–	–
3053	Before	6.3	3.9		
	After	2.5*	3.8	5	40
Average	Before	5.1	3.8		
	After	2.7*	4.8*	36	69

*Significant from 'before' value.

Adapted from Faulkner *et al.* (1968).

to increase horse and donkey sperm fertility, especially following cryopreservation (Neuhauser *et al.* 2015). Recently, lipidomics has identified novel lipids in horse SP including phosphatidylethanolamines (PtdEs) that are absent in sperm (Wood *et al.* 2016). Whereas the authors conclude that PtdEs may protect sperm or contribute to capacitation, it is important to understand that these lipids are a common component of bacterial and mitochondrial membranes and secretory vesicles (Dowhan 1997, Marsh 2007, van Meer *et al.* 2008), suggesting either potential contamination of semen by bacteria or the potential for vesicle signaling in SP.

The horse has a unique problem of insemination-induced endometritis, which has increased with the use of SP-free AI. Interestingly, when endometrial inflammation was measured in response to SP in horse, similar increases in leukocyte infiltrate and immune mediator expression were observed when compared with infusion of sterile PBS (Palm *et al.* 2008), suggesting mechanical stimulation of inflammation in horse, possibly due to the application of excess fluid volume in the uterus. It has been suggested that SP regulates inflammation in the equine to mediate inflammation resolution. When SP is infused at AI, the duration of post-insemination inflammation is shortened compared with horses undergoing standard SP-free AI (Troedsson *et al.* 2001). Similarly, when SP is added to semen for AI and used in animals with induced inflammation using heat-killed sperm, pregnancy rates of 77% were achieved, while only 5% of mares became pregnant in the absence of SP (Alghamdi *et al.* 2004). Whereas the properties of SP to protect sperm during inflammation in the equine reproductive tract are novel when compared with other species, the ability for SP to improve conception rates in horse mirrors that of other species. When sperm are resuspended into SP after cryopreservation, conception rates in the donkey were increased 166% compared with commercial extender (Rota *et al.* 2012). However, there exist a number of studies that suggest a negative effect of SP on the cryosurvival of equine sperm at higher concentrations (above 5%) (Brinsko *et al.* 2000, Aurich 2008). The conflicting data between improved

and decreased sperm parameters may be in part due to variation of SP composition between stallions and/or preservation protocols (Aurich *et al.* 1996). Anecdotally, it would appear that semen preparation for freezing and storage uses the near-complete removal of SP by centrifugation as the industry standard. A greater depth of exploration is required in horse reproductive capabilities to improve reproductive efficiency and the contribution that SP may play in reproductive outcomes.

SP signaling and ovine reproduction

Currently, natural conception using live ram cover is the standard practice in the sheep industry. However, following the advent of genetic selection AI is becoming increasingly popular among sheep producers. In addition, sheep are seasonal breeders (breeding in the autumn months), which places additional constraints on the producers to breed their animals quickly and efficiently over a short time, something which could potentially be improved by AI. However, AI in sheep is currently technically challenging due to the anatomical structure of the cervix that is restrictive to the passage of an intrauterine inseminating device. Currently, AI in sheep requires the use of highly skilled laparoscopic insemination of semen directly into the uterus.

Following live cover of ewes with fertile rams, acute changes in the cellular distribution and abundance of leukocytes in the cervix and uterus is observed compared with non-mated ewes at estrus (Mattner 1969, Scott *et al.* 2006). Neutrophil density is increased in the cervix and uterus 6 h after insemination, while macrophage number in the uterus peaks 24 h following insemination, mirroring cellular distribution seen in rodents and humans. Interestingly, inflammatory response of the ewe endometrium to semen or SP is dependent on the estrous cycle stage (Scott *et al.* 2006). Whole semen, SP and sperm induces an inflammatory response in the endometrium of ewes at estrus, increasing neutrophils, CSF2 and IL-8 secretion, while no inflammation is observed in ewes exposed to SP at diestus. It is interesting to note, however, that when antibiotics

where administered in combination with semen or SP, inflammation was reduced, suggesting a confounding influence of contaminating bacteria. The fact that ewes respond differently to the ejaculate depending on estrous cycle stage corresponds to the changes in the proteome of ram SP, and may have implications in the seasonal breeding of sheep (Smith *et al.* 1997, Perez-Pe *et al.* 2001, Cardozo *et al.* 2006). Additional proteomic analysis of ram SP has revealed striking similarities with the most abundant proteins of bovine SP, namely the presence of BSP A1/A2-like protein accounting for 34% of protein in ram SP (Jobim *et al.* 2005).

With the increased advancements of sperm freezing and application of AI in sheep, further research is required to determine if SP-induced endometrial inflammation can benefit pregnancy outcomes in domestic sheep breeds.

Current reproductive strategies in domestic species and the potential role for SP

As noted above, the application of reproductive technologies in agricultural production of domestic species is implemented to ensure high productivity, maximize efficiency and increase genetic merit of offspring. The use of exogenous hormones for estrous cycle regulation and ovarian stimulation, semen collection, AI, gamete freezing, IVF, embryo transfer (ET) and sperm sex sorting are now common place in many species; none more so than in the dairy cow industry where all of these technologies are used routinely and many of which were first developed and later implemented in human-assisted reproduction. The successful implementation of IVF/ET and AI demonstrate that SP is not required to achieve pregnancy. In fact, it has been demonstrated many times that IVF/ET pregnancy rates exceed those of timed AI in the dairy industry (Vasconcelos *et al.* 2006, Demetrio *et al.* 2007, Vasconcelos *et al.* 2011, Pellegrino *et al.* 2016). However, it is known that early pregnancy loss, including fertilization failure and embryonic death are leading causes of pregnancy failure in the dairy industry (reviewed in Wiltbank *et al.* (2016)). The technology of ET itself bypasses very early pregnancy loss in cow by ensuring fertilization and the first 5–7 days of embryonic development have been completed before introducing the embryo into the female reproductive tract, giving a plausible reason why ET generates higher pregnancy rates than traditional or timed AI. Interestingly, gene expression studies of the oviduct in which the early embryo develops show little change due to the presence of an embryo following transfer in the absence of SP (Maillo *et al.* 2015). In fact, further studies reveal that gene expression in the oviduct isthmus following AI is unchanged by the presence of an embryo when compared with oviductal cells at estrus (Maillo *et al.* 2016). The authors conclude from these studies that gene expression in the oviduct, and

subsequent production of embryokines, is dependent only on estrous cycle stage. However, no studies in cow have shown responsiveness of oviductal cells to SP or its active components; conversely in the mouse oviductal gene expression of embryokines is significantly upregulated following coital exposure to SP (Bromfield *et al.* 2014). The potential of SP to modulate the early embryonic developmental environment of the oviduct remains to be suitably investigated.

The application of sex-sorting sperm by flow cytometry in the dairy industry is common place and used to increase the potential of female offspring to maximize milk production of the herd (giving rise to milking cows). The negative impact of SP on sex sorting is interesting and important to such a valuable technology. Burroughs and colleagues have shown that the inclusion of SP from 5 to 20% has negative consequences on the sort rate of X-sperm in addition to reducing post-thaw motility of sperm compared with sperm washed free of SP (Burroughs *et al.* 2013). In this regard, it may be a best practice to remove SP before sex sorting and potentially supplement SP at the time of AI.

Recapitulating the normal developmental environment of the embryo, implantation, placentation and fetal development is key to optimizing currently used reproductive technologies in domestic species. Whereas SP is not required for pregnancy, small improvements to reproductive performance in domestic species could have large economic (and potentially environmental) impacts on production efficiency. More research is required to determine if the application or supplementation of SP, or its active components, could improve reproductive efficiency for domestic species production and potentially even alter postnatal development of offspring as described in the rodent (Bromfield *et al.* 2014).

Conclusions

The mechanistic role of SP in rodent reproduction continues to be defined at a cellular and molecular level and suggests that SP can improve reproductive outcomes and potentially even the health and development of offspring. However, it remains to be seen whether a similar role for SP exists in domestic species where it is becoming common practice to remove SP from the reproductive equation. Whereas it is clear that SP is not required for successful pregnancy, incremental improvements to reproductive outputs in domestic species can bear financial and environmental impacts. If the influence of SP on programming health in offspring is recapitulated in domestic species, increased growth, production, sustainability and efficiency in agriculture could potentiate astonishing global impacts. It is our aim to continue to expand on the knowledge of how SP can help us improve the reproductive efficiency of our

domestic species for the benefit of human health and well-being.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

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