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Review: The potential of seminal fluid mediated paternal–maternal communication to optimise pregnancy success

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Artificial insemination has been a landmark procedure in improving animal agriculture over the past 150 years. The utility of artificial insemination has facilitated a rapid improvement in animal genetics across agricultural species, leading to improvements of growth, health and productivity in poultry, swine, equine and cattle species. The utility of artificial insemination, as with all assisted reproductive technologies side-steps thousands of years of evolution that has led to the development of physiological systems to ensure the transmission of genetics from generation to generation. The perceived manipulation of these physiological systems as a consequence of assisted reproduction are points of interest in which research could potentially improve the success of these technologies. Indeed, seminal fluid is either removed or substantially diluted when semen is prepared for artificial insemination in domestic species. Although seminal fluid is not a requirement for pregnancy, could the removal of seminal fluid from the ejaculate have negative consequences on reproductive outcomes that could be improved to further the economic benefit of artificial insemination? One such potential influence of seminal fluid on reproduction stems from the question; how does the allogeneic foetus survive gestation in the face of the maternal immune system? Observation of the maternal immune system during pregnancy has noted maternal immune tolerance to paternal-specific antigens; a mechanism by which the maternal immune system tolerates specific paternal antigens expressed on the foetus. In species like human or rodent, implantation occurs days after fertilisation and as such the mechanisms to establish antigen-specific tolerance must be initiated very early during pregnancy. We and others propose that these mechanisms are initiated at the time of insemination when paternal antigens are first introduced to the maternal immune system. It is unclear whether such mechanisms would also be involved in domestic species, such as cattle, where implantation occurs weeks later in gestation. A new paradigm detailing the importance of paternal–maternal communication at the time of insemination is becoming evident as it relates to maternal tolerance to foetal antigen and ultimately pregnancy success.

Keywords: artificial insemination, immune tolerance, pregnancy success, seminal fluid, semen

Implications

The utility of artificial insemination in animal agriculture has dramatically improved production due to selective breeding. As with many reproductive technologies, artificial insemination bypasses the requirement for seminal fluid as a transport medium for sperm. These technologies demonstrate that seminal fluid is not required for pregnancy; however, it is curious that seminal fluid has a substantial effect on the female reproductive tract at insemination. This article discusses the role of seminal fluid in modulating the maternal environment during early pregnancy. Recapitulation of these events during artificial insemination may further improve pregnancy outcomes and offspring performance of domestic species.

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Introduction

Transmission of sperm through the male reproductive tract, ascension up the female reproductive tract to the awaiting oocyte is the primary role for seminal fluid. However, studies dating back to the 1920s have suggested a secondary role of seminal fluid in the reproductive process (Long and Evans, 1922). Pioneers in reproductive biology, Ryuzo Yanagimachi and MC Chang investigated the importance of seminal fluid in the golden hamster stating, 'One also wonders whether there are other functions of leucocytes in the uterus [resulting from seminal fluid exposure], besides elimination of bacteria and spermatozoa' (Yanagimachi and Chang, 1963). In parallel to this interesting postulation, the immunological paradox of pregnancy has been a source of debate for decades. How does the allogeneic foetus survive the immunologically hostile maternal environment during pregnancy?

A number of hypothesis were postulated by the Nobel laurate Sir Peter Medawar in the 1950s designed to explain how a foetus could survive in an immunologically disparate host (Medawar, 1953). One of Medawar's hypotheses suggested 'immunological indolence or inertness of the mother', however this would leave the mother vulnerable to infection or autoimmunity. Although Medawar's hypothesis of separation of foetus and mother may be a predominate reason for foetal survival, it has become evident that there is not immunological indolence of the mother and there is in fact maternal modulation (or tolerance) of the immune system which aids in the survival of the conceptus. The question remains, how is maternal immune tolerance to the conceptus (or conceptus antigens) established so as to be active at the time of embryo implantation? One potential mechanism of establishing maternal tolerance to the conceptus may involve the assistance of the father at the time of conception. Here we discuss the potential of seminal fluid mediated paternal–maternal communication to optimize pregnancy success.

A brief history of artificial insemination

Approximately 100 years after the invention of the microscope, Antonie van Leeuwenhoek was the first to describe the observation of living spermatozoa in 1677 using his own microscope design. Leeuwenhoek describes observing a fresh human ejaculate 'before six beats of the pulse had intervened' containing what he describes as 'a great number of living *animalcules*'; referring to sperm (Letter to William Brouncker of the Royal Society, November 1677). Leeuwenhoek's collection of fresh semen was produced 'without sinfully defiling myself, [*sic*] what remains after conjugal coitus'. It would take another 100 years before the first successful attempt at artificial insemination was achieved by the Italian physiologist Lazzaro Spallanzani in 1784. Although Spallanzani considered sperm cells to be parasites contained within semen, he successfully executed artificial insemination in a bitch in heat that subsequently gave birth to three puppies. The success of this procedure was likely associated with the protracted oestrus observed in dogs as little was understand about ovulation and the oestrous cycle at the time. It was not until the end of the 19th century that practical approaches for artificial insemination were developed in Russia by Ilya Ivanovich Ivanoff and continued by Milovanov who refined artificial insemination practices and developed the artificial vagina for semen collection. It was reported that the growth of artificial insemination in the Russian cattle industry grew from 19 970 insemination in 1930 to over 1.5 million insemination in 1939 (Pincus, 1938). The development of sperm cryopreservation techniques by Christopher Polge further increased the capacity to transport semen long distance and dramatically improved domestic animal genetics (Polge *et al*., 1949; Polge, 1952). Currently, it is estimated that 72% of all dairy cows in the USA are bred by artificial insemination (United States Department of Agriculture – National Institute of Food and Agriculture). A consistency throughout these later advances in artificial insemination was the utilization of semen extenders to increase viability of semen, and increase the number of potential inseminations from a single ejaculate. As a consequences of semen extension, seminal fluid has been diluted in semen used for artificial insemination since the 19th century. Could replacement or enrichment of seminal fluid components enhance the success of artificial insemination in domestic species?

The role of insemination beyond sperm delivery

Of course the specific objective of insemination is the delivery of male gametes into the female reproductive tract to facilitate fertilisation of female gametes. However it is interesting to consider the cellular and biochemical content of semen as a whole. Indeed seminal fluid (the acellular fraction of semen) derived from the male accessory glands is rich in simple sugars, buffers, antioxidants, hormones and proteins of unknown function presumed to be present simply to facilitate sperm survival and transport through the female reproductive tract. Research has now begun to highlight the importance of some of these seminal fluid proteins as potential mediators of paternal–maternal communication delivered at the time of insemination. Consider briefly lower-order organisms such as crickets, mosquitoes and flies where physiological and behavioural changes associated with reproductive outcomes have been demonstrated in females after exposure to seminal fluid (Avila *et al*., 2011). Observations dating back to the 1960s have demonstrated the acute potential of semen to modulate the cellular environment of the female reproductive tract of mice, human, cattle, swine, horse and sheep. Following insemination in rodents an acute influx of leucocytes is observed for the proceeding 72 h (Yanagimachi and Chang, 1963; Mattner, 1968; De *et al*., 1991; McMaster *et al*., 1992; Robertson *et al*., 1996). This influx of leucocytes is paralleled by an increase in the expression of inflammatory mediators by the endometrium, including C–C motif ligand *(CCL)2, CCL3, CCL5* and colony-stimulating factor *(CSF)2*. (Robertson and Seamark, 1992; Robertson *et al*., 1997). Further studies in the rodent have been able to demonstrate that seminal fluid is the active component of the ejaculate to elicit these changes observed in the maternal tissues, whereas specifically seminal vesicle derived transforming growth factor beta (TGFβ) has been shown to be the active compound in seminal fluid responsible for the increased expression of endometrial inflammatory mediators and ultimately post-insemination inflammation (Robertson *et al*., 1996; Tremellen *et al*., 1998). Similarly in humans a post coital inflammatory reaction has been observed in the cervix following exposure to semen where no inflammation is observed following condom protected intercourse (Sharkey *et al*., 2012b). In parallel with the mouse, seminal fluid derived TGFβ is responsible for inducing increased expression of the inflammatory mediators *interleukin-6* and *CSF-2* in human cervical epithelial cells (Sharkey *et al*., 2012a). In cattle a similar inflammatory response to semen has been demonstrated (Mahajan and

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Table 1 *Pregnancy rates in cattle treated with seminal fluid at the time of artificial insemination*

	Pregnancy rate (%)		
	Control	Seminal fluid	TGF-β1
Beef	55.7	62.4	51.0
Dairy	33.2	37.8	36.3

Adapted from Odhiambo *et al.* (2009). Seminal fluid was collected from a single bull for beef studies and six Holstein bulls and combined for dairy studies. Pregnancy was diagnosed at 35 to 40 days post insemination.

Menge, 1967; Mattner, 1968); however it is important to note that the seminal vesicles of the bull contribute roughly half the volume of ejaculated semen. In fact when the seminal vesicle glands are surgically removed from bulls, natural fertility remains high at approximately 65% conception (Faulkner *et al.*, 1968). A more recent study aimed to evaluate the benefit of seminal fluid (or TGFβ) supplementation at the time of artificial insemination on pregnancy rates in cattle. Although statistically underpowered, the study suggests that artificial insemination supplemented with seminal fluid or TGFβ can improve pregnancy rates, particularly in poor performing herds (Table 1) (Odhiambo *et al.*, 2009). Seminal fluid infusion into the porcine uterus induces significant cellular inflammation 36 h after infusion that was still evident 8 days later, considerably different than the acute inflammation observed in other species (O'Leary *et al.*, 2004). The same research team described a significant increase in the number of total and viable embryos collected from sows following seminal fluid supplementation (O'Leary *et al.*, 2004). The horse and sheep also show increased acute inflammation of the endometrium after the application of seminal fluid or semen (Mattner, 1969; Scott *et al.*, 2006; Palm *et al.*, 2008).

The question remains, what is the relevance of this post-insemination, seminal fluid induced inflammatory reaction? A proposed role would be the prophylactic clean-up of sexually transmitted pathogens, or non-viable sperm cells. It is interesting to note that many inflammatory mediators upregulated in the endometrium or oviduct by seminal fluid are also embryotrophic in nature, specifically CSF-2, leukemia inhibitory factor and IL-6 (Lavranos *et al.*, 1995; de Moraes and Hansen, 1997; Gutsche *et al.*, 2003; Bromfield *et al.*, 2014; Hansen *et al.*, 2014). The temporal expression of these so-called embryokines may be in part regulated by seminal fluid exposure to orchestrate embryo development coordinate with insemination. An even more intriguing relevance of this inflammatory event relates to the induction of maternal immune modulation required for pregnancy success in viviparous species.

Immune modulating capacity of semen

As mentioned previously, Medawar hypothesized a requirement for suppression or modulation of maternal immunity to facilitate the survival of the allogeneic conceptus. There is potential for this immune modulation to be orchestrated by ovarian or placental hormones, or the conceptus itself. However, neither of these scenarios allow for the potential maternal immune adaptions to be specific toward the paternal antigens expressed by the conceptus and/or be in place at the time of embryo implantation (at least in rodents and humans). An intriguing possibility remains that insemination could act as a first 'priming' event of the maternal immune system to paternal antigen potentially expressed by the conceptus. The underlying mechanisms of immune tolerance required for pregnancy are proposed to be clonal deletion, anergy and clonal unresponsiveness of alloreactive T lymphocytes. These mechanisms would prevent the cytotoxic actions of specific alloreactive lymphocytes within the peripheral circulation during pregnancy (Piazzon *et al.*, 1985). In many mucosal tissues, the prevalence of a Th2 skewed immune response is associated with a state of functional tolerance and this is likely to also be the case in pregnancy (Chaouat *et al.*, 1997).

Seminal fluid has been demonstrated to potentiate changes in immune function of T cells, B cells, NK cells and macrophages in the mouse, bovine and human (Anderson and Tarter, 1982; Fahmi *et al.*, 1985; Saxena *et al.*, 1985). It is evident that exposure to semen, and indeed seminal fluid, drives an acute hypertrophy in the spleen and lymph nodes draining the uterus in the mouse (Maroni and de Sousa, 1973; Beer and Billingham, 1974; Johansson *et al.*, 2004). The quality of any immune response, including the phenotypes of effector T cells and state of the cytokine profile is determined at the time of primary antigen exposure and is dependent on the activation state of antigen presenting cells (Constant and Bottomly, 1997; Kapsenberg *et al.*, 1999). It has been suggested that the site of lymphocyte activation is of major significance to the functionality downstream effector cells (Harper *et al.*, 1996). However, the majority of data supports the idea that antigen presenting cells play a fundamental role in the programming of lymphocytes and that local cytokine expression is the key factor in regulating antigen presenting cell behaviour (Harper *et al.*, 1996; Constant and Bottomly, 1997; Kapsenberg *et al.*, 1999; Egan *et al.*, 2000). Could it be that activation of specific cells in the draining lymph nodes of the reproductive tract may help to prime the maternal immune system with paternal antigen?

The frequency of antigen exposure is thought to work in conjunction with dose in the generation of mucosal tolerance. One-off high dose exposures or small repeated doses of antigen has been shown to be most beneficial in the development of tolerance (Garside and Mowat, 2001); a paradigm that fits with the exposure of the uterine epithelium to seminal antigens during intercourse. Although 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 *et al.* demonstrated that tumour growth in female mice could be induced if mated to males with a matching major histocompatibility complex (MHC) haplotype to that of the introduced tumour cell line. The utilization of

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uterine ligation before mating in this model also excluded the possibility that the conceptus was responsible for the systemic changes to immune tolerance observed. Tumour growth in virgin mice or those mated to a disparate MHC haplotype to the tumour was inhibited (Robertson *et al.*, 1997). This provides direct evidence that exposure to semen can induce systemic immune tolerance to potential paternal antigens.

Seminal fluid is rich in immune-deviating cytokines such as TGFβ and PGE2 which can lead to the alteration of the cytokine profile of a T cell population in the Th2 direction thought to be beneficial to pregnancy success (Tafuri *et al.*, 1995). In a landmark experiment depletion of forkhead box P3 (FOXP3) positive T regulatory cells lead to a complete failure in pregnancy (Aluvihare *et al.*, 2004). TGFβ has been demonstrated to activate FOXP3 positive T regulatory cells *in vitro* (Fantini *et al.*, 2005). Interestingly our own studies have demonstrated that seminal fluid exposure plays a significant role in the generation and recruitment of FOXP3 cells into female reproductive tissues (Robertson *et al.*, 2009; Guerin *et al.*, 2011).

The impact of semen on pregnancy outcomes: a role in assisted reproduction and pathology

It is clear that seminal fluid is not required for pregnancy. With the advent of artificial insemination, *in vitro* fertilisation and intracytoplasmic sperm injection, the sperm cell is the only requirement of the ejaculate to achieve a viable pregnancy. With that being said and the preceding discussion, it has come to light that seminal fluid may play a role in improving pregnancy outcomes and potentially staving off particular pathologies of pregnancy. Recently, we have been able to demonstrate in mice that an absence of seminal fluid exposure during mating results in reduced embryo development, poor placentation and metabolic perturbations in offspring (Bromfield *et al.*, 2014). We conclude that an absence of seminal fluid resulted in foetal programming due to reduced secretion of seminal fluid induced embryokines in the oviduct, altered tissue remodelling resulting in poor placentation, and perturbed maternal tolerance toward the allogeneic conceptus, all culminating in altered offspring phenotype. The immunomodulatory properties of seminal fluid have been demonstrated to be detrimental in an experimental model of endometriosis. It was demonstrated that human endometriosis lesion growth was increased in the nude mouse after exposure to seminal fluid (McGuane *et al.*, 2015). Epidemiological evidence in humans has suggested a potential role for semen exposure in modulating pathologies of pregnancy with suspected immunological aetiologies. Data suggests that semen exposure in a partner specific manner can be beneficial in reducing preeclampsia, a pathology with suspected immune aetiology. Reducing semen exposure with the use of barrier contraception or by short term cohabitation increased the risk of women developing preeclampsia (Klonoff-Cohen *et al.*, 1989; Robillard *et al.*, 1995). Even more compelling, a randomized controlled trial in 87 women with recurrent spontaneous abortion suggests that pregnancy rates can be significantly improved by the administration of vaginal capsules containing seminal fluid (Coulam and Stern, 1995). The addition of seminal fluid during artificial insemination in cattle was shown to increase pregnancy rates by nearly 5%, albeit not significantly (Table 1) (Odhiambo *et al.*, 2009). It is important to consider that an increase in pregnancy rate of 5% in an agricultural context could have enormous economic and production impacts to producers.

As the utility of IVF increases in human medicine and agricultural practice it is easy to overlook the understudied effects of *in vitro* culture on offspring health. Indeed, IVF and embryo transfer technologies exist in the absence of semen or seminal fluid. In both humans and cattle the impacts of *in vitro* embryo culture appear to carry negative consequences including increased risk of premature birth, very low birth weight, complications during delivery, serious birth defects in humans and overgrowth in cattle resulting in major organ defects (Young *et al.*, 1998; Perri *et al.*, 2001; Hansen *et al.*, 2002; Schieve *et al.*, 2002; Wang *et al.*, 2002; Ochsenkuhn *et al.*, 2003). Collectively these perturbations of *in vitro* culture are a consequence of our failure to recapitulate the maternal developmental environment of the embryo. It is interesting to surmise that the developmental environment of the embryo can be altered by exposure to semen. The inflammatory mediator CSF-2 is an example of a well-studied embryokine with the potential to increase embryonic development in rodents, cattle and humans (Sjoblom *et al.*, 1999 and 2005; Ziebe *et al.*, 2013; Siqueira *et al.*, 2017). In parallel, CSF-2 is also one of the most highly upregulated molecules in the endometrium or oviduct following seminal fluid exposure (Robertson *et al.*, 1996; Sharkey *et al.*, 2012a and 2012b; Bromfield *et al.*, 2014). Two small studies have even suggested that exposure to semen by intercourse around the time of embryo transfer can improve pregnancy rates in women (Marconi *et al.*, 1989; Tremellen *et al.*, 2000). The implication for a simple intervention to potentiate positive reproductive or production measures should be considered for use in agricultural industries like dairy and swine where artificial insemination with minimal seminal fluid exposure is routine.

Potential manipulation of paternal–maternal communication for agriculture

Assisted reproductive technologies including ovarian synchronization, semen collection, artificial insemination, *in vitro* fertilisation and embryo transfer have been extremely important to the economic and productive success of a number of domestic species. These technologies have allowed producers to rapidly improve genetic merit of animals and increase productivity in an ever demanding climate. The utility of these technologies is so well utilized now that a number of studies have demonstrated that *in vitro* fertilisation and embryo transfer technology outperform artificial insemination in regard to pregnancy rates in the dairy cow

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(Vasconcelos *et al*., 2011; Pellegrino *et al*., 2016). In regard to these studies it is important to consider that much of the reported embryo loss in the dairy cow occurs within the 1st week of pregnancy (Wiltbank *et al*., 2016), and therefore *in vitro* fertilisation and embryo transfer may be a simple means to bypass this period of embryo vulnerability. Nevertheless, it is interesting to note that studies in rodents suggest that seminal fluid can alter the developmental environment of the oviduct by increasing expression of embryokines (Bromfield *et al*., 2014). In cattle, gene expression of the oviduct does not appear to be responsive to the presence of a developing embryo or even change from that described at oestrus (Maillo *et al*., 2015; Maillo *et al*., 2016). However neither of these studies considered the potential implications of seminal fluid in modulating the environment of the oviduct.

If we aim to recapitulate the natural developmental environment of the oviduct and uterus in domestic species to optimize reproductive technologies and postnatal development of offspring, we must endure to remember that such an environment is not that of artificial insemination but that of live cover where the female reproductive tract is exposed to male derived factors including seminal fluid. We hope to expand our understanding of how seminal fluid contributes to pregnancy success in domestic species by better understanding the potential of paternal–maternal communication as it pertains to embryo development, foetal growth and immune modulation required for pregnancy success.

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Declaration of interest

None.

Ethics statement

None.

Software and data repository resources

None.

References

Aluvihare VR, Kallikourdis M and Betz AG 2004. Regulatory T cells mediate maternal tolerance to the fetus. Nature Immunology 5, 266–271.

Anderson DJ and Tarter TH 1982. Immunosuppressive effects of mouse seminal plasma components *in vivo* and *in vitro*. Journal of Immunology 128, 535–539.

Avila FW, Sirot LK, LaFlamme BA, Rubinstein CD and Wolfner MF 2011. Insect seminal fluid proteins: identification and function. Annual Review of Entomology 56, 21–40.

Beer AE and Billingham RE 1974. Host responses to intra-uterine tissue, cellular and fetal allografts. Journal of Reproduction and Fertility Supplement 21, 59–88.

Bromfield JJ, Schjenken JE, Chin PY, Care AS, Jasper MJ and Robertson SA 2014. Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. Proceedings of the National Academy of Sciences of the United States of America 111, 2200–2205.

Chaouat G, Tranchot Diallo J, Volumenie JL, Menu E, Gras G, Delage G and Mognetti B 1997. Immune suppression and Th1/Th2 balance in pregnancy revisited: a (very) personal tribute to Tom Wegmann. American Journal of Reproductive Immunology 37, 427–434.

Constant SL and Bottomly K 1997. Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. Annual Review of Immunology 15, 297–322.

Coulam CB and Stern JJ 1995. Effect of seminal plasma on implantation rates. Early Pregnancy 1, 33–36.

De M, Choudhuri R and Wood GW 1991. Determination of the number and distribution of macrophages, lymphocytes and granulocytes in the mouse uterus from mating through implantation. Journal of Leukocyte Biology 50, 252–262.

de Moraes AA and Hansen PJ 1997. Granulocyte-macrophage colony-stimulating factor promotes development of *in vitro* produced bovine embryos. Biology of Reproduction 57, 1060–1065.

Egan RM, Yorkey C, Black R, Loh WK, Stevens JL, Storozynsky E, Lord EM, Frelinger JG and Woodward JG 2000. *In vivo* behavior of peptide-specific T cells during mucosal tolerance induction: Antigen introduced through the mucosa of the conjunctiva elicits prolonged antigen-specific T cell priming followed by anergy. Journal of Immunology 164, 4543–4550.

Fahmi HA, Hunter AG, Markham RJ and Seguin BE 1985. Immunosuppressive activity of bovine seminal plasma on bovine lymphocytes *in vitro*. Journal of Dairy Science 68, 2315–2321.

Fantini MC, Becker C, Tubbe I, Nikolaev A, Lehr HA, Galle PR and Neurath MF 2005. Transforming growth factor beta induced FOXP3+ regulatory T cells suppress Th1 mediated experimental colitis. Gut 55, 671–680.

Faulkner LC, Hopwood ML and Wiltbank JN 1968. Seminal vesiculectomy in bulls. II. Seminal characteristics and breeding trials. Journal of Reproduction and Fertility 16, 179–182.

Garside P and Mowat AM 2001. Oral tolerance. Seminars in Immunology 13, 177–185.

Guerin LR, Moldenhauer LM, Prins JR, Bromfield JJ, Hayball JD and Robertson SA 2011. Seminal fluid regulates accumulation of FOXP3+ regulatory T cells in the preimplantation mouse uterus through expanding the FOXP3+ cell pool and CCL19-mediated recruitment. Biology of Reproduction 85, 397–408.

Gutsche S, von Wolff M, Strowitzki T and Thaler CJ 2003. Seminal plasma induces mRNA expression of IL-1beta, IL-6 and LIF in endometrial epithelial cells *in vitro*. Molecular Human Reproduction 9, 785–791.

Hansen M, Kurinczuk JJ, Bower C and Webb S 2002. The risk of major birth defects after intracytoplasmic sperm injection and *in vitro* fertilization. New England Journal of Medicine 346, 725–730.

Hansen PJ, Dobbs KB and Denicol AC 2014. Programming of the preimplantation embryo by the embryokine colony stimulating factor 2. Animal Reproduction Science 149, 59–66.

Harper HM, Cochrane L and Williams NA 1996. The role of small intestinal antigen-presenting cells in the induction of T-cell reactivity to soluble protein antigens: association between aberrant presentation in the lamina propria and oral tolerance. Immunology 89, 449–456.

Johansson M, Bromfield JJ, Jasper MJ and Robertson SA 2004. Semen activates the female immune response during early pregnancy in mice. Immunology 112, 290–300.

Kapsenberg ML, Hilkens CM, Wierenga EA and Kalinski P 1999. The paradigm of type 1 and type 2 antigen-presenting cells. Implications for atopic allergy. Clinical and Experimental Allergy 29, 33–36.

Klonoff-Cohen HS, Savitz DA, Celafo RC and McCann MF 1989. An epidemiologic study of contraception and preeclampsia. Journal of the American Medical Association 262, 3143–3147.

Lavranos TC, Rathjen PD and Seamark RF 1995. Trophic effects of myeloid leukaemia inhibitory factor (LIF) on mouse embryos. Journal of Reproduction and Fertility 105, 331–338.

Long JA and Evans HM 1922. The oestrous cycle in the rat and its associated phenomena. University of California Press, Berkeley, CA, USA.

Mahajan SC and Menge AC 1967. Influence of reproductive phase on the inflammatory response and rate of sperm removal in the uterus and oviduct of the cow. American Journal of Veterinary Research 28, 1037–1041.

Maillo V, de Frutos C, O'Gaora P, Forde N, Burns GW, Spencer TE, Gutierrez-Adan A, Lonergan P and Rizos D 2016. Spatial differences in gene expression in the bovine oviduct. Reproduction 152, 37–46.

https://doi.org/10.1017/S1751731118000083 Published online by Cambridge University Press

Maillo V, Gaora PO, Forde N, Besenfelder U, Havlicek V, Burns GW, Spencer TE, Gutierrez-Adan A, Lonergan P and Rizos D 2015. Oviduct-embryo interactions in cattle: two-way traffic or a one-way street? Biology of Reproduction 92, 144.

Marconi G, Auge L, Oses R, Quintana R, Raffo F and Young E 1989. Does sexual intercourse improve pregnancy rates in gamete intrafallopian transfer? Fertility and Sterility 51, 357–359.

Maroni ES and de Sousa MA 1973. The lymphoid organs during pregnancy in the mouse. A comparison between a syngeneic and an allogeneic mating. Clinical and Experimental Immunology 13, 107–124.

Mattner PE 1968. The distribution of spermatozoa and leucocytes in the female genital tract in goats and cattle. Journal of Reproduction and Fertility 17, 253–261.

Mattner PE 1969. Differential leucocytic responses to spermatozoa in the cervix and the uterus in ewes. Journal of Reproduction and Fertility 18, 297–303.

McGuane JT, Watson KM, Zhang J, Johan MZ, Wang Z, Kuo G, Sharkey DJ, Robertson SA and Hull ML 2015. Seminal plasma promotes lesion development in a xenograft model of endometriosis. American Journal of Pathology 185, 1409–1422.

McMaster MT, Newton RC, Dey SK and Andrews GK 1992. Activation and distribution of inflammatory cells in the mouse uterus during the preimplantation period. Journal of Immunology 148, 1699–1705.

Medawar P 1953. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symposia of the Society for Experimental Biology 7, 320–338.

O'Leary S, Jasper MJ, Warnes GM, Armstrong DT and Robertson SA 2004. Seminal plasma regulates endometrial cytokine expression, leukocyte recruitment and embryo development in the pig. Reproduction 128, 237–247.

Ochsenkuhn R, Strowitzki T, Gurtner M, Strauss A, Schulze A, Hepp H and Hillemanns P 2003. Pregnancy complications, obstetric risks, and neonatal outcome in singleton and twin pregnancies after GIFT and IVF. Archives of Gynecology and Obstetrics 268, 256–261.

Odhiambo JF, Poole DH, Hughes L, Dejarnette JM, Inskeep EK and Dailey RA 2009. Pregnancy outcome in dairy and beef cattle after artificial insemination and treatment with seminal plasma or transforming growth factor beta-1. Theriogenology 72, 566–571.

Palm F, Walter I, Budik S, Kolodziejek J, Nowotny N and Aurich C 2008. Influence of different semen extenders and seminal plasma on PMN migration and on expression of IL-1beta, IL-6, TNF-alpha and COX-2 mRNA in the equine endometrium. Theriogenology 70, 843–851.

Pellegrino CA, Morotti F, Untura RM, Pontes JH, Pellegrino MF, Campolina JP, Seneda MM, Barbosa FA and Henry M 2016. Use of sexed sorted semen for fixed-time artificial insemination or fixed-time embryo transfer of *in vitro*-produced embryos in cattle. Theriogenology 86, 888–893.

Perri T, Chen R, Yoeli R, Merlob P, Orvieto R, Shalev Y, Ben-Rafael Z and Bar-Hava I 2001. Are singleton assisted reproductive technology pregnancies at risk of prematurity? Journal of Assisted Reproduction and Genetics 18, 245–249.

Piazzon I, Matusevich M, Deroche A, Nepomnaschy I and Pasqualini CD 1985. Early increase in graft-versus-host reactivity during pregnancy in the mouse. Journal of Reproductive Immunology 8, 129–137.

Pincus JW 1938. Artifical insemination in Russia. The Journal of Heredity 29, 391–392.

Polge C 1952. Fertilizing capacity of bull spermatozoa after freezing at 79 degrees C. Nature 169, 626–627.

Polge C, Smith AU and Parkes AS 1949. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 164, 666.

Robertson SA and Seamark RF 1992. Granulocyte-macrophage colony stimulating factor (GM-CSF): One of a family of epithelial cell-derived cytokines in the preimplantation uterus. Reproduction, Fertility and Development 4, 435–448.

Robertson SA, Mau VJ, Tremellen KP and Seamark RF 1996. Role of high molecular weight seminal vesicle proteins in eliciting the uterine inflammatory response to semen in mice. Journal of Reproduction and Fertility 107, 265–277.

Robertson SA, Mau VJ, Hudson SN and Tremellen KP 1997. Cytokine-leukocyte networks and the establishment of pregnancy. American Journal of Reproductive Immunology 37, 438–442.

Robertson SA, Guerin LR, Bromfield JJ, Branson KM, Ahlstrom AC and Care AS 2009. Seminal fluid drives expansion of the CD4 + CD25 + T regulatory cell pool and induces tolerance to paternal alloantigens in mice. Biology of Reproduction 80, 1036–1045.

Robillard PY, Hulsey TC, Perianin J, Janky E, Miri EH and Papiernik E 1995. Association of pregnancy-induced hypertension with duration of sexual cohabitation before conception. The Lancet 344, 973–975.

Saxena S, Jha P and Farooq A 1985. Immunosuppression by human seminal plasma. Immunological Investigations 14, 255–269.

Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G and Wilcox LS 2002. Low and very low birth weight in infants conceived with use of assisted reproductive technology. New England Journal of Medicine 346, 731–737.

Scott JL, Ketheesan N and Summers PM 2006. Leucocyte population changes in the reproductive tract of the ewe in response to insemination. Reproduction, Fertility and Development 18, 627–634.

Sharkey DJ, Macpherson AM, Tremellen KP, Mottershead DG, Gilchrist RB and Robertson SA 2012a. TGF-beta mediates proinflammatory seminal fluid signaling in human cervical epithelial cells. Journal of Immunology 189, 1024–1035.

Sharkey DJ, Tremellen KP, Jasper MJ, Gemzell-Danielsson K and Robertson SA 2012b. Seminal fluid induces leukocyte recruitment and cytokine and chemokine mRNA expression in the human cervix after coitus. Journal of Immunology 188, 2445–2454.

Siqueira LG, Tribulo P, Chen Z, Denicol AC, Ortega MS, Negron-Perez VM, Kannampuzha-Francis J, Pohler KG, Rivera RM and Hansen PJ 2017. Colony-stimulating factor 2 acts from days 5 to 7 of development to modify programming of the bovine conceptus at day 86 of gestation. Biology of Reproduction 96, 743–757.

Sjoblom C, Roberts CT, Wikland M and Robertson SA 2005. Granulocyte-macrophage colony-stimulating factor alleviates adverse consequences of embryo culture on fetal growth trajectory and placental morphogenesis. Endocrinology 146, 2142–2153.

Sjoblom C, Wikland M and Robertson SA 1999. Granulocyte-macrophage colony-stimulating factor promotes human blastocyst development *in vitro*. Human Reproduction 14, 3069–3076.

Tafuri A, Alferink J, Moller P, Hammerling GJ and Arnold B 1995. T cell awareness of paternal alloantigens during pregnancy. Science 270, 630–633.

Tremellen KP, Seamark RF and Robertson SA 1998. Seminal transforming growth factor beta1 stimulates granulocyte-macrophage colony-stimulating factor production and inflammatory cell recruitment in the murine uterus. Biology of Reproduction 58, 1217–1225.

Tremellen KP, Valbuena D, Landeras J, Ballesteros A, Martinez J, Mendoza S, Norman RJ, Robertson SA and Simon C 2000. The effect of intercourse on pregnancy rates during assisted human reproduction. Human Reproduction 15, 2653–2658.

Vasconcelos JL, Jardina DT, Sa Filho OG, Aragon FL and Veras MB 2011. Comparison of progesterone-based protocols with gonadotropin-releasing hormone or estradiol benzoate for timed artificial insemination or embryo transfer in lactating dairy cows. Theriogenology 75, 1153–1160.

Wang JX, Norman RJ and Kristiansson P 2002. The effect of various infertility treatments on the risk of preterm birth. Human Reproduction 17, 945–949.

Wiltbank MC, Baez GM, Garcia-Guerra A, Toledo MZ, Monteiro PL, Melo LF, Ochoa JC, Santos JE and Sartori R 2016. Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. Theriogenology 86, 239–253.

Yanagimachi R and Chang MC 1963. Infiltration of leucocytes into the uterine lumen of the golden hamster during the oestrous cycle and following mating. Journal of Reproduction and Fertility 5, 389–396.

Young LE, Sinclair KD and Wilmut I 1998. Large offspring syndrome in cattle and sheep. Reviews of Reproduction 3, 155–163.

Ziebe S, Loft A, Povlsen BB, Erb K, Agerholm I, Aasted M, Gabrielsen A, Hnida C, Zobel DP, Munding B, Bendz SH and Robertson SA 2013. A randomized clinical trial to evaluate the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) in embryo culture medium for *in vitro* fertilization. Fertility and Sterility 99, 1600–1609.

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