Communication requested: Boar semen transport through the uterus and possible consequences for insemination

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ABSTRACT

Recent insemination techniques bypass the interactions between sperm and the uterine wall because the semen is deposited deep into the tip of uterine horn or directly into the oviduct. Such techniques allow high dilution of the ejaculates. After normal mating, semen entering the uterus communicates with the uterine milieu. Intact sperm of high mitochondrial membrane potential bind to uterine epithelial cells, whereas most of the unbound sperm in the uterine lumen have damaged membranes. Lectins are the most likely factors to mediate these sperm–uterine interactions. The lectin wheat germ agglutinin is known to induce the strongest binding of sperm, whereas binding is impaired when sialic acid receptors are blocked by wheat germ agglutinin. This suggests that sialic acid is involved in porcine sperm–endometrium interactions, and it is hypothesized that the use of a semen extender supplemented with sialidase would allow insemination with reduced sperm numbers. A lack of contact of sperm and seminal plasma with the uterine wall, as a result of deep insemination, may adversely affect (1) events during ovulation, (2) induction of immunologic tolerance against paternal antigens, (3) preparation of the endometrium for implantation and placentation, and (4) immunologic support required for the fetus during pregnancy. Seminal plasma is known to signal post-insemination changes in the uterine endometrium involving the redistribution of leukocytes. This may involve migration of leukocytes from the uterine wall to the ovary, as seminal plasma particularly increases the appearance of the major histocompatibility complex class II-positive cells. Uterine epithelial cells respond to sperm binding by the production of pro- or anti-inflammatory cytokines. These cytokines may include synchronizing substances, transferred through a counter-current pathway to the ipsilateral ovary, thereby accelerating the final maturation of pre-ovulatory follicles and advancing time of ovulation. In several species, an ovulation-inducing factor exists in seminal plasma, first identified as ß-nerve growth factor in camelid semen, indicating another pathway that influences the hypothalamic-pituitary-gonadal axis. In summary, low-dose inseminations may not necessarily require semen deposition deep into the uterine horn, as binding inhibitors can circumvent the binding of sperm to the uterine wall. However, subsequent immune-relevant events that control ovulation and prepare the uterine milieu for the developing embryo should be taken into account.

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1. Introduction

Pork production is based on high breeding performance and requires intensive boar selection. However, partly due to the anatomy and physiology of the female genital tract

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and partly because of suboptimal artificial insemination (AI) techniques, ejaculates of fertile boars are used inefficiently when 2 to 3 billion sperm are inseminated twice during estrus [1]. Many of the sperm and most of the seminal plasma are lost during transport from the site of insemination, which normally is the proximal cervix or uterine body, to the oviduct [2,3]. In consequence, besides negative economic aspects, new biotechnologies such as insemination with sex-sorted boar sperm, which require the utilization of very small insemination doses, are very difficult to introduce into the breeding system [4]. At least in Europe, it would be of importance to establish an effective method to predetermine sex ratios and produce females, as boar castration has become a legal animal welfare issue [5,6]. Therefore, a prerequisite for future sustainable pork production would be a low-dose insemination concept. During the last 4 decades, there have been many new insights on the physiological interdependence of gametes and the female genital tract, but major questions remain to be answered. Although the interactions of sperm with oviduct cells and the zona pellucida of the ovulated oocyte are well understood, information about the fate of sperm and their interactions within the uterus is relatively incomplete. However, it is important to elucidate the events and cellular interactions that occur during sperm transport, as fertilization failure results mainly from insufficient competent sperm arriving at the oviducts [7].

2. Sperm transport

Reproduction in all animals with internal fertilization requires sperm to leave the male body, migrate to the site of fertilization in the female, and meet with the mature oocyte at the optimum time for fertilization. Because the quality of ejaculated sperm is variable among boars, and ejaculates containing billions of sperm enter the female genital tract after mating, a strict selection process is necessary to ensure fully competent sperm fertilize the oocyte and to avoid polyspermic fertilization [8–11].

During mating, boars deposit about 250 mL of ejaculate, containing 50 to 60 billion sperm and seminal plasma, into the proximal part of the cervix and the distal part of the uterine body of sows, which is then plugged with the gel-like secretions of the bulbourethral gland. Subsequent sperm transport through the uterus requires coordinated contractions of the myometrium [12]. Zerobin [13] described different wave patterns of the myometrium depending on the stage of the estrous cycle. At the onset and the end of estrus, waves are directed toward the cervix, while they run from the cervix toward the uterotubal junction (UTJ) on the second day of estrus. Myometrial wave activity is stimulated by the introduction to the uterus of the voluminous inseminate and by the physical action of mating or insemination [14,15]. Progesterone and estrogens control the activity of the myometrium, whereby the frequency and amplitude of contractions reach a maximum during estrus, and daily variations depend on varying hormone levels including estrogens, oxytocin, and prostaglandins [16]. As porcine seminal plasma contains high levels of estrogen, it promotes uterine contractility followed by a significant increase of prostaglandins [17–20].

Only a very few, highly selected sperm finally reach the site of fertilization [21]. For transcervical AI in the sow, usually 1.5 to 5 billion sperm are diluted in a total volume of 80 to 100 mL extender [1,22,23]. This is different for example to bovine insemination. At natural mating, bulls place the ejaculate into the vagina, and the cervix acts as a major selective barrier for sperm integrity [24,25]. In that species, bypassing the cervix during AI avoids or at least reduces sperm losses, and many more inseminated sperm reach the UTJ and the oviduct compared with cervical insemination. Accordingly, interactions between sperm and the uterus of cows and heifers after insemination remain very limited. Wendt [26] for example found no increase of polymorphonuclear leukocytes (PMN) located at the uterine epithelial cells (UECs) and in the uterine lumen of cows after insemination.

In sows, sperm losses by semen backflow seem to be a normal process, which occur through the vestibule to the outside in almost all inseminated females [12]. These losses can be up to 70% of the volume [27,28] and up to 40% of the inseminated sperm [29]. Interestingly, all attempts to prevent backflow by cervical tamponade failed [30], although after natural mating, bulbourethral plugs deposited at the end of ejaculation into the sow's vagina prevent semen backflow.

During transport, the volume of the ejaculate or insemination dose reduces quickly and, soon after insemination, almost no seminal fluid/extender is present in the uterus [31]. A small group of sperm is transported very quickly after insemination and reach the oviducts within less than an hour [32]. Overstreet and Tom [33] and Baker et al. [27] postulated that sperm entering the oviduct by fast transport through the uterus are of no importance for fertilization, and it may either be a random event, or a kind of security measure to provide sperm in the oviduct for early arriving oocytes before the main sperm depot has been established.

Most of the sperm are transported slowly, and a significant proportion undergoes a series of interactions with the uterine milieu, where further losses are caused by sperm binding to endometrial epithelial cells, and to neutrophilic granulocytes, being present in the uterine lumen very soon after the arrival of sperm [31,34–39]. It is unknown whether sperm are selected randomly or whether certain characteristics enable them to pass into the oviduct, but it is generally accepted that significant selection happens when spermatozoa pass the UTJ to enter the oviduct [40,41]. Dead [42], immotile [43,44] or morphologically abnormal sperm have a lower chance of reaching the oviduct than those that are motile and normal [45]. In this context, a study by Ardon et al. [46] indicated that chromatin instability has an impact on sperm transport through the genial tract and final penetration rates into oocytes, where the ultimate selection and recognition happens during contact of sperm with the zona pellucida [47,48].

2.1. Low-dose insemination

In the 1960s, First et al. [40,41] recommended 40 billion sperm as the minimum dose suitable for AI of a sow. With the development of standardized commercial extenders in the 1980s and 1990s, the average number of sperm for