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Effects of female bovine plasma collected at different days of the estrous cycle on epididymal spermatozoa motility

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ABSTRACT

The aim of this study was to assess the effects of female bovine plasma collected at different days of the reproductive cycle on epididymal spermatozoa motility and to test hypothesis that the subpopulations pattern of motile spermatozoa is affected by this treatment. Blood plasma samples were collected from five Holstein Friesian cows at different stages of the estrous cycle (days 0, 5, 10, 12 and 18), one pregnant cow and one adult bull and were diluted 1:9 (V/V) with normal saline. Female charcoal-treated plasma, Bull plasma and saline were used as controls. Semen samples were obtained from cauda epididymidis through retrograde flushing and diluted in saline to approximately 60 × 106 sperm/ml. The extended semen was diluted 1:2 (V/V) with tested media and motility was evaluated at 15 min and then every hour for 6 h using a computer-assisted semen analysis. Multivariate clustering procedure was applied to identify and quantify specific subpopulations within the semen samples. The statistical analysis clustered all the motile spermatozoa into three separate subpopulations with defined patterns of movement: Subpopulation 1 poorly motile and non-progressive spermatozoa (39.3%), subpopulation 2 including the fastest and the most vigorous spermatozoa (46.4%) and subpopulation 3 represented by slow, non-vigorous but linear spermatozoa (14.3%). Initially, sperm samples supplemented with female, male or female charcoal-treated plasma stimulated equally total motility and spermatozoa belonging to subpopulation 2 regardless of the estrous cycle stage. After 1-h incubation, the motility of these both categories of spermatozoa (total motile and those assigned to subpopulation 2) is enhanced and maintained more in day 12, 18 and pregnant cow plasma than in female plasma from earlier stage of the estrous cycle (day 0, 5 and 10), male plasma and female-charcoal treated plasma. In conclusion, the overall results showed that female plasma stimulated significantly sperm motility, especially at the late stage of the estrous cycle. Additionally, to the diverse compounds contained in blood plasma, progesterone may play a key role in such motility activation.

1. Introduction

It is well established that poor sperm motility impacts negatively the outcomes of natural and assisted reproduction techniques [1]. Therefore, a number of strategies have been explored to improve sperm motility including but not limited to: caffeine [2], pentoxifylline [3], follicular fluid [4], oviductal fluid [5] and cumulus oocyte complex [6]. In the same way, a number of studies have reported the beneficial effect of homologous or heterologous blood serum or blood plasma on sperm motility in various species including human [7–9], dog [10], goat [11], hamster [12], ram [13] and buffalo [14].

In bovine species, Brown and Senger [15] showed that motility of ejaculated spermatozoa, rendered non-motile by *in vitro* aging (dilution in 2.9% sodium citrate and incubation for 8 h), is restored at levels equal to or exceeding the motility estimated at collection by addition of 20% heifer serum. Chen et al. [16], exploring the impacts of divers substances showed the positive impact of bovine serum on bull sperm motility after freezing-thawing process, which

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however remains bull dependent. Moreover, a number of authors reported that addition of cow [17], heifer [18] or bull [15,18] blood serum induced a marked head-to-head spermatozoa agglutination (HHA). According to these authors, such supplementation does not damage spermatozoa and does not affect adversely the maintenance of the acrosomal cap.

In contrast, according to Li and Funahashi [19], fresh blood serum supplementation, in opposition to heat-treated serum or to the fresh serum in presence of caffeine or heparin, increased chemotactic activity of polymorphonuclear leucocytes and stimulated their phagocytotic activity of bull sperm cells. In addition, Verberckmoes et al. [20] hypothesized that prolonged HHA induced by blood or serum may interfere with *in vivo* fertility by preventing the spermatozoa from reaching the sperm reservoir.

Otherwise, the stage dependent effect of blood serum supplementation on sperm functions has been investigated in previous studies on hamster [21], human [8] and dog [10] species. Barros et al. [21] reported a cycle-dependent effect of blood sera collected at different stages of the estrous cycle on the incidence of acrosome reaction of golden hamster spermatozoa. In human, Akerlöf et al. [8] have mentioned that no difference was noted between the effects of male and female serum, collected during the follicular or luteal phases, on sperm motility assessed subjectively. In dog, supplementation of sperm samples with female plasma showed that hyper-activated spermatozoa percentage increased linearly from proestrus to reach the highest values at estrus, whereas these effects were not observed in steroid free female and male plasma [10].

In bovine species, Lapointe et al. [22] reported that oviductal cells stimulate survival and motility of bovine sperm *in vitro* in a hormone-dependent manner. Grippo et al. [5] demonstrated that oviductal fluid collected from the isthmus during the nonluteal stage causes a lowering in sperm motility but optimizes the ability of spermatozoa to undergo the acrosome reaction, without diminishing their viability. In addition, Boquest and Summers [23], reported that pre-treatment of isthmic and ampullary bovine oviductal epithelial cell with estrous-stage specific cow serum, enhanced their ability to prolong spermatozoal motility.

Against this background, and owing to the resemblances in composition between serum and the oviductal secretions [24–26] and the fact that the effects of blood plasma collected at different days of estrous cycle on sperm motility and sperm subpopulations distribution have not been reported previously for cattle, the aim of this study was to test the hypothesis that, beyond the fact that the co-incubation of bull spermatozoa with blood plasma affect their motility, this effect change with the days of the female reproductive cycle and, therefore, with the hormonal status of cow.

In order to increase detection power of such effect, multivariate analysis approach was used. In fact, according to Holt et al. [27], the adoption of this approach would allow to derive the maximum benefit from the use of CASA system and to take advantage of the large quantity of data that it generates. One of the several advantages offered by this kind of analysis that has been widely reported [27–30] is the fact that it take into account internal variability and the wide ranges in semen samples that the classical approach based on the average values neglect by considering these samples homogeneous.

2. Materials and methods

2.1. Animal selection

Five healthy, normally cycling, with observed heat signs, Holstein Friesian cows at the early (days 0 and 5), middle (days 10 and 12) and late (day 18) stages of estrous cycle, one pregnant cow and one adult bull were used in this study. Sexual behavior was observed, to assess the onset of estrus, which was designed as day 0 of the estrous cycle. For pregnant cow, pregnancy was confirmed by transrectal palpation 60 days after artificial insemination.

2.2. Blood samples

Blood samples were taken from the coccygeal vein of each animal into heparinized vacutainer tubes. Samples were immediately placed in a cooler box with ice packs, and within an hour, centrifuged at 1200 × g for 20 min for plasma collection. Aliquots of plasma were stored into labeled eppendorf tubes at −20 °C until used.

2.3. Tested media

Table 1 below summarizes the different tested media.

2.3.1. Female plasma samples

Plasma aliquots collected from pregnant (P) and normally cycling cows (d0, d5, d10, d12 and d18) were diluted 1:9 (V/V) in physiological saline.

2.3.2. Control groups

The control groups included three different media: 0.9% sodium chloride (saline) only; male plasma (MP) and female charcoal-treated plasma (FTP). MP and FTP was diluted 1:9 (V/V) in physiological saline.

2.3.3. Female charcoal treated plasma preparation

Part of pregnant cow plasma aliquot was stirred with charcoal (Norit A) to extract steroids [31]. Charcoal extraction of endogenous plasma hormones was carried out at room temperature with 1% (wt/vol) activated charcoal. The mixture was stirred for 15 min at moderate speed (10 000 × g), then, charcoal was removed by centrifugation at 10 000 × g for 15 min at 4 °C. This adsorption procedure was repeated three times. After the final centrifugation, adsorbed plasma was sterilized by Millipore filtration (0.22 μm) and stored at - 20 °C until used [32].

2.4. Semen collection and processing

2.4.1. Collection of epididymal sperm

Three testes of mature and healthy bulls were obtained from a local abattoir soon after slaughter the males, transported to the laboratory at room temperature (18 °C) within ½ to 1 h [33] and semen was collected immediately at arrival. The epididymis was isolated from testes and from the surrounding connective tissue and cleaned. Cauda epididymidis and vas deferens were carefully isolated from the rest of the epididymis by making a cut with a scalpel near the junction of the corpus and the proximal cauda. A clamp was placed between the distal corpus and the cauda epididymis. Then, the lumen of the ductus deferens was cannulated with a blunted 22G needle. Semen from the cauda epididymidis was obtained through retrograde flushing by applying air pressure with a 5 ml syringe in the proximal scrotal segment of the vas deferens [33,34]. Semen was collected into 1.5 ml conical tubes after making a small slit on the cauda epididymitis with a razor blade, taking care to avoid cutting blood vessels. The collected semen was pooled in order to reduce individual variation among the evaluated samples and to allow for the availability of large semen volume [35,36]. The pooled semen was diluted in physiological saline solution [37] to approximatively 60 × 10^6 sperm/ml.