Chapter

The Relationship of Sperm Motility Pattern and Its Ability to Agglutinate with Vaginal Sperm Selection, Uptake in Sperm Storage Tubules and Competitiveness

Mohamed A.M. Sayed, Hanan H. Abd Elhafeez, Catrin S. Rutland and Taymour M. El-Sherry

Abstract

To ensure survival, some unique features can be distinguished in birds that help them maintain reproduction. These features include the ability to store sperm for long periods within the utero-vaginal junction, a high sperm concentration per ejaculate, and polyspermy fertilization. Sperm face many challenges prior to fertilization. After copulation, most ejaculated sperm exit the female reproductive tract, and less than 1% continue in an attempt to achieve fertilization. In addition, egg size is substantially larger than sperm size because of the presence of the egg yolk. This results in a large number of sperm penetrating the egg away from the oocyte. These challenges have triggered evolutionary changes to maintain the existence of many species, such as the enormous relative size of the testis, which produces billions of sperm each day, and the ability to store viable sperm for long periods in the oviduct to ensure asynchronous fertilization. This chapter discusses several contemporary and sometimes controversial points regarding sperm behavior and their storage in the oviduct.

Keywords: microfluid device, rheotaxis, Sharkasi and danderwai chickens, sperm mobility, sperm selection, sperm storage tubules, sperm agglutination

1. Introduction

During natural mating, the rooster deposits its sperm into the hen's vagina, but a large number of sperm (more than 80%) are ejected shortly after copulation [1]. Furthermore, it has also been reported that only a small number of sperm (< 1%) inseminated into the vagina pass through and enter the sperm storage tubules (SSTs) [2]. Therefore, the vagina appears to be the primary site for sperm selection in avian species. It is believed that the sperm selection process is of utmost importance as it sorts the fittest sperm, allowing them to traverse and eliminate the non-fit sperm. This process is beneficial as it reduces embryonic mortality relative to what it could be

without this selection. Deep artificial insemination close to the utero-vaginal junction, performed shortly after oviposition, where the vaginal wall is flaccid, deprives the vagina of sperm selection. This has been reported to be associated with high embryonic mortality [3]. A number of researchers have described vaginal selection by limiting sperm migration to those sperm capable of progressive motility, eliminating dead and immobile sperm [4]. However, the mechanism by which the selection process takes place is still unknown.

1.1 Sperm motility and mobility

In chickens and turkeys, it was reported that the migration of spermatozoa, from the entrance to the vagina where they are deposited to the uterovaginal junction where they are stored, is achieved through their active motility [5]. However, this motility is not needed between the uterovaginal junction and the infundibulum because sperm are transported by passive displacement. According to this assumption, sperm transport through the vagina is critical and requires energy expenditure (for the flagellum oscillatory movement) to reach the uterovaginal junction and penetrate the sperm storage tubules. While other means such as the peristalsis movement of the oviduct and/or the movement of cilia may be responsible for the passive transport of the released sperm from the SSTs to the infundibulum. This has been demonstrated as dead spermatozoa inserted in the uterus are transported along the reproductive tract on inert particles, such as carbon powder [6, 7].

Moreover, hundreds of millions of sperm compete to traverse the vagina and motility is not the only determinant in winning this competition. Other factors such as velocity and progressive motility are included. Spermatozoa that move linearly but at a slow velocity, and those swimming in circles at high velocity, might not eventually achieve significant mobility and are unlikely to be competitive [8]. Therefore, it can be stated that not all motile sperm are mobile. Froman and McLean [9] developed an assay to measure sperm mobility in chickens using Accudenz solution in a cuvette with the spermatozoa layered on top of the solution and incubated at 41°C for 5 minutes. The researchers found that the sperm straight-line velocity (VSL) must exceed 30 μm/s in order for the sperm to penetrate the Accudenz solution [10]. Sperm mobility is therefore defined as the net forward movement of sperm against resistance at body temperature [11]. This may indicate that spermatozoa must demonstrate progressive motility with VSL > 30 μm/s to be capable of reaching the sperm storage tubules. Froman and coauthors [12] surmised that sperm mobility is the dominant factor in sperm selection within the vagina.

In heterospermic insemination trials, when a female is inseminated with ejaculates containing high or low sperm mobility from different males, the sperm cells from the first type of ejaculate fertilized most of the ova [5]. This means that a small number of low-mobility sperm are capable of reaching the SSTs and fertilizing a few ova. This proves that another factor interacts with sperm mobility in regulating vaginal sperm selection.

1.2 Mechanisms behind sperm transport

Rheotaxis has recently been considered an important factor controlling sperm transport in mammalian genitalia. Miki and Clapham reported that the sperm's ability to orient themselves in oviductal fluid flow secreted post-copulation to align against the flow direction and swim upstream is considered a significant factor responsible

for sperm guidance in mice [13]. How the fluid flow guides the sperm is still controversial. Some researchers have proposed that rheotaxis is an actively sensed process because fluid flow is sensed by mechanosensing channels on the sperm, while others have proposed that rheotaxis is a passive process and can be explained by the models of fluid mechanics. Sperm adjust their flagellar beating patterns in response to external stimuli during active reorientation. In response to the stimulus attractant, sperm bend their flagella asymmetrically and swim towards it. The asymmetric flagellar beating patterns are a result of sliding microtubules that are regulated by calcium and calmodulin. Thus, active sperm responses are always accompanied by calcium signaling and oscillations in intracellular Ca2+ concentrations. Zhang and colleagues [14] undertook quantitative analysis of human sperm flagellar behavior during rheotaxis-turning. The researchers did not observe significant differences in flagellar beating amplitude and asymmetry between rheotaxis-turning and freely swimming sperm in the absence of fluid flow. According to these observations, human sperm rheotaxis occurs passively through hydrodynamic interactions between the sperm flagellum and the surrounding fluid flow; therefore, no flow sensing is involved. Zaferani et al. [15] exploited the ability of viable sperm to swim against the flow and passively isolated motile sperm inside a corral from the semen sample using a microfluidic corral system. Medical infertility treatments and clinical trials require this kind of sperm sorting, which does not harm sperm structure and morphology. Unlike conventional methods which are labor- and time-consuming and involve more risks to sperm, the technique used by Zaferani et al. [15] eases the process of sperm sorting.

In birds, Parker [16] assumed that sperm pass through the oviduct by swimming against the ciliary current. Although it was proposed as the mechanism by which sperm ascend the oviduct in 1895 by Verworn [17] and its observation was noted in vitro in 1906 by Adolphi [18], studies on avian sperm rheotaxis are still lacking in the literature. In 1906 Adolphi observed that avian sperm exhibit positive rheotaxis when a slow current is generated in a thin layer of fluid contained between a coverslip and a glass slide [18]. Also, Wishart and Ross [19] observed in 1985 that chicken and turkey sperm show rheotaxic properties by aligning themselves along the axis of a fluid current. More recently, El-Sherry and colleagues [20] fabricated a microfluidic device with a narrow channel cross-section approaching close to that of the sperm gland and forced tiny amounts of liquid inside the microchannel by applying hydrostatic pressure to generate a fluid flow (fluid flow = 33 μm/s) to study the behavior of chicken sperm (**Figure 1**). The researchers observed that nearly half of spermatozoa showed positive rheotaxis [20].

Bakst et al. [2] reported that the cilia lining the lumen of the vagina beat in an abovarian direction. Through their activity, cilia direct luminal secretions to the cloaca. Sperm located in the troughs created between apposed mucosal folds get trapped in this secretory material and as a result only motile sperm propel themselves and/or are transported in an adovarian direction. A counter-current mechanism may facilitate the transport of sperm by moving oviducal fluid between longitudinally oriented folds towards the uterovaginal junction, while secretory material in the central vaginal luminal area is transported towards the cloaca [21].

To avoid inbreeding after mating, promiscuous birds can improve the genetic diversity of their offspring by selecting against related male sperm within the reproductive system [21]. When artificial insemination is used, the female's ability to prefer non-relative males disappears, which suggests that male phenotype as well as eyesighting may influence sperm selection [21]. The female's tendency to bias her sperm selection in favor of nonrelative males by using a mechanism referred to as cryptic

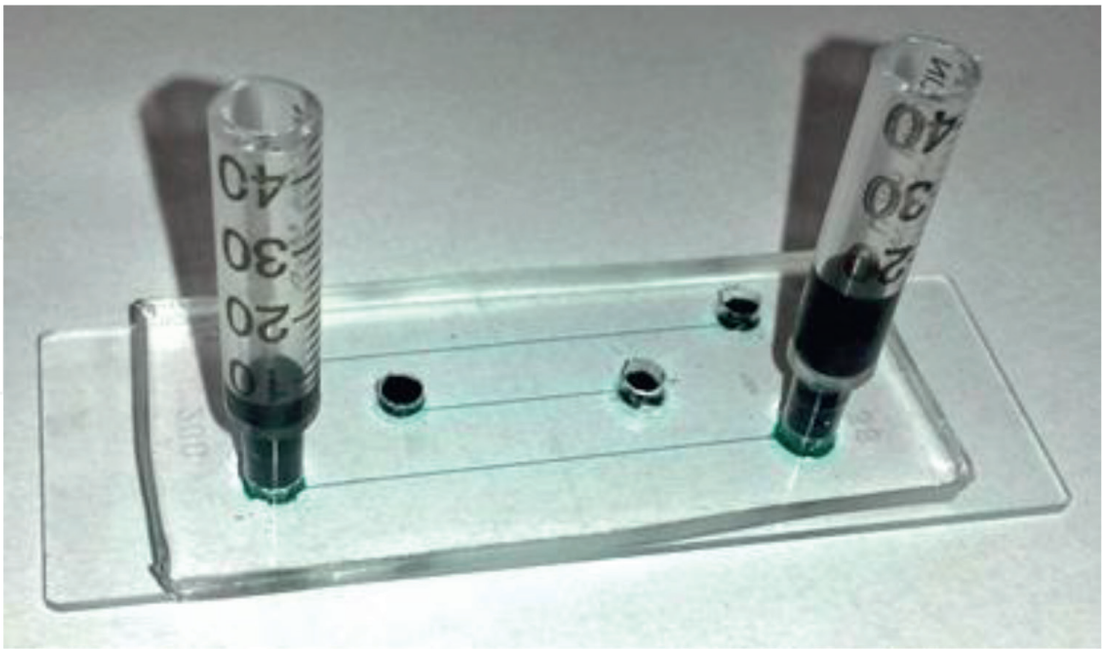

Figure 1.
Hydrostatic pressure was applied to create fluid flow inside the microchannel where sperm rheotaxis was studied. Microchannels with dimensions of 200 μm × 20 μm (W × H) and a length of 3.6 μm were employed [20].

female choice may be regulated through controlling the characteristics of the luminal oviductal fluid. It was reported that the efficiency of in vitro sperm rheotaxis is affected by fluid flow velocity, shear stress, and fluid viscosity. Increasing fluid flow velocity and shear stress induces more sperm to display rheotactic behavior, while increasing fluid viscosity act to decrease rheotaxis efficiency [14]. Consequently, a promiscuous female may bias her sperm selection in favor of genetically dissimilar males by decreasing the viscosity of her luminal vaginal fluid, thus causing it to flow more fluidly and increase rheotaxis behavior, which allows the sperm to swim across the vagina against the flow. The exact opposite happens in the case of genetically related males.

Once sperm cross the vagina and reach the uterovaginal junction, they enter the SSTs, where they are stored for a period of time which varies by species (from 2 to 10 weeks). This feature is unique to birds as it ensures the continuation of the fertilization process for a long time without the need for a number of repeated copulations, in the case of the absence of males, and also, if fertilization takes place a few hours before the egg is released, it ensures that the sperm remain alive inside the oviduct and are not expelled with the descent of the egg. How sperm remain alive without losing their fertilizing capacity for long periods inside the SSTs is still questioned.

Froman [22] proposed that sperm residency and egress from the SST can be explained on the basis of sperm cell motility. In accordance with the author's theory, sperm maintain their position by swimming against a fluid current generated by the epithelium of the SST, and egress when their velocity drops below the point at which retreated movement begins due to a lack of sperm's energy which makes the sperm swept by the fluid current force. Zaniboni and Bakst [23] confirmed the presence of aquaporin 2, 3, and 9 within the apical portion of the SSTs epithelial cells by immunocytochemistry. The authors reported that their findings support Froman's model of sperm residence in the SSTs. However, rather than Froman's assumption that SST fluid secretion is constant, the authors suggested that factors accompanying active

egg production modulate either the volume or the velocity of SST fluid secretion which would regulate the sperm residence and egress from the SSTs. None of the authors demonstrated the mechanism responsible for SSTs uptake of sperm, but we believe that rheotaxis is involved in this process.

On the other hand, Bakst [24] suggested that the resident sperm in the SSTs are metabolically inactive due to reduced oxygen consumption, which inhibits sperm motility and prolongs sperm storage duration within the SSTs. The authors attributed the decreased sperm oxygen uptake to an increased zinc concentration in the SSTs which acts as a metabolic inhibitor in turkey sperm. Similarly, but in a different way, Matsuzaki and Sasanami [25] proposed that avian sperm motility is suppressed within the SSTs and the resident sperm remain in a quiescent state which explains the prolonged sperm storage. After release from the SSTs, sperm motility is restored. Matsuzaki and coauthors [26] observed increased production and release of lactic acid in the SSTs under hypoxic conditions, which may suppress the motility of resident sperm. In this case, the significance of sperm rheotaxis is manifested during sperm selection and uptake but not during storage.

There has been evidence that resident sperm cluster together in agglutinated bundles. Head-to-head agglutinated sperm have been observed in the SSTs of chickens [27, 28], quails [2], and turkeys [29]. Because this pattern of sperm residency is common among domestic birds, the sperm agglutination mode was suggested as a plausible explanation for the prolonged storage period of sperm within the SST.

How does avian sperm agglutination occur? Is there a biochemical substance responsible for agglutination? Do all sperm agglutinate? Does sperm agglutination constrain sperm motility? Does sperm agglutination occur after arrival at the SSTs? These questions have been difficult to answer because it is not easy to monitor sperm inside the opaque oviduct.

The spermatozoal glycocalyx (glycoprotein glycolipid coating the sperm) is essential for gamete recognition and agglutination [30]. Froman reported that manipulating the spermatozoal glycocalyx by treating fowl spermatozoa with neuraminidase that hydrolyzes the α-glycosidic bonds resulted in decreased fertility without affecting sperm vitality [28]. It was suggested that neuraminidase manipulation of the glycocalyx perturbed sperm sequestration in the SSTs, which in turn decreased fertility. However, the authors could not overlook the possibility that neuraminidase treatment may have reduced sperm-oocyte recognition. To negate this possibility, they performed intramagnal insemination with neuraminidase-treated spermatozoa and found that fertility was not decreased compared to the controls. The authors concluded that manipulation of the sperm glycoprotein-glycolipid coat reduces fertility by increasing the rate at which sperm are lost from the SSTs through perturbing sperm sequestration in the SSTs but not through decreasing sperm-oocyte recognition.

Bakst and Bauchan [29] found small vesicles and membrane fragments in the SST lumen of turkeys, some of which were fused with the sperm membrane. The authors speculated that these particles may be involved in prolongs sperm storage. These particles are either secreted from the epithelium of SSTs, or produced and secreted from the male reproductive system, but their source remains unclear, as well as whether or not these particles are responsible for agglutination.

Grützner and coresearchers [31] reported that the epididymal epithelium of monotremes produces and secretes a specific protein(s) that is required for the formation of sperm bundles. In both short-beaked echidnas and platypus, Nixon et al., [32] found that an epididymal secreted protein, acidic cysteine-rich osteonectin; SPARC,

contributes to sperm bundle formation and that the dispersal of these bundles is associated with the loss of this protein.

El-Sherry and coauthors [20] provided the first detailed description of the sperm bundle characteristics in chickens. To overcome the difficulty of observing sperm behavior in the opaque oviduct, and to model chicken sperm motility inside the genitalia of chickens, the researchers used a microfluidic device which had a microchannel with a cross-section similar to that of sperm glands, and generated a flowing fluid with a flow velocity of 33 μm/s to mimic the flowing secretions in the vaginal lumen area [20]. The authors observed that Sharkasi chicken spermatozoa form thread-like bundles composed of dozens of individuals immediately after ejaculation (**Figure 2**). A bundle is formed when a few sperm get close together, they start moving synchronously and wrap around one another, and then they adhere to an adhesive substance. This agglutinating substance is evident using scanning and transmission electron microscope (SEM and TEM; **Figures 3** and **4**) and also when Acridine orange-stained semen smears were examined under a fluorescence microscope (**Figure 5**). The agglutinated sperm bundles grew with time and could remain in vitro for hours before dispersing. The sperm bundles had a unique pattern of motility and were capable of sticking to any static or adjacent surface. The researchers found that the sperm bundle consists of two segments: the initial segment, which consists of the free heads of the agglutinated sperm, and the terminal segment, which consists of their tails and distal sperm (**Figure 6** and Video 1, https://www.nature.com/articles/s41598-022-17037-x#MOESM4). The free heads, at the initial part of the bundle, were observed to be responsible for the bundle motility due to their oscillatory movements, which drag the adhered distal segment of the bundle in a spiral-like movement. Long bundles had some free heads of adhered sperm

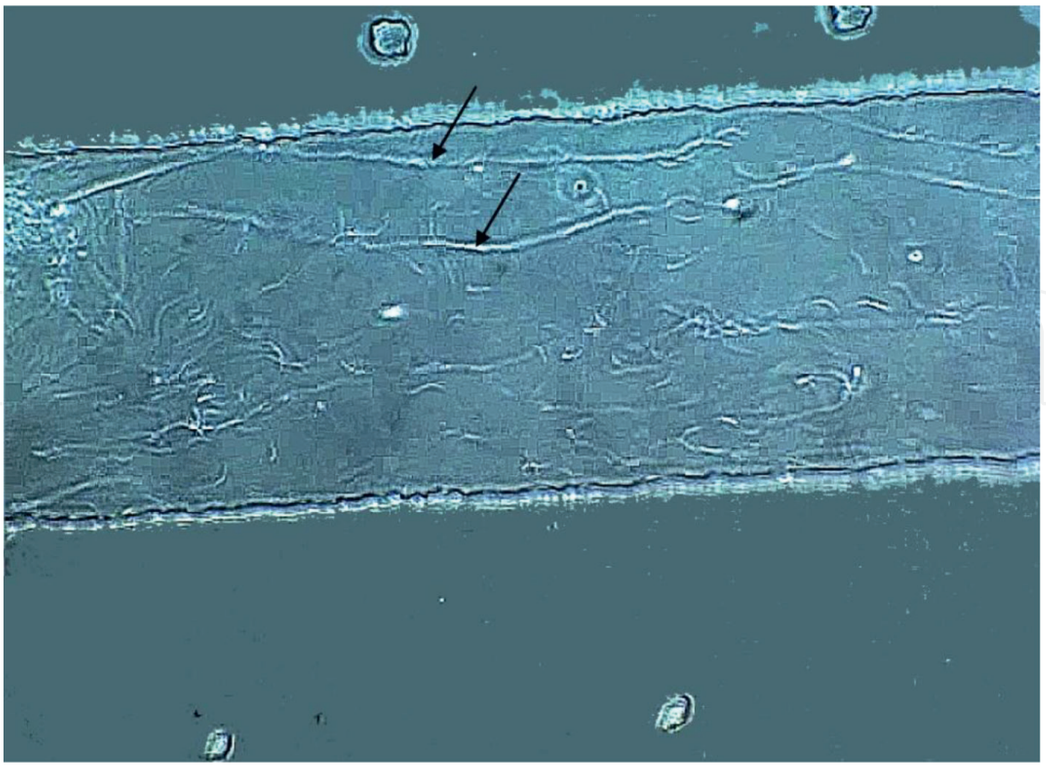

Figure 2.
Two Sharkasi sperm thread-like bundles swimming in a dynamic environment parallel to the sidewall of a microfluidic channel of 200 μm. 20 μm dimensions (W, H) under phase contrast microscope. A magnification of x400 was used [20].

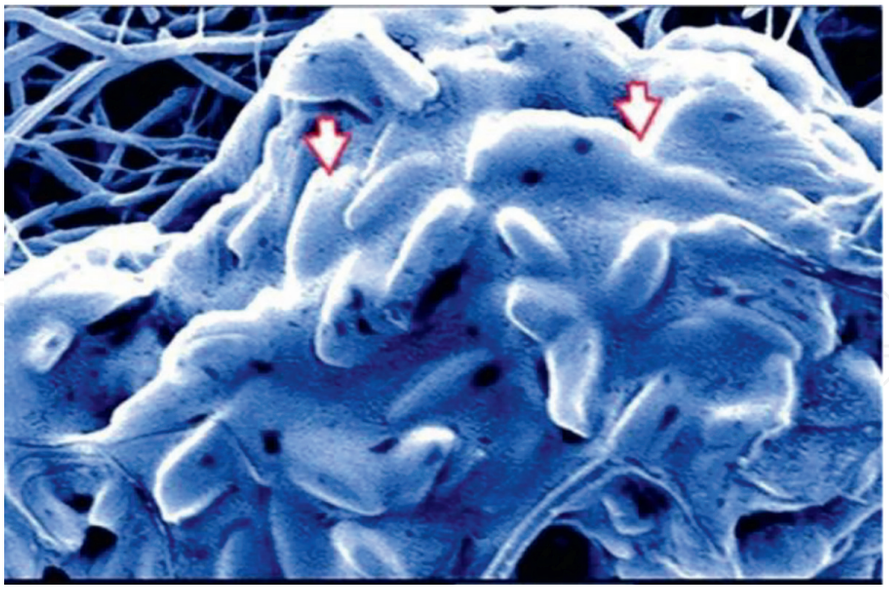

Figure 3.
Scanning electron micrographs of agglutinating sperm. Multiple sperm showing adhered heads and wrapped tails with evident agglutinating substance [20].

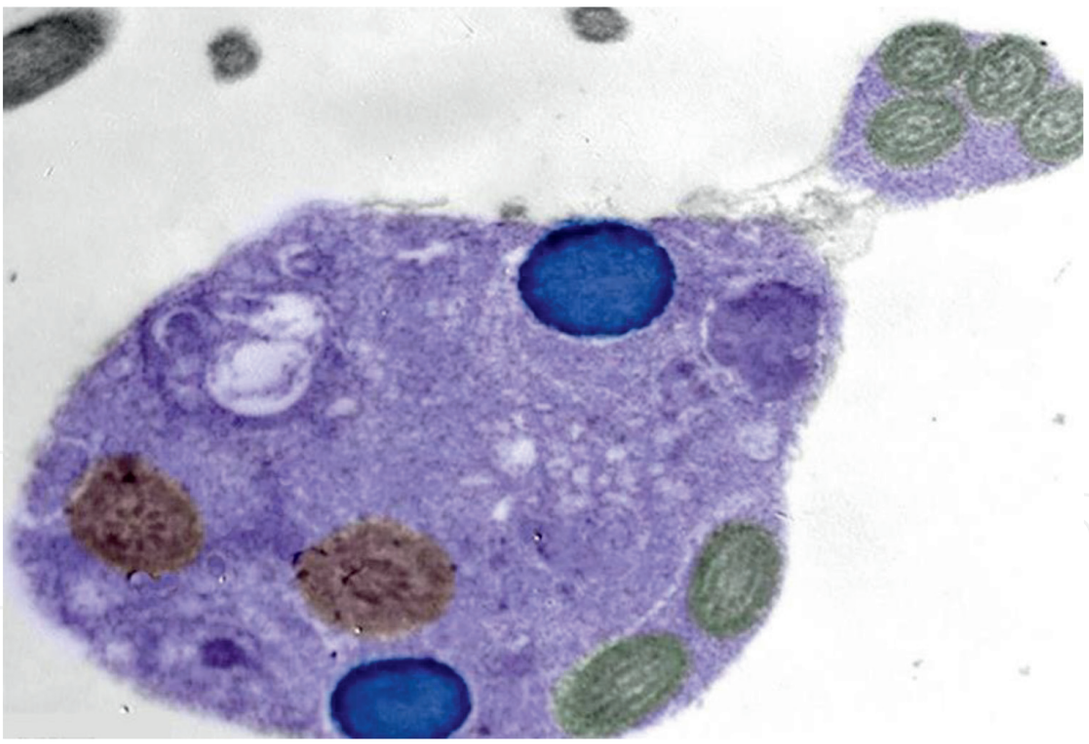

Figure 4.
Digitally colored transmission electron micrograph of a sperm bundle in a cut section show the sperm heads with two nuclei (blue color) and flagellum (green color) present with the agglutinating material shown in a light purple color [20].

at the terminal segment that act as paddles and aid in bundle movement. Furthermore, the authors reported that sperm bundles showed rheotactic behavior and swam parallel to each other when present in a slow fluid flow; however, as the velocity of the flow increased they started to overlap and stick to any stationary object (microchannel sidewall) so as not to be swept away with the flowing current [20]. These findings are

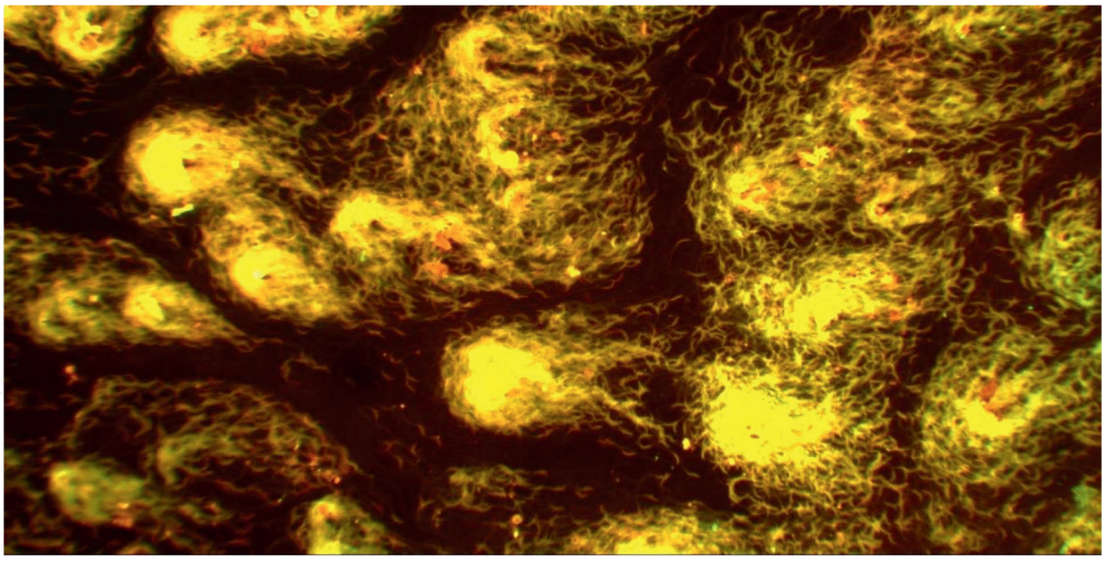

Figure 5.
Micrographs of sperm smears stained with Acridine orange showing sperm head aggregates coated with agglutinating material [20].

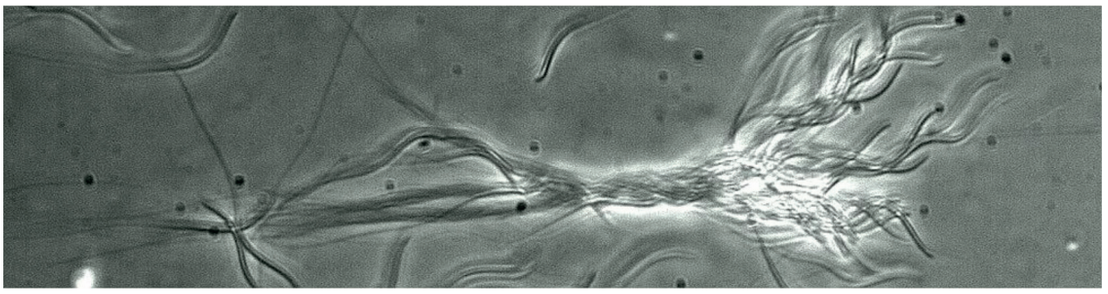

Figure 6.
An image of a developing sperm bundle under phase contrast microscope showing the initial segment of free heads and the terminal segment of adhered tails [20].

important as they proves that sperm agglutination can occur before mating and that the agglutinating substance originates from the male reproductive tract. Consequently, the bundle formation is not subject to being confined in a small area (SSTs) due to pressure as suggested previously [33]. Additionally, sperm bundles are motile, showing positive rheotaxis, and are capable of sticking firmly in a dynamic environment with high flow velocity. Also, scanning electron microscopy of sperm bundles revealed that the sperm were coated with copious amounts of an adhesive substance particularly in their head region. This indicates that sperm heads adhere in immobile bundles that occur after reaching the storage site (SSTs).

In another study, Sayed et al. [34] demonstrated that the tendency of sperm to agglutinate varies between chicken breeds. This might be attributed to variations in the amounts of the agglutinating substance secreted from the male's reproductive tract. Through artificial insemination of Sharkasi and Dandarawi hens with semen pools containing equal numbers of sperm from Sharaksi (showing high sperm agglutination) and Dandarawi (showing low sperm agglutination), the authors studied the relationship between sperm competitiveness and sperm tendency to agglutinate. There were no significant differences between Dandarawi and Sharkasi in terms of sperm morphometric measurements, straight-line and curvilinear velocities, and progressive motility, but Sharkasi roosters fathered the majority of the offspring. It

was suggested that the higher tendency of Sharkasi sperm to agglutinate inside the SSTs, ensures longer periods of residency within the female, which in turn increases their chances of fertizing more ova than Dandarawi sperm. Furthermore, Sayed et al. [34] reported longer fertility period in Sharkasi compared to Dandarawi chickens (22 vs. 14 days, respectively). It was suggested that Sharkasi sperm bundles remain in the SSTs for a longer time because they will spend more time completely dispersing compared to those in Dandarawi.

2. Conclusions

From the above-mentioned information, it can be concluded that intense sperm selection occurs in the vagina and that sperm mobility and rheotaxis are the determinant factors on the basis of which sperm selection in the vagina and sperm uptake in SSTs takes place. Sperm are capable of agglutinating in motile bundles having distinctive motility behaviors making them capable of clinging to adjacent surfaces. In the lumen of SSTs, sperm agglutinate in stationary bundles which prolongs sperm storage duration. Sperm gradually detach from the agglutinated sperm bundle and egress from the SSTs to ascend the oviduct and fertilize the ova. Therefore, sperm agglutination influences paternity outcomes when sperm from different males are present in a competitive situation because sperm bundles from males with a high tendency of sperm agglutination will remain in the SSTs for longer durations, and this gives them increased opportunities to fertilize more ova.

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Conflict of interest

The authors declare no conflict of interest.

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