

Post-Coital Sperm Competence in Polygamous Animals: The Role of Sperm Traits in Species – Specific Strategies

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Abstract

In some species, the females may mate with more than one male. In these polygamous animals, both pre-coital and post-coital strategies are required for reproductive success. This mini review aims at providing an update of the role of sperm traits in species-specific strategies for post-coital sperm competence. According to the sperm-competition theory, in such species the reproductive success of the males then depends upon both the magnitude of inter-male rivalry and covert sperm competition. The number of inseminated sperm, the percentage of sperm without morphological abnormalities in the ejaculates, and the sperm velocity variables play a significant role in sperm competition. Therefore, males that transfer more sperm per ejaculate or who copulate more often, are more likely to sire offspring. The transfer of large numbers of sperm is also guaranteed by ejaculates with high sperm concentrations. Usually, males of polygamous species have large testes and produce ejaculates with high sperm concentrations. Sperm competition acts as a selective force driving evolutionary change in sperm morphology and function. The degree of sperm pleiomorphy shown by a species varies according to the degree of sperm competition its behaviour entails. For example, the ejaculates of very polygamous species have much more homogenous sperm with lower sperm abnormalities. Finally, recent studies in birds have shown that the straight-line velocity (VSL) value, the most accurate estimate of sperm cell velocity, is of great importance in competition scenarios. Thus, sperm with a higher VSL affords a male fertilization advantages over his rivals.

Keywords: Sperm competence; Fertilization; Post-coital; Pre-coital; Polygamous animals

Introduction

Sperm selection mechanisms depend on both the self-selective propensity of sperm and on its ability to overcome the obstacles faced in the female reproductive system. The sperm of some individuals appears to enjoy advantages over that of their rivals [1]. This is particularly important in polygamous species that practice polyandry, including many ruminants and several species of bird (e.g., the chicken and capercaillie). However, a certain degree of polyandry is seen even in theoretically monogamous species, e.g., in partridges, finches and primate species, including humans.

Pre-coital and Post-coital Strategies for Reproductive Success in Polygamous Animals

In many non-mammalian species, in which female preference is important in mate selection, a preference for males showing exaggerated sexual selection traits has been reported [2-4]. Most studies on these groups have focused on the ornaments to which female attention is paid. Research into mate selection in mammals, however, has focused mainly on the role played by weaponised male secondary sexual characteristics. Antlers and horns are employed in the pre-coital strategies of several ruminant species, the males using them to establish their dominance. A relationship between horn development and seasonal changes in breeding activity is clearly evident, with wild ruminants attaining maximum horn development just before the onset of the breeding season [5]. However, this weaponry also appears to be a sensitive indicator of genetic stress [6,7] and a means of providing signals of male vigour for attracting females [8]. The role of symmetry (a characteristic of genetic vigour) in the attraction of females is well established in many species, including humans [9]. Thus, dominant males with larger and more symmetrical horns are likely to be selected for reproduction via easier access to receptive females, but also via the latter's preference for them.

However, in some species, the females may mate with more than one male [10]. According to the "sperm-competition" theory, in such species the reproductive success of the males then depends upon both the magnitude of inter-male rivalry and covert sperm competition [11]. Reproductive success is therefore a function of both pre- and post-copulatory strategies [11]. Certainly, larger weaponry is often associated with better sperm quality. For example, in red deer (*Cervus elaphus*), antler size and complexity is associated with testis size and sperm velocity [12], and in ibexes, those with the largest and best-developed ornamentation produce better quality semen (the proportion of motile spermatozoa is larger and the number of primary morphological sperm abnormalities smaller) [13]. This provides some evidence to support the phenotype-linked fertility insurance hypothesis [14]. It may even be that wild bovid and cervid females use horn/antler quality as an index of male genetic and sperm quality [12,15]. However, when the ejaculates of two or more males must compete to fertilize a set of ova, the quality of the sperm they carry becomes of great importance. For example, chicken (*Gallus gallus*) females commonly copulate with multiple males and store their sperm in storage tubules for two-three weeks [16]. A rooster's chances of fathering offspring therefore depend not just on his ability to fight with his spurs, but on the ability of his ejaculate to outrun the stored sperm of his rivals [17,18]. Dominant males may have more

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copulation opportunities and thus deposit relatively more sperm in females [19], but at the time of egg fertilization, having fast sperm is vital in helping them finally beat their competitors [20]. A background for sexual selection among males both before and after copulation is thus established [21].

Roosters also have comparatively large testes, a trait shared with many species with multi-male mating systems, including butterflies [22], fish [23], birds [24,25], frogs [26], primates [27,28], terrestrial mammal's carnivores [29] and ungulates [30]. In some wild ruminants there is even a correlation between testis size and horn quality (size, symmetry) [31], again highlighting that pre-coital and post-coital sperm selection strategies can be inextricably linked.

Sperm Competition: The Race for Fertilization, a Question of Quantity

Sperm competition occurs when spermatozoa from more than one male have the chance of fertilizing the eggs of a polyandrous female [32]. A number of hypotheses attempt to explain the evolutionary benefits that females may derive from polyandry and sperm competition [33]. The females ensure their eggs are fertilized by the best sperm, thus maximizing offspring fitness ("good sperm/genes hypothesis") [34], and they may minimize the risks associated with foeto-maternal immunological incompatibility or maternal vs. paternal genomic conflicts ("genetic incompatibility avoidance" hypothesis) [35]. Further, in the context of a socially monogamous pair, extra-pair copulations compensate for having chosen a poor quality 'regular' mate (the "trading-up" hypothesis) [36].

The transfer of large numbers of spermatozoa in competitive situations is an effective strategy; males that transfer more sperm per ejaculate, or who copulate more often, are more likely to sire offspring. In wild ruminants, the number of matings during the short rutting season (about three months in most species) is extremely high, thus allowing more sperm to be delivered to the females. However, males will make special attempts to mount females that they have seen serviced by other males - and produce larger than normal ejaculates when they do. They also produce larger ejaculates after periods of separation from females [10] (as do human males after long periods away from their normal mates [37]). Similarly, roosters deliver different numbers of sperms into different hens depending on the perceived degree of their promiscuity and the development of their sexual ornamentation [38].

The transfer of large numbers of sperm is also guaranteed by ejaculates with high sperm concentrations. Usually, males of polygamous species have large testes and produce ejaculates with high sperm concentrations [39,40]; larger testes produce more sperm than smaller testes, allowing males to either deliver larger or more frequent ejaculates [32].

Paternity identification techniques have revealed unsuspected levels of extra-pair matings across many species. For example, in the red legged partridge (*Alectoris rufa*), classically considered a monogamous species in which a pair remains together during the reproductive period, the extra-pair paternity rate is some 61%. Polygamy is even more extended in the chukar partridge (*Alectoris chukar*). These species and their crosses therefore provide good experimental models for studying sperm competition in birds. It is already known that sperm concentration in the more polygamous chukar partridge is higher (mean 1480 million sperm/mL) than in its less polyandrous red-legged cousin (mean 750 million sperm / mL) [40]. Male partridges that show

a stronger trend towards polygamy (i.e., those with a tendency to be unfaithful to their mate) may produce more sperm than others.

Sperm Abnormalities and Competition Capacity

Sperm competition acts as a selective force driving evolutionary change in sperm morphology and function [41]. Thus, sperm morphology may predict sperm competition success (i.e. paternity) in some species [42]. Certainly, it has been demonstrated that sperm cells with longer tails have a competitive advantage over shorter-tailed sperm [43]. The degree of sperm pleiomorphy (within-male variation in sperm morphology) shown by a species varies according to the degree of sperm competition its behaviour entails. For example, the natural ejaculates of the golden eagle (*Aquila chrysaetos*) contain very heterogeneous spermatozoa (over 37% of their sperms may show head abnormalities) as well as large numbers of spermatocytes and spermatids [44]. This species shows a degree of monogamy unusual even among birds - a male may never stray from its mate during the course of their reproductive life together, perhaps because breeding success requires the investment of considerable energy in caring for offspring and mate. In the peregrine falcon, another monogamous species, spermatogonia and spermatids in ejaculates can be even more common [45]. For example, spermatids make up a mean 37% of all spermatozoa in a falcon's ejaculate, but can account for as many as 75% (Figure 1). It has been suggested that these abnormalities in sperm morphology are the result of suppression of the final stages of spermiogenesis and the retention of an immature morphology [46]. In contrast, the ejaculates of very polygamous birds, such as the chicken, have much more homogenous sperm, with far fewer (<20%) showing head abnormalities [47] (Figure 2). Differences in the degrees of pleiomorphy are also seen between smaller birds. In the Eurasian bullfinch (*Pyrrhula pyrrhula*), a more monogamous species in which sperm competition is low, 8-18% of sperms may be immature/show head abnormalities, while in the polygamous dunnoek (*Prunella modularis*), in which sperm competition is intense, only 4-5% may be so affected [48]. Indeed, the percentage of sperm morphological abnormalities would appear to indicate the degree of monogamy in most species that have been studied. Human sperm show a significant incidence of pleomorphism, with high percentage of sperm abnormalities and presence of immature germinal cells, unlike primate

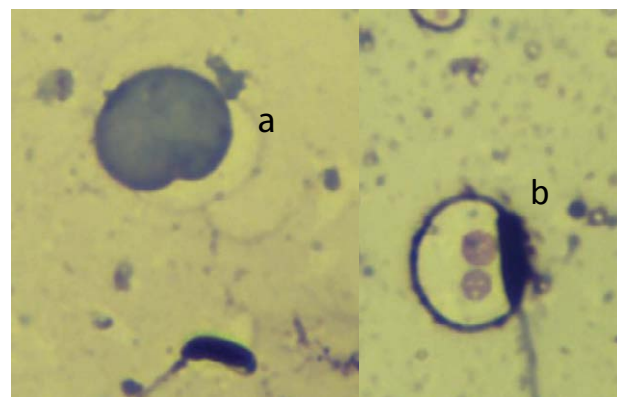


Figure 1: The ejaculates of monogamous species, such as the peregrine falcon (*Falco peregrinus*), are usually heterogeneous and contain high percentages of immature cells (spermatocytes [a] and spermatids [b]) (magnification 1000x, cells stained with aniline blue).



Figure 2: Homogeneous sperms are characteristic of polygynous species (e.g., the chicken) (magnification 1000x, sperm stained with Hemacolor®).

species highly polygamous (e.g. chimpanzees, *Pan troglodytes*). Since one of the most important sperm quality variables is the normal sperm morphology, a major problem of morphological analysis in human is related pleomorphism spermatozoa, and sometimes, precise definition of limits of normal human spermatozoa may be confused [49]. Humans and gorillas (*Gorilla* spp) share a high incidence of pleomorphism [50], which may be associated to the evolution of sperm morphology under conditions of low levels of sperm competition [46].

The question arises, however: *what is the function of these immature cells in the ejaculates of monogamous species?* In certain invertebrate species, such as *Bombyx mori*, it has been suggested that non-fertile sperm aid the successful transfer of the fertile sperm to the site of sperm storage or fertilization in the female [51]. It has been suggested that some morphological forms of human sperm have evolved to perform different roles in relation to sperm competition (e.g. kamikaze and egg-getter sperm [52]). However, the role of these cells in bird ejaculates remains unknown. It has been suggested that alterations in sperm morphology are largely due to errors during spermatogenesis, and that these errors may be increased in species with low level sperm competition.

Sperm Velocity Variables and their Importance in Competition

A number of key questions have been addressed to determine the diagnostic significance of sperm movement characteristics on male fertility [53]. Certainly, sperm assessment methods are used extensively in clinical studies of infertility, and computer-assisted methods (CASA) are available for examining sperm motility and morphology [49]. The latter are also useful for detecting heterogeneity among ejaculates affecting sperm competition capacity. CASA methods can record the percentage of motile spermatozoa, and the percentage showing progressive motility (spermatozoa swimming forward quickly in a straight line), as well as the movement characteristics of spermatozoa, i.e., their curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), amplitude of lateral head displacement (ALH), and beat-cross frequency (BCF).

Recent studies in birds have shown that the VSL value, the most accurate estimate of sperm cell velocity, is of great importance in competition scenarios: the higher the VSL of a male's sperm, the more likely it is to outcompete that of rival males. In a competitive scenario in which equal numbers of sperm from high- and low-motility males were mixed, that of the high-motility males fertilized the majority of the eggs. The use of a multi-breed mating model has shown that the sperm of some chicken breeds, traditionally thought to be of moderate fertility, such as the Blue Andalusian breed, compensate for this drawback by having sperm with better movement characteristics, including a high VSL [54]. Differences in VSL values (which may be heritable) are attributable to the rate of ATP synthesis. Social status may also affect VSL [20].

However, the VSL is not the only important variable in avian sperm competition. Motile sperm actively ascend the vagina and enter the sperm storage tubules (SSTs) (from where they progress through the oviduct by antiperistalsis to reach the infundibulum where fertilization occurs), but the mechanisms of SST filling and emptying are not well known. It is known, however, that when a hen mates with multiple roosters, it is the sperm of the last male that usually fertilizes most of the eggs [55]. Similarly, when hens are artificially inseminated in a sequential fashion with the semen of two roosters, the larger proportion of offspring is fathered by the second male [56]. This phenomenon is known as "last male precedence". It has several possible explanations, including sperm stratification in the SSTs, the displacement of previous ejaculates, passive sperm loss [57], and the sequential maturation of the SSTs according to the time of the breeding cycle [58]. While sperm quality varies among males within a breed [59], it may also vary between breeds [60]. Breed differences in sperm quality might determine the importance and grade of intensity of last male precedence mechanisms. The influence of sperm variables (especially sperm motility variables) on fertility and on last male precedence have been examined using sperm mobility phenotypes within breeds [61]. In a multibreed mating scenario, breeds with high VSL values afford them a competitive advantage. These findings suggest that VSL play the most important role in securing last male precedence.

In mammals, sperm length has been positively correlated with maximum sperm velocity [62]. Although larger sperm heads might be thought a handicap to rapid swimming, several reports show that sperms with longer heads may swim faster [62,63], affording them a competitive advantage. Several authors indicate that males of species with polyandrous females have longer and faster sperms than those with sexually monogamous females [64]. Membrane potential of mitochondria (a trait associated with sperm velocity) in human's sperm is lower than in chimpanzees, a species with multiple partner mating system [65]. The comparison of sperm functionality in these species reveals an influence of sperm competition on sperm functionality during capacitation. Whereas chimpanzee sperm show maintenance or increase of membrane potential after capacitation, sperm from humans show reduction in membrane potential [65]. Competition between spermatozoa from rival males continues at the site of fertilization. Thus, differences between species in levels of sperm competition are related with the proportion of spermatozoa that undergo capacitation and with the proportion of spermatozoa that respond to ovum-associated signal [66]. Although sperm competition seems to have influence on post-testicular sperm maturation and capacitation in mammals, avian sperm need little post-testicular maturation and do not require capacitation before they are capable of fertilizing an ovum.

In a recent study, the sperm traits of native Iberian red-legged partridges (*A. rufa*) were compared to those of sperm from red-legged partridges with introgression from *A. chukar* (an Asiatic more polygamous partridge species) [40]. Since *A. chukar* is more promiscuous than *A. rufa*, more polyandry can be expected in their hybrids than in pure *A. rufa* birds. In agreement with the above, the sperm heads of the pure birds were shorter. Further, the coefficients of variation for all the morphometric sperm head variables examined (length, width, area) were greater in the pure than in the hybrid partridges. At least in passerine birds, smaller intra-specific variation in sperm morphometry is related to an increased risk of sperm competition [67]. In addition, the VSL was higher in the more polygamous hybrid partridges [40]. As in the chicken, the VSL therefore appears to be the most important sperm trait in competition scenarios.

Sperm velocity can differ between ejaculates of the same male, depending upon the risk of sperm competition [48]. The presence or absence of females can also affect this variable, highlighting its dependence on different stimuli. Roosters housed with females produce sperm with higher VSL and lower non-progressive motility values, and have fewer slow sperms than do those housed without females; sperm quantity (volume and concentration), in contrast, is unaffected [68]. The presence of females might lead to endocrine changes in the males (e.g., in testosterone and luteinizing hormone levels), leading to alterations in motility. In conclusion, in many species the VSL varies depending on their degree of polygamy. This variable plays an important role in sperm competition: sperm with a higher VSL affords a male fertilization advantages over his rivals.

This review has provided an update of the role of sperm traits in species-specific strategies for post-coital sperm competence. Sperm competition is a complex process that involves many factors. Recent advances in spermatology of avian species have allowed identifying the role of certain traits, such as sperm morphology and the movement characteristics of spermatozoa. Because it has been reported that ejaculates from several species are composed of well-defined subpopulations, further studies should be performed to determine the role of sperm subpopulations, characterized by precise values of the motion parameters or morphometric characteristics, on sperm competition.

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