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Physiology

Female nutritional condition affects ovarian fluid quality in guppies

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Male and female gametes are often embedded in fluids that are produced by gonads and other reproductive tissues. Female reproductive fluids, usually called ovarian fluid (OF), which often constitute a relevant volumetric component of the egg mass, are rich in ions, sugars and proteins, and are involved in several functions, from protecting gametes to facilitating fertilization, and often act as mediators of post-mating sexual selection. Despite their applied and evolutionary importance, we know virtually nothing about the costs of female reproductive fluid production. We investigated the effect of nutritional condition on OF quality by experimentally manipulating the diet of two groups of female guppies (*Poecilia reticulata*) which were maintained for 20 days either on a restricted diet or had ad libitum access to food. In this species, OF enhances sperm swimming longevity and velocity (a predictor of sperm competition success) and mediates post-copulatory inbreeding avoidance. We found that sperm velocity was significantly lower in the OF of diet-restricted females, indicating that OF quality is dependent on female nutritional condition. Our results demonstrate that OF represents a non-trivial component of female reproductive investment and provides a tool to investigate which OF constituents are involved in modulating OF–sperm interactions and fertilization.

1. Introduction

Eggs are larger than sperm, and female reproductive investment per unit gamete is, therefore, larger than in males [1]. At fertilization, gametes are usually embedded within fluids (ovarian fluid, OF, in the females and seminal fluid, SF, in males), produced by the gonads or by accessory glands. These fluids can in some cases exceed in volume that of the gametes, as in humans, where SF accounts for 95% of the volume of the ejaculate [2]. OF volume is usually smaller, but can represent up to 30% of the spawned reproductive material in several fish species [3]. Therefore, SF and OF likely represent an important component of male and female reproductive investment. While costs and benefits of SF production have been intensely studied in recent decades [4], less is known about the OF. OFs are rich in ions, sugars, hormones and proteins, whose concentration can be 10 times higher than that observed in the SF [5]. The diversity of OF proteins is also impressive, with hundreds of different proteins identified (e.g. [6,7]). The OF has several functions, from enhancing sperm performance to protecting sperm and eggs from adverse environmental conditions [8–10]. It is increasingly evident that female reproductive fluids are also involved in post-mating sexual selection, by differentially affecting sperm performance and mediating individual male's fertilization success [11]. Despite their importance in female reproduction, the costs of OF production, and, more generally, the effect of female condition on OF quality has been relatively little investigated. For example, it is known

Table 1. Effect of the ovarian fluid on sperm velocity in relation to female diet. Effect size and Cohen's *d* calculation was based on the paired *t*-test statistics. Each male's ($n = 17$) sperm velocity was measured in the OF of two different individual females, one per each diet treatment (ad libitum, $n = 17$; restricted, $n = 17$).

sperm velocity	diet	mean (95% CI)	mean difference AL − R (95% CI)	paired *t* (d.f. = 16)	*p*-value	effect size *r*	Cohen's *d*
VAP ($\mu m\ s^{-1}$)	AL	94.4 (88.1–100.7)	9.38 (1.8–16.9)	2.64	0.018	0.551	0.766
	R	85.0 (78.3–91.7)					
VCL ($\mu m\ s^{-1}$)	AL	120.2 (115.0–125.5)	10.99 (4.0–18.0)	3.31	0.004	0.637	1.070
	R	109.3 (104.0–114.5)					

that female nutritional conditions influence OF composition in mammals (e.g. [12]), but it is less well understood whether and how these changes in composition affect the capability of the OF to accomplish its functions (but see [13]).

In this study, we experimentally investigated the relationship between a female's nutritional condition and the effect that her OF has on sperm performance in the guppy *Poecilia reticulata*, an internally fertilizing freshwater fish. In this species, OF enhances sperm velocity [14], protects sperm cells from temporal viability decline [10] and mediates post-mating inbreeding avoidance by differentially enhancing sperm velocity of related and unrelated males [15]. The capability of the OF to modulate sperm performance, by increasing sperm longevity and/or velocity, has significant consequences on female reproductive fitness in this species [15]. To investigate if female condition influences the effect of OF on sperm performance, we assigned two groups of female guppies to either an ad libitum (AL) or a restricted (R) food diet for 20 days, and subsequently, measured the enhancing effect of their OF on sperm velocity. We predicted that the OF of R-diet females has a reduced capability to enhance sperm velocity.

2. Methods

The guppies used in this experiment were descendants of wild-caught fish collected from the lower part of Tacarigua River in Trinidad, and were maintained in large stock tanks (150 l) (see [15] for details on fish maintenance). Females were randomly assigned either to AL diet or to R diet (150 and 50 nauplii twice a day for 20 days, respectively), following an established protocol [16]. This type of diet treatment has a measurable effect on female condition [17]. During the diet treatment, females were isolated from males and, starting 5 days after the beginning of the diet treatment, were checked daily for brood production. Since the effect of the OF on sperm velocity varies according to female reproductive stage [14], we used only females in late pregnancy (see the electronic supplementary material). OF was collected, blind of diet treatment, from anaesthetized females following an established procedure [15] (see the electronic supplementary material for more details). Sperm swimming velocity was measured in a solution containing 3 µl of the OF sample retrieved from the female and 2 µl of sperm activating solution (150 mM KCl and 4 mg ml^{-1} BSA) [14,15]. Males used in the experiment were anaesthetized as above and sperm bundles were collected following a standard procedure [15]. To control for intrinsic differences in sperm velocity among males, each male's ejaculate was split into two aliquots and sperm velocity was measured in the OF of one AL and one R female (final sample size included 17 males, 17 R and 17 AL females, electronic supplementary material, figure 1S). To this end, intact sperm bundles were placed on a multi-well slide containing the OF solution and the swimming velocity of the sperm leaving the bundles was recorded using a CEROS sperm tracker [15]. We obtained two sperm velocity estimates, namely VAP (average velocity over a smoothed path) and VCL (curvilinear velocity). These measures are significantly repeatable within male [18] and predict competitive fertilization success in guppies [19]. Mean sperm velocity in the OF of AL and R females were compared in a paired *t*-test (SPSS v. 24). For more details on methodology, see the electronic supplementary material. Within-male order of analysis was alternated between diet groups.

3. Results

VAP and VCL were significantly lower in the OF of R females than in the OF of AL females (table 1 and figure 1; electronic supplementary material, table 2S). Taking mean sperm velocity in AL OF as reference, the mean reduction in sperm swimming velocity associated with diet restriction was −12.8% (±4.62 s.e.) and −10.9% (±3.28 s.e.), for VAP and VCL, respectively (electronic supplementary material, figure 2S). In particular, sperm velocity was slower in the OF retrieved from R females, than that in the OF retrieved from AL females, in 13 (VAP) and 14 (VCL) of the 17 males used. The observed differences in sperm velocity measured in the OF samples of AL and R females were not associated with the within-male order of analysis nor with individual differences in female body size (electronic supplementary material, figures 2S, 3S and table 2S).

4. Discussion

Female diet restriction significantly affected OF capability to enhance sperm velocity. A previous study found that sperm swim on average 10.3% faster in the OF of AL females as compared to the velocity expressed in the control solution only [15]. This difference is similar to that observed here between AL and R females' OF, suggesting that the OF produced by R females may have completely lost its capability to enhance sperm velocity. In fish, the characteristics of OF that seem to have the largest effect on sperm swimming are pH, inorganic and protein composition, and viscosity [13,20,21]. The analysis of these OF characteristics (including OF volume and viscosity) would be necessary to shed light on the physiological mechanism linking a guppy female's nutritional status with the effect that her OF has on sperm performance [10,14]. It would also be interesting to extend this investigation to external fertilizers, as female nutritional

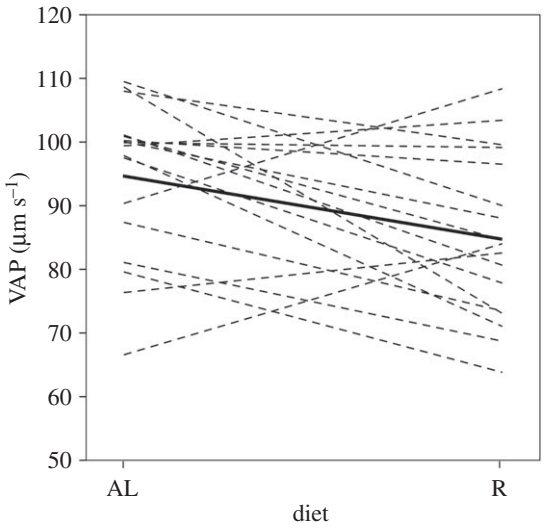

Figure 1. *In vitro* sperm velocity (VAP) in the ovarian fluid (OF) of females that were maintained on restricted (R) or ad libitum (AL) diets. Dotted lines connect sperm velocity measures from the same individual male in the OF of two different individual females. Solid line represents the average velocity in the two conditions.

condition may contribute to explaining, for example, the low quality of the OF of farmed fish [21].

Our findings have several important implications. It is increasingly evident that OF–sperm interactions are pivotal in the fertilization process [22] and subsequent embryo development [7]. The OF–sperm interaction may have important applications, from aquaculture [5] to conservation [21]. The role of OF in post-mating sexual selection has become increasingly apparent [11], for example, in reducing the risk of inbreeding [15,23] or favouring genetically compatible males in externally fertilizing species [24]. While the knowledge of OF composition is rapidly accumulating [5,6], we know relatively little about the specific role that different OF components have in OF–sperm interactions [13] and how they are implicated in mediating post-mating sexual selection [11] and offspring fitness. Our results indicate that female condition should be considered in future studies on OF–sperm interaction. They also suggest that the phenotypic manipulation of female condition represents a powerful tool to identify the OF components that are primarily involved in the interaction with sperm at fertilization and in the subsequent events, from fertilization to embryo development, and that can ultimately influence offspring fitness [25].

Ethics. The experimental procedures used in this study were approved by the Ethics Committee of the University of Padova, according to the Italian legal requirements (permit no. CEASA 178739, 23/09/2014 Tit. III Cl. 13 Fasc. 55).

Data accessibility. Data are available from the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.n3q5t28) [26].

Authors' contributions. G.C. performed the diet manipulation, OF collection and sperm velocity assays. The authors equally contributed to the conception and design of the experiment and to analysis and interpretation of data. They both contributed to draft the article. They approved the version to be published and agreed to be accountable for all aspects of the work in ensuring that all questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Competing interests. The authors declare no conflict of interest.

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