

Effect of Anserine and Carnosine on Sperm Motility in the Japanese Quail

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Sperm motility is considered as one of the most important traits for successful fertilization, but the motility of an ejaculated sperm decreases with time when stored as liquid. It is reported that seminal plasma serves as a nutrient rich medium for sperm and plays an important role in sperm motility and its fertilization ability. Several studies have reported that imidazole dipeptides such as anserine and carnosine affect sperm motility and its fertilization ability in mammals. In this study, we report the presence of anserine and carnosine in the male reproductive tract of the Japanese quail. Abundant levels of anserine (44.46 μ M) and carnosine (41.75 μ M) were detected in the testicular fluid and seminal plasma respectively using the amino acid analyzer; however, seminal plasma solely contained carnosine. When the ejaculates were incubated with anserine or carnosine, we found that both the dipeptides improve sperm motility parameters such as straight line velocity, curvilinear velocity, average path velocity and amplitude of lateral head displacement after *in vitro* sperm storage at 15°C. These results indicate that imidazole dipeptides are present in the male reproductive tract and may improve sperm quality during *in vitro* sperm storage in the liquid states.

Key words: anserine, carnosine, imidazole dipeptide, male reproductive tract, the Japanese quail

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Introduction

In animals, sperm motility is an important trait for successful fertilization (Florman and Ducibella, 2006). In birds, ejaculated sperms are stored in sperm storage tubules (SSTs) of the female reproductive tract before fertilization (Sasanami *et al.*, 2013); motility is considered to be an important factor responsible for sperm uptake into the SSTs (Froman, 2003). Essentially, an avian sperm undergoes an intense selection process in the female reproductive tract; immotile sperms are incapable of fertilizing the eggs (Allen and Grigg, 1957).

The modern poultry industry promises to provide superior quality meat and eggs. To propagate the next generation, artificial insemination (AI) is now widely being used in

breeder farms (Łukaszewicz, 2010). Although cryopreservation has long been recognized for germplasm preservation in mammals including livestock and laboratory animals, the technique is not reliable in birds due to low fertilization ability of frozen/thawed sperms (Long, 2006; Long *et al.*, 2014). It is reported that cryopreservation damages sperm plasma membrane (Bakst and Sexton, 1979) and decreases the morphological integrity of the sperm (Blesbois *et al.*, 2005). To minimize fertility loss, sperm may be stored as liquid in birds, but the motility of an ejaculated sperm decreases with time upon storage as liquid *in vitro* (Kotłowska *et al.*, 2007).

Seminal plasma (SP), a complex fluid added to the sperm in the male reproductive tract (Duncan and Thompson, 2007) serves as a nutrient rich medium (Juyena and Stelletta, 2012) and plays an important role in sperm maintenance, viability and transportation into female reproductive tract (Poiani, 2006). The role of SP proteins, peptides and amino acids has been widely investigated in mammals (Manjunath *et al.*, 2007; Koppers *et al.*, 2011; Crawford *et al.*, 2015; Viana *et al.*, 2018). Several studies have emphasized the important effects of imidazole dipeptides such as anserine and carnosine on sperm motility and its fertilization ability in vari-

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ous species (Siems *et al.*, 2003; Tareq *et al.*, 2008; Bosler *et al.*, 2014). Carnosine is a naturally occurring dipeptide consisting of β -alanine and histidine while anserine is a methylated form of carnosine (Boldyrev *et al.*, 2013). Because both anserine and carnosine are histidine-containing dipeptides, it is thought that they play similar physiological roles (Boldyrev *et al.*, 2013) such as exhibiting buffering capacity at neutral pH (Tanokura, 1983; Sale *et al.*, 2010). Essentially, both these imidazole dipeptides have been reported to maintain muscle buffering capacity and muscle functions (Begum *et al.*, 2005). In domestic birds such as chicken, imidazole dipeptides are reported to be present in the skeletal muscles and are known for their antioxidant capacity (Sato *et al.*, 2008); however, to our knowledge, there are no reports on the presence of these dipeptides in the male reproductive tract of birds. It was therefore interesting to investigate their existence in the male reproductive tract. Here, we report the presence of imidazole dipeptides, anserine and carnosine in the male reproductive tract of the Japanese quail and demonstrate that the dipeptides improve sperm motility even when stored *in vitro* in the liquid state.

Materials and Methods

Animal Care and Management

Male Japanese quails (*Coturnix japonica*), 40–60 weeks of age (Quail cosmos, Toyohashi, Japan) were maintained in individual cages under photoperiods of 14h light and 10h dark cycle (lights went on at 05:00). Birds were allowed to eat commercial feed (Toyohashi Feed Mills, Toyohashi, Japan) and drinking water *ad libitum*. Animal management and all experimental procedures were carried out in accordance with the approved guidelines of the Animal Care Committees of Shizuoka University (Approval number: 2018A-5).

Preparation of Testicular Fluid, Seminal Plasma, Cloacal Gland Secretion and Blood Serum

Three male quails were decapitated and dissected to harvest the testis. Briefly, the testis was cut and fluid was collected from inside the testis using a micropipette. The fluid (100 μ l) from each of the testis was pooled and the mixture was centrifuged at 15,000 $\times g$ for 10 min at 4°C; the supernatant was collected as testicular fluid. For the preparation of SP, ejaculates from 10 mature male quails were pooled, centrifuged at 5,000 $\times g$ for 3 min at 4°C, and supernatant was collected. For the preparation of cloacal gland secretion, cloacal foam was obtained from 10 male quails by pressing the gland. The foam was centrifuged at 20,400 $\times g$ for 5 min at 21°C. Blood was collected from the wing vein of 3 males and centrifuged at 2,000 $\times g$ for 20 min at 4°C for collection of serum; 100 μ l of each sample was pooled. The isolated testicular fluid, SP, cloacal gland secretion and blood serum were stored at –20°C until further use.

Semen Collection and Processing

Hanks balanced salt solution (HBSS) containing 136 mM NaCl, 5.4 mM KCl, 0.8 mM MgSO₄, 1.26 mM CaCl₂, 4.2 mM NaHCO₃ and 5.6 mM glucose was used as sperm diluent. HBSS was incubated at 37°C for 10 min before semen

collection. Semen was obtained during mating from mature male quails prior to ejaculation, in accordance with the procedure by Kuroki and Mori (1997) and suspended in 500 μ l HBSS. Sperm cells were counted microscopically using a hemocytometer (BX 51, Olympus Optics, Tokyo, Japan) and prepared to reach a final concentration of 1×10^8 sperm/ml. 100 μ l of HBSS containing varying concentrations of anserine (L-Anserine Nitrate, Fujifilm Wako Pure Chemical Co., Osaka, Japan) or carnosine (L-Carnosine, Wako Pure Chemical Corporation) were added to the sperm suspension to achieve a final concentration of 2×10^7 sperm/ml. Suspensions were incubated at 15°C upto 12 h. The optimum concentration of anserine and carnosine was determined from our preliminary experiments in which the ejaculated spermatozoa were incubated with either 1 μ M, 3 μ M, 10 μ M, 30 μ M or 100 μ M of the dipeptides. We found that more than 3 μ M of anserine and carnosine had no further potentiation effect on sperm motility (data not shown).

Sperm Motility Test Using Computer Assisted Semen Analysis (CASA)

Sperm suspensions were collected from the droplets at varying incubation times: 1 h, 3 h, 6 h, 9 h and 12 h. Sperm motility was analyzed using the CASA system (SMAS, Ver. 3, DITECT Digital Image Technology, Japan). Glass slides were coated with 2% BSA and warmed on a hot plate at 39°C. Sperm suspensions were placed on the warm glass slide, covered with cover glass, and observed microscopically (Nikon ECLIPSE E200, Nikon, Tokyo, Japan). Sperm motility parameters such as straight line velocity (VSL μ m/s), curvilinear velocity (VCL μ m/s), average path velocity (VAP μ m/s), linearity (LIN) (LIN = VSL/VCL), straightness (STR) (STR = VSL/VAP), wobble (WOB) (WOB = VAP/VCL), amplitude of lateral head displacement (ALH μ m), beat-cross frequency (BCF Hz) and motility rate (%) were recorded.

Detection of Anserine and Carnosine in the Male Reproductive Tract

The testicular fluid, SP, cloacal gland secretion and blood serum were diluted 2 times with water and mixed with 3% sulfosalicylic acid in a ratio of 1:1. The mixture was centrifuged at 20,400 $\times g$ for 10 min at 4°C and the supernatant was analyzed using an amino acid analyzer (JCL-500/V2; JEOL, Tokyo, Japan), as previously described (Imanari *et al.*, 2010). In brief, free amino acids were separated on a strong cation exchange column using lithium citrate buffers of different pH for elution. The amino acid derivatives were detected using ninhydrin at 440 nm or 570 nm at a reference wavelength of 690 nm. A solution of amino acid standards (Fujifilm Wako Pure Chemical Co.) was used as an external standard.

Data Analysis

All the data analyses were performed using the statistical package R version 3.3.3 (The R Foundation for Statistical Computing Platform, <https://cran.r-project.org/bin/macosx/>). Shapiro-Wilk test was performed to confirm the distribution normality. Percentage data were arcsine transformed before analysis and significance was based on the transformed mean value. Data was analyzed for significant differences using

one-way analysis of variance (ANOVA). Differences between treatments were analyzed with Tukey's honestly significant difference test. Declaration of significance were based on $P < 0.05$.

Results

Presence of Anserine and Carnosine in the Male Reproductive Tract

Male reproductive tract of the Japanese quail was investigated for the presence of anserine, carnosine and their constituents— β -alanine, 1-methylhistidine, and histidine (Table 1). In the testicular fluid, anserine was detected but carnosine was below detection limits; seminal plasma solely contained carnosine. The constituents histidine, β -alanine, and 1-methylhistidine were present in both the samples. In case of cloacal gland secretion, neither anserine nor carnosine was detected. Blood serum contained both imidazole dipeptides as well as their constituents except for β -alanine.

Effects of Anserine and Carnosine on Sperm Motility

In the next set of experiments, we incubated the sperm with different concentrations of either anserine or carnosine: 0 μ M, 0.1 μ M, 0.3 μ M, 1 μ M or 3 μ M (Table 2). No significant difference was observed in sperm motility at 0.1 μ M concentration of anserine or carnosine compared to controls, but a significant increase in sperm motility parameters was observed at 0.3 μ M, 1 μ M or 3 μ M of anserine or carnosine. The most effective dose of anserine and carnosine for sperm motility improvement was found to be 1 μ M and 0.3 μ M,

respectively. Next, the sperms were treated with 1 μ M anserine or 0.3 μ M carnosine for varying incubation time. Upon storage, a decreasing trend in sperm motility was noted with time and no significant change was observed upto 3 h of incubation; however, 6 h onwards, a significant improvement in motility parameters was observed in comparison to controls (Fig. 1). The beneficial effects of anserine or carnosine on sperm motility were observed even after 9 h of incubation; the sperm motility parameters continued to improve after 12 h of incubation with carnosine.

Discussion

In this study, we observed that imidazole dipeptides, anserine and carnosine are present in the male reproductive tract of the Japanese quail. Although the levels were far lower than that reported in the human (Mannion *et al.*, 1992) or chicken (Crush, 1970) skeletal muscle, this is the first report demonstrating the presence of imidazole dipeptides in the reproductive system of birds.

Analysis of biological fluid derived from the male reproductive tract revealed that the testicular fluid contains anserine, but not carnosine. In chickens, carnosine is reportedly produced from β -alanine and histidine by carnosine synthase and is further converted to anserine by the action of histamine N-methyltransferase-like protein (Baldyrev *et al.*, 2013). In addition, it is also known that carnosine is sensitive to carnosinase such as CNDP1 and 2 responsible for the degradation of carnosine to β -alanine and histidine

Table 1. Presence of imidazole dipeptides in the male reproductive tract of the Japanese quail*

Sources	Anserine (μ M)	Carnosine (μ M)	β -alanine (μ M)	Histidine (μ M)	1-methylhistidine (μ M)
Testicular fluid	44.46	—	54.06	210.00	60.65
Seminal plasma	—	41.75	109.67	50.63	52.98
Cloacal gland secretion	—	—	51.30	18.70	7.48
Blood serum	41.91	2.56	—	75.16	43.88

* single analysis was performed
- indicates value under detection limit

Table 2. Effect of various concentrations of anserine and carnosine on sperm motility after 6h of sperm storage

Parameters	Control	Anserine (μ M)				Carnosine (μ M)			
		0.1	0.3	1	3	0.1	0.3	1	3
VSL (μ m/s)	5.5 \pm 2.7 ^a	5.8 \pm 2.6 ^{ab}	10.5 \pm 2.4 ^{ab}	11.6 \pm 3.6 ^b	9.7 \pm 4.6 ^{ab}	6.9 \pm 2.1 ^a	9.7 \pm 3.4 ^a	8.9 \pm 1.8 ^a	9.6 \pm 4.5 ^a
VCL (μ m/s)	38.4 \pm 9.9 ^a	44.9 \pm 5.9 ^{ab}	56.1 \pm 10.3 ^{ab}	61.9 \pm 7.6 ^b	56.5 \pm 12.5 ^{ab}	54.9 \pm 3.7 ^{ab}	60.1 \pm 11.7 ^b	56.8 \pm 10.6 ^b	60.3 \pm 12.8 ^b
VAP (μ m/s)	12.7 \pm 3.8 ^a	14.0 \pm 2.2 ^{ab}	22.3 \pm 4.5 ^b	23.6 \pm 4.4 ^b	21.7 \pm 8.5 ^b	17.6 \pm 3.1 ^{ab}	22.5 \pm 6.7 ^b	20.1 \pm 3.9 ^{ab}	22.8 \pm 8.5 ^b
LIN	0.2 \pm 0.1 ^a	0.2 \pm 0.1 ^a	0.2 \pm 0.1 ^a	0.2 \pm 0.1 ^a	0.2 \pm 0.1 ^a	0.1 \pm 0.02 ^a	0.2 \pm 0.03 ^a	0.2 \pm 0.1 ^a	0.1 \pm 0.03 ^a
STR	0.4 \pm 0.1 ^a	0.3 \pm 0.1 ^a	0.4 \pm 0.1 ^a	0.4 \pm 0.1 ^a	0.4 \pm 0.1 ^a	0.3 \pm 0.04 ^a	0.4 \pm 0.04 ^a	0.4 \pm 0.1 ^a	0.3 \pm 0.03 ^a
WOB	0.4 \pm 0.1 ^a	0.3 \pm 0.1 ^a	0.4 \pm 0.1 ^a	0.4 \pm 0.1 ^a	0.4 \pm 0.1 ^a	0.3 \pm 0.03 ^a	0.4 \pm 0.04 ^a	0.4 \pm 0.02 ^a	0.4 \pm 0.1 ^a
ALH (μ m)	1.1 \pm 0.2 ^a	1.1 \pm 0.2 ^{ab}	1.6 \pm 0.3 ^b	1.7 \pm 0.3 ^b	1.5 \pm 0.5 ^{ab}	1.6 \pm 0.2 ^{ab}	1.8 \pm 0.4 ^b	1.6 \pm 0.3 ^b	1.7 \pm 0.4 ^b
BCF (Hz)	8.2 \pm 1.0 ^a	7.9 \pm 1.3 ^a	8.9 \pm 1.1 ^a	8.2 \pm 0.7 ^a	8.2 \pm 0.9 ^a	7.7 \pm 0.5 ^a	7.8 \pm 0.4 ^a	8.1 \pm 0.9 ^a	7.7 \pm 0.6 ^a
Motility rate (%)	59.7 \pm 12.3 ^a	58.6 \pm 11.3 ^a	68.1 \pm 19.4 ^a	74.2 \pm 12.9 ^a	71.1 \pm 20.6 ^a	64.4 \pm 12.7 ^a	72.1 \pm 16.9 ^a	74.4 \pm 9.6 ^a	77.6 \pm 11.7 ^a

Data ($n=5$ independent experiments) is expressed as mean \pm SD. Different superscript letters suggesting significant differences at $P < 0.05$ among treatments within the same row.

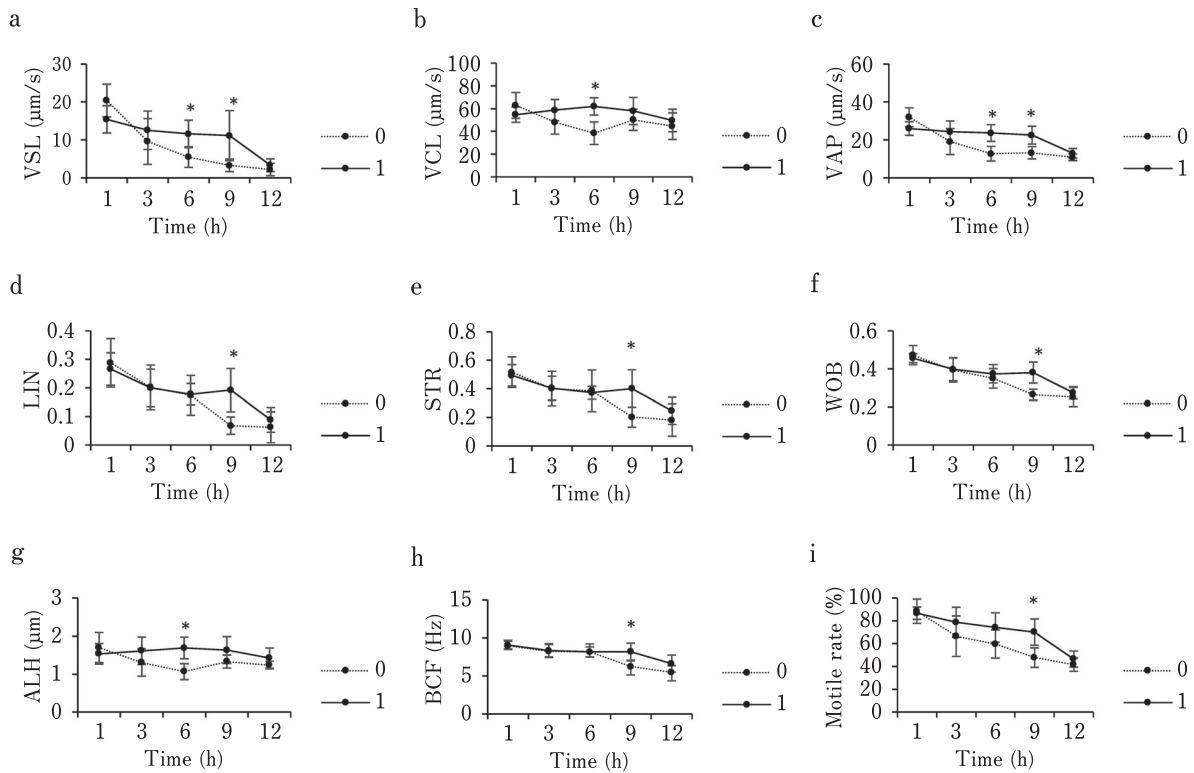


Fig. 1. Time-dependent study of anserine on sperm motility parameters. Sperm samples were incubated with ($1 \mu\text{M}$) or without anserine. Sperm motility was recorded at varying incubation times—1 h, 3 h, 6 h, 9 h and 12 h using CASA. The graph shows (a) straight line velocity (VSL); (b) curvilinear velocity (VCL); (c) average path velocity (VAP); (d) linearity (LIN); (e) straightness (STR); (f) wobble (WOB); (g) amplitude of lateral head displacement (ALH); (h) beat-cross frequency (BCF); and (i) motile rate. Data ($n=5$ independent experiments) is expressed as mean \pm SD. * indicates statistically significant difference at $P < 0.05$.

(Boldyrev *et al.*, 2013). It is therefore assumed that testicular anserine is a product of carnosine methylation and the remaining carnosine is probably degraded by the action of CNDPs or similar enzymes. To ascertain this, further expression studies and localization experiments of these testicular enzymes is warranted. If the testicular fluid is transported to epididymis and vas deferens when the sperm passes through the male reproductive tract, anserine should also be present in the SP; however, as per our findings, SP contains only carnosine (Table 1). The presence of carnosine in SP suggests that it is not derived from the testis but is newly synthesized and added later in the male reproductive tract; the enzymes responsible for either carnosine methylation or degradation are absent. Similarly, dominant localization of carnosine was reported in the muscles of horses and humans (Harris *et al.*, 1990), though the exact mechanism of its stabilization is unknown. Another possibility is that demethylation of methylhistidine occurs in the latter part of the testis in the reproductive tract and testicular anserine is again converted to carnosine during its passage through the male reproductive tract. Although the enzyme responsible for demethylation of anserine has not been demonstrated in ani-

mals yet, it would be interesting to explore a novel demethylase in the male reproductive tract of birds.

In this study, carnosine ($0.3 \mu\text{M}$) and its derivative—anserine ($1 \mu\text{M}$) improved sperm motility upon *in vitro* storage. In physiological condition, avian species may uplift the production of antioxidant enzymes to protect the sperm (Mavi *et al.*, 2020) but the antioxidant defense mechanism may be disturbed *in vitro* due to oxidative stress (Sikka, 1996). Avian sperm contains high amounts of polyunsaturated fatty acids (Surai *et al.*, 1998). The sperm integrity becomes susceptible with increased reactive oxygen species and lipid peroxidation (Khan, 2011). Several studies revealed the antioxidant properties of anserine and carnosine. The antioxidant activity of histidine and its derivatives is due to their imidazole moiety which decreases the rate of oxidation (Kohen *et al.*, 1988). A histidine containing compound is linked to having antioxidant ability and the peptide linkage between β -alanine, histidine, and 1-methylhistidine is associated with the antioxidant properties (Wu *et al.*, 2003). In addition, the antioxidant activity of carnosine is facilitated by chelation of metal ions, scavenging of reactive oxygen species and peroxy radicals (Kohen *et al.*, 1988; Boldyrev

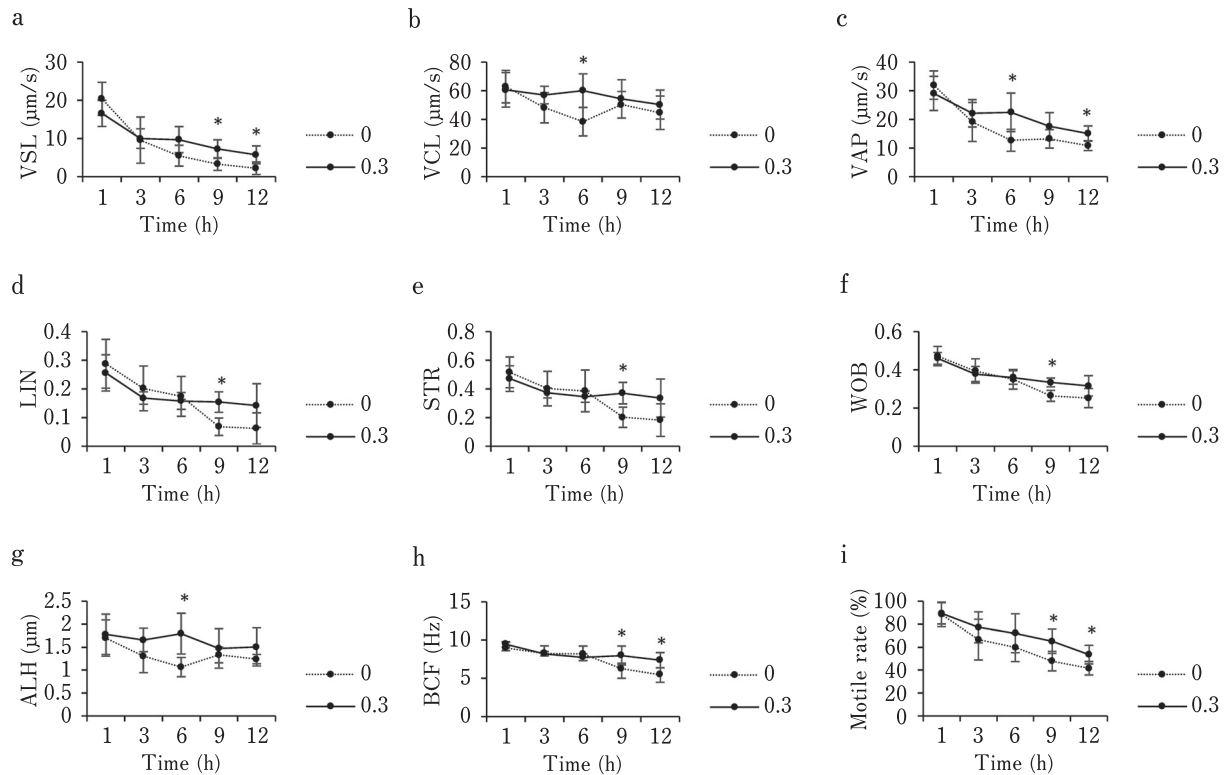


Fig. 2. **Time-dependent study of carnosine on sperm motility parameters.** Sperm samples were incubated with (0.3 μM) or without carnosine. Sperm motility was recorded at varying incubation times—1 h, 3 h, 6 h, 9 h and 12 h using CASA. The graph shows (a) straight line velocity (VSL); (b) curvilinear velocity (VCL); (c) average path velocity (VAP); (d) linearity (LIN); (e) straightness (STR); (f) wobble (WOB); (g) amplitude of lateral head displacement (ALH); (h) beat-cross frequency (BCF); and (i) motile rate. Data ($n=5$ independent experiments) is expressed as mean \pm SD. * indicates statistically significant difference at $P < 0.05$.

et al., 2013). The effect of carnosine on sperm motility has been investigated in mammals. For instance, carnosine improves the VSL of sperm in rams (Tan and Han, 1995), prevents oxidative stress in testis of rats (Aydın *et al.*, 2018), and improves the sperm mitochondrial activity in humans (Adami *et al.*, 2020). Carnosine acts as a malondialdehyde scavenger—lower malondialdehyde concentration was observed in stallion semen with higher carnosine content (Rocha *et al.*, 2018). From the above mentioned studies, we believe that anserine or carnosine addition may improve sperm motility by reducing oxidative stress during its storage.

To conclude, our study indicates that imidazole dipeptides exist in the male reproductive tract and may improve sperm quality during its *in vitro* storage as liquid. Although we did not examine the membrane integrity of stored sperms, imidazole dipeptides could be potential additives for sperm storage *in vitro* to minimize membrane damage.

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Conflicts of Interest

The authors declare no conflict of interest.

References

- Adami LNG, de Lima BT, Andretta RR, Bertolla RP and Nichi M. Carnosine treatment during human semen processing by discontinuous density gradient. *Andrologia*, 52: e13497. 2020.
- Allen TE and Grigg GW. Sperm transport in the fowl. *Australian Journal of Agricultural Research*, 8: 788-789. 1957.
- Aydın AF, Küçükgergin C, Çoban J, Doğan-Ekici I, Doğru-Abbasoğlu S, Uysal M and Koçak-Toker N. Carnosine prevents testicular oxidative stress and advanced glycation end product formation in D-galactose-induced aged rats. *Andrologia*, 50: e12939. 2018.
- Bakst MR and Sexton TJ. Fertilizing capacity and ultrastructure of fowl and turkey spermatozoa before and after freezing. *Journal of Reproduction and Fertility*, 55: 1-7. 1979.
- Begum G, Cunliffe A and Leveritt M. Physiological role of carnosine in contracting muscle. *International Journal of Sport Nutrition and Exercise Metabolism*, 15: 493-514. 2005.
- Blesbois E, Grasseau I and Segineurin F. Membrane fluidity and the

- ability of domestic bird spermatozoa to survive cryopreservation. *Reproduction*, 129: 371–378. 2005.
- Boldyrev AA, Aldini G and Derave W. Physiology and pathophysiology of carnosine. *Physiological Reviews*, 93: 1803–1845. 2013.
- Bosler JS, Davies KP and Neal-Perry GS. Peptides in seminal fluid and their role in infertility: A potential role for opiorphin inhibition of neutral endopeptidase activity as a clinically relevant modulator of sperm motility: A review. *Reproductive Sciences*, 21: 1334–1340. 2014.
- Crawford G, Ray A, Gudi A, Shah A and Homburg R. The role of seminal plasma for improved outcomes during in vitro fertilization treatment: review of the literature and meta-analysis. *Human Reproduction Update*, 21: 275–284. 2015.
- Crush KG. Carnosine and related substances in animal tissues. *Comparative Biochemistry and Physiology*, 34: 3–30. 1970.
- Duncan MW and Thompson HS. Proteomics of semen and its constituents. *Proteomics Clinical Applications*, 1: 861–875. 2007.
- Florman HM and Ducibella T. In *Physiology of Reproduction*, 3 edn, Vol 1, pp 55–112. Ed. JD Neill. St Louis, MO, USA: Elsevier Academic Press. 2006.
- Froman D. Deduction of a model for sperm storage in the oviduct of the domestic fowl (*Gallus domesticus*). *Biology of Reproduction*, 69: 248–253. 2003.
- Harris RC, Marlin DJ, Dunnett M, Snow DH and Hultman E. Muscle buffering capacity and dipeptide content in the Thoroughbred horse, Greyhound dog and man. *Comparative Biochemistry and Physiology Part A: Physiology*, 97: 249–251. 1990.
- Imanari M, Higuchi M, Shiba N and Watanabe A. Accurate analysis of taurine, anserine, carnosine and free amino acids in a cattle muscle biopsy sample. *Animal Science Journal*, 81: 369–376. 2010.
- Juyena NS and Stelletta C. Seminal plasma: An essential attribute to spermatozoa. *Journal of Andrology*, 33: 536–551. 2012.
- Khan RU. Antioxidants and poultry semen quality. *World's Poultry Science Journal*, 67: 297–308. 2011.
- Kohen R, Yamamoto Y, Cundy KC and Ames BN. Antioxidant activity of carnosine, homocarnosine, and anserine present in muscle and brain. *Proceedings of the National Academy of Sciences of the United States of America*, 85: 3175–3179. 1988.
- Koppers AJ, Reddy T and O'Bryan MK. The role of cysteine-rich secretory proteins in male fertility. *Asian Journal of Andrology*, 13: 111–117. 2011.
- Kotłowska M, Dietrich G, Wojtczak M, Karol H and Ciereszko A. Effects of liquid storage on amidase activity, DNA fragmentation and motility of turkey spermatozoa. *Theriogenology*, 67: 276–286. 2007.
- Kuroki M and Mori M. Binding of spermatozoa to the perivitelline layer in the presence of a protease inhibitor. *Poultry Science*, 76: 748–752. 1997.
- Long JA. Avian semen cryopreservation: What are the biological challenges? *Poultry Science*. 85: 232–236. 2006.
- Long JA, Purdy PH, Zuidberg K, Hiemstra SJ, Velleman SG and Woelders H. Cryopreservation of turkey semen: Effect of breeding line and freezing method on post-thaw sperm quality, fertilization, and hatching. *Cryobiology*, 68: 371–378. 2014.
- Łukaszewicz E. Artificial insemination in geese. *World's Poultry Science Journal*, 66: 647–658. 2010.
- Manjunath P, Bergeron A and Fan J. Seminal plasma proteins: functions and interaction with protective agents during semen preservation. *Society of Reproduction and Fertility Supplement*, 65: 217–228. 2007.
- Mannion AF, Jakeman PM, Dunnett M, Harris RC and Willan PLT. Carnosine and anserine concentrations in the quadriceps femoris muscle of healthy humans. *European Journal of Applied Physiology and Occupational Physiology*, 64: 47–50. 1992.
- Mavi GK, Dubey PP and Cheema RS. Association of antioxidant defense system with semen attributes vis a vis fertility in exotic and indigenous chicken breeds. *Theriogenology*, 144: 158–163. 2020.
- Poiani A. Complexity of seminal fluid: A review. *Behavioral Ecology and Sociobiology*, 60: 289–310. 2006.
- Rocha CC, Kawai GK, de Agostini Losano JD, Angrimani D de SR, Rui BR, de Cássia Bicudo L, da Silva B do CS, Alonso MA, Mendes CM, Ortiz D'Avila Assumpção ME, Pereira RJG, Barnabe VH and Nichi M. Carnosine as malondialdehyde scavenger in stallion seminal plasma and its role in sperm function and oxidative status. *Theriogenology*, 119: 10–17. 2018.
- Sale C, Saunders B and Harris RC. Effect of beta-alanine supplementation on muscle carnosine concentrations and exercise performance. *Amino Acids*, 39: 321–333. 2010.
- Sasanami T, Matsuzaki M, Mizushima S and Hiyama G. Sperm storage in the female reproductive tract in birds. *Journal of Reproduction and Development*, 59: 334–338. 2013.
- Sato M, Karasawa N, Shimizu M, Morimatsu F and Yamada R. Safety evaluation of chicken breast extract containing carnosine and anserine. *Food and Chemical Toxicology*, 46: 480–489. 2008.
- Siems WE, Maul B, Wiesner B, Becker M, Walther T, Rothe L and Winkler A. Effects of kinins on mammalian spermatozoa and the impact of peptidolytic enzymes. *Andrologia*, 35: 44–54. 2003.
- Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. *Frontiers in Bioscience*, 1: e78–86. 1996.
- Surai PF, Blesbois E, Grasseau I, Chalah T, Brillard JP, Wishart GJ, Cerolini S and Sparks NHC. Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 120: 527–533. 1998.
- Tan Z-J and Han Y-L. Effects of carnosine and gossypol on the motility and ultrastructure of ram spermatozoa. *Zoological Research*, 16: 119–125. 1995.
- Tanokura M. 1H-NMR study on the tautomerism of the imidazole ring of histidine residues. I. Microscopic pK values and molar ratios of tautomers in histidine-containing peptides. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, 742: 576–585. 1983.
- Tareq KMA, Hossain MDS, Akter QS, Sawada T, Afrose S, Hamano KI and Tsujii H. Effect of amino acids and dipeptides on the acrosome reaction and accumulation of ammonia in porcine spermatozoa. *Reproductive Medicine and Biology*, 7: 123–131. 2008.
- Viana AGA, Martins AMA, Pontes AH, Fontes W, Castro MS, Ricart CAO, Sousa MV, Kaya A, Topper E, Memili E and Moura AA. Proteomic landscape of seminal plasma associated with dairy bull fertility. *Scientific Reports*, 8: 16323. 2018.
- Wu HC, Shiao CY, Chen HM and Chiou TK. Antioxidant activities of carnosine, anserine, some free amino acids and their combination. *Journal of Food and Drug Analysis*, 11: 148–153. 2003.