



Hormones orchestrated pre- and post-copulatory sexual traits in male Mongolian gerbils



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HIGHLIGHTS

- Dominant gerbils had more and better quality of sperm than subordinate gerbils.
- Dominant gerbils had the same energy intake and RMR as subordinate gerbils.
- There was no trade-off between pre- and post-copulatory sexually selected traits.
- Aggression level and sperm quality increased with elevated T but reduced CORT.
- CORT and T orchestrated relationship between pre- and post-copulatory sexual traits.

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ABSTRACT

Parker's sperm competition model predicts a negative relationship between pre-copulatory (social status) and post-copulatory (sperm quality and quantity) sexually selected traits, however, empirical studies have revealed considerable inconsistency in this relationship. We hypothesized that there was a trade-off between pre- and post-copulatory sexually selected traits, and hormones (corticosterone, CORT; testosterone, T) orchestrate this relationship. In this study, we measured energetic parameters in the dominant–subordinate Mongolian gerbils (*Meriones unguiculatus*), whose relationship was established under chronic social encounters in a neutral arena, and then tested the relationship between their social status and sperm quality and quantity. Our results showed that dominant males initiated attack sooner and displayed more aggression, self-grooming and locomotion behaviors in daily social encounters across seven consecutive days. Dominant gerbils also had more and better quality of sperm than that of subordinate males, yet showed no significant differences in energy intake and RMR in comparison with subordinate individuals. In addition, dominant males had higher concentrations of serum T than subordinate males, whereas the concentrations of CORT showed a reverse pattern. The frequency and duration of aggression (indicative of social status) increased with elevated T concentrations. Sperm quality in terms of number and activity were associated with higher concentrations of serum T in dominant gerbils, whereas small sperm counts and poor-quality sperm were associated with relatively higher concentrations of serum CORT in subordinate gerbils. Together, our data indicated that there was no trade-off between pre- and post-copulatory sexually selected traits but hormones orchestrated the relationship between these traits in male Mongolian gerbils.

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1. Introduction

Darwin's theory of sexual selection suggests that male mating competition (pre-copulatory competition) is a potent selective pressure for the evolution of secondary sexual traits such as ornaments, weapons or chemical signals. However, when females mate with more than one male, this male–male competition extends to post-copulation which

affects paternity through sperm competition [1]. Sperm competition can also be an important selective pressure for the evolution of ejaculate quality and genital morphology [2]. Consequently, a male's overall fitness was affected by pre- and post-copulatory competitions, and the relationship has crucial implications for the evolution of male reproductive strategies.

Males can allocate only a limited amount of resources to reproduction as almost all life-history traits depend upon the same resources [3]. The expression and maintenance of sexual traits involved in both pre- and post-copulatory competitions are both energy and time consuming [4,5]. Therefore, Parker's sperm competition model predicted

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that investment into elaboration in ejaculate quality (such as testes, sperm quantity and quality) could reduce the availability of resources for all other reproductive traits (such as social status and scent-marking behavior) [3]. Given this, we might predict that subordinate males could counteract an initial disadvantage in pre-copulatory competition by adopting less-obvious post-copulatory tactics that enhance sperm competitive success [1,3]. However, empirical studies have revealed considerable inconsistency in the direction and strength of relationship among these sexual traits [6,7]. Because energy is a crucial resource for all life-history traits, variation in the rate of energy acquisition and transformation (the metabolic rate) is an important issue within the framework of evolution and ecological physiology [8,9]. Moreover, few studies have illustrated the costs of social status and sexual traits from the aspect of energetics.

Sex steroids (testosterone, T) and stress hormones (glucocorticoids, GCs; corticosterone, CORT, in rodents) are ubiquitous among male vertebrates and profoundly affect both reproductive behavior and reproductive physiology [10,11]. Circulating concentrations of T are elevated during the breeding season (especially during the courtship and mating phase) in many vertebrate species [12]. Gonadal T controls seasonal changes in reproductive processes such as sexual (vocalization, courtship, copulation) and aggressive (mate-guarding, territorial) behaviors, as well as the expression of numerous sexually selected traits and sperm production and maturation [12,13]. GCs suppress reproductive functions along the hypothalamic–pituitary–gonadal (HPG) axis at multiple levels: 1) the testis/ovary (to modulate steroidogenesis and/or gametogenesis directly); 2) the pituitary gland (to inhibit the release and synthesis of follicle-stimulating hormone and luteinizing hormone); and 3) the hypothalamus (to decrease the release and synthesis of gonadotropin-releasing hormone) [14]. To our knowledge, no study examined how hormones orchestrate the relationship between pre- and post-copulatory sexually selected traits.

Mongolian gerbils (*Meriones unguiculatus*) are distributed throughout the agricultural land and desert grassland of Mongolia, Northern China and Russia [15]. They are small non-hibernating, seasonally breeding, and granivorous rodents which live in burrow systems or social groups consisting of multiple females and males year-round [16,17]. In the field the scent marking behavior of Mongolian gerbils was done by rubbing mid-ventral sebaceous glands against substrates within their territory and was found to be modulated by T [18,19]. Male gerbils, especially the largest dominant males, mark their territory to attack intruder males and to attract females in estrus [20]. During the breeding period Mongolian gerbils overlapped more home ranges which allows male gerbils to enter neighboring burrow systems to mate with extra-pair females [21] and female gerbils to access male mates of neighboring colonies [20], suggesting that they possessed a characteristic of promiscuous species. In the present study, we examined the individual difference in energy intake, resting metabolic rate (RMR), gland mass, sperm quality and quantity, serum CORT and serum T concentrations to test the hypothesis that there was a trade-off between pre- and post-copulatory sexually selected traits, and CORT and T orchestrate the relationship of these traits in male Mongolian gerbils.

2. Materials and methods

2.1. Animals

The animals were the offspring of Mongolian gerbils trapped in 1999 in Inner Mongolian grasslands. They were housed in plastic cages (3 or 4 male gerbils/cage, 30 × 15 × 20 cm) with sawdust as bedding prior to experiment, and maintained under a constant photoperiod of 16 h:8 h light–dark cycle and at a temperature of 23 ± 1 °C. They were provided with commercial standard rat pellets (Beijing HFK Bioscience Co.) and water ad libitum. Sixteen adult male gerbils (weight-matched) were selected from different cages. Energy intake and RMR were measured in these gerbils. They were paired randomly (never met before), and

then their dominant–subordinate relationship was established under chronic social encounters in a neutral arena. Finally, serum hormone concentrations and sperm quality and quantity were evaluated. All experimental procedures complied with the guidelines of the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences.

2.2. Energy intake

Food intake was measured in metabolism cages as previously described [22]. Briefly, food was quantitatively provided then feces and food residues were collected at same time in the fourth day, and then dried to constant mass at 60 °C. The difference between the food given and residue was defined as dry matter intake (DMI). A Parr 1281 oxygen bomb calorimeter (Parr Instrument, USA) was used to determine the caloric value of food and feces. The following equations were used to calculate gross energy intake (GEI, kJ/g), digestible energy intake (DEI, kJ/g) and digestibility of energy [22,23]:

GEI = DMI × caloric value of food;

DEI = GEI – (dry mass of feces × caloric value of feces);

Digestibility(%) = DEI/GEI × 100%.

2.3. RMR

RMR was measured using an open-flow respiratory system (TSE LabMaster Calorimetry System, Germany), following previous studies [24]. Briefly individual gerbils were transferred into a respiratory chamber (TSE, type I for mice, volume 2.7 L) housed inside a constant-temperature incubator (30 ± 0.5 °C, Sanyo, MIR-554). Fresh air was warmed by a copper tube to 30 °C and then pumped through the animal chamber at a flow rate of 800 mL/min. Subsequently, air leaving the chamber passed through a gas analyzer at the rate of 390 mL/min. Each measurement lasted 3 h with data recorded every 6 min. RMR was quantified as the oxygen consumption (VO₂; mL/min) calculated as the average of 12-min stable lowest values. Body mass was recorded before the measurement.

2.4. Behavioral procedures

Sixteen adult males were selected and assigned into 8 fixed pairs with similar body weights (within 10% difference). The dominant–subordinate relationship was formed very quickly in a neutral arena (40 × 27.5 × 60 cm Plexiglas box). The arena was divided into equal compartments using a removable opaque partition following the previous protocols [25]. Briefly, each gerbil was acclimated for 5-min period in each compartment before the partition was removed, and then they were allowed to interact freely for 10 min during which their encounters were recorded using a digital video. The arena was thoroughly cleaned with water and 75% ethanol before or between trials. Frequency and duration of aggression (chasing, sideways posture, attack, biting and latency of the initial attack), defense (cowering, upright posture, threatening, fleeing, and lying on the back on the ground), sniffing, self-grooming, locomotion displayed by each animal were quantified, as defined previously [25–27]. Behaviors were continuously recorded by hand on a data sheet with a pre-calibrated time scale in units of 10 s. Behaviors that lasted 10 s or less was treated as 1 unit, if the duration was between 10 s and 20 s which was considered to be 2 units, and so on [26]. Behavioral procedures were repeated for 7 consecutive days once each day for each pair.

2.5. Blood sample and organ mass

Following behavioral procedures, animals were sacrificed by CO₂ overdose. Blood samples were collected and then centrifuged at 4 °C

for 30 min at 4000 rpm after 30 min at room temperature; then the serum was collected and stored at -80°C until assayed. The mid-ventral sebaceous glands, paired seminal vesicles, testes and epididymis were dissected and weighed (to 1 mg). Finally, the left cauda epididymis was weighed and immediately stored at 37°C in 0.9% saline.

2.6. Sperm evaluation

The excised left cauda epididymis was immediately minced using a glass homogenizer in 37°C saline solution (10 mL/g tissue). The preparation of sperm suspension and sperm concentration, motility and morphology were performed according to the method of Seed [28].

The sperm concentration and motility were assessed by hemocytometers (Qiujiang, Zhejiang) under a light microscope. The sperm concentration was expressed as the number of sperm per gram of cauda epididymis and sperm motility was quantified as the percentage of sperm showing a progressive movement among 200 counted spermatozoa from each male.

Sperm morphology was assessed by eosine staining. Sperm suspension was smeared onto a glass slide, fixed in 95% ethanol for 5 min after air-dried, stained with 1% eosine and rinsed with distilled water. A minimum of 200 sperms were examined from each sample.

2.7. Serum T and CORT assays

Serum T and CORT concentrations were determined by ^{125}I -labeled RIA kit (Beifang, Beijing, China) and ELISA (enzyme-linked immunosorbent assay) kit (Jiancheng, Nanjing, China), respectively. The detailed procedure followed the manufacturers' instructions. The T and CORT concentrations range detected by these assays were 2–2000 ng/dL and 5–1000 ng/mL; inter-assay variations were <8.7% and 7%; intra-assay variations were <3.6% and 5%, respectively.

2.8. Statistical analysis

All analyses between subordinate and dominant animals were analyzed by the non-parametric Wilcoxon matched pair test (if data were not normally distributed) or two-tailed paired *t*-tests (if data were normally distributed) using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Correlations between frequency of aggression, DEI, sperm quantity and T concentration were analyzed using either Spearman correlation (if data were not normally distributed) or Pearson correlation (if data were normally distributed). Results were presented as mean \pm SE. $P < 0.05$ and $P < 0.01$ was considered to be statistical significance and very significance.

3. Results

3.1. Behaviors

The dominant–subordinate relationship was formed quickly in every encounter, and then remained consistent throughout the experiment (Fig. 1). Gerbils showing higher duration ($P < 0.05$, Fig. 1A) and frequency ($P < 0.05$, Fig. 1A) of defensive behavior were classified as subordinate males. Whereas their opponents as the dominant ones who had a shorter latency to attack ($P < 0.05$, Fig. 1C) and higher duration ($P < 0.05$) and frequency ($P < 0.05$) of aggression (Fig. 1A and B) displayed higher levels of aggression. Frequency of aggression was highly correlated with the serum T concentrations ($r = 0.577$, $P = 0.019$, Fig. 1D).

3.2. Serum T and CORT concentration

The serum T concentration in the dominant males was about 1.14 times higher than that in their subordinate counterparts ($t = 3.028$, $df = 7$, $P = 0.019$, Fig. 2A), while the CORT concentration in the

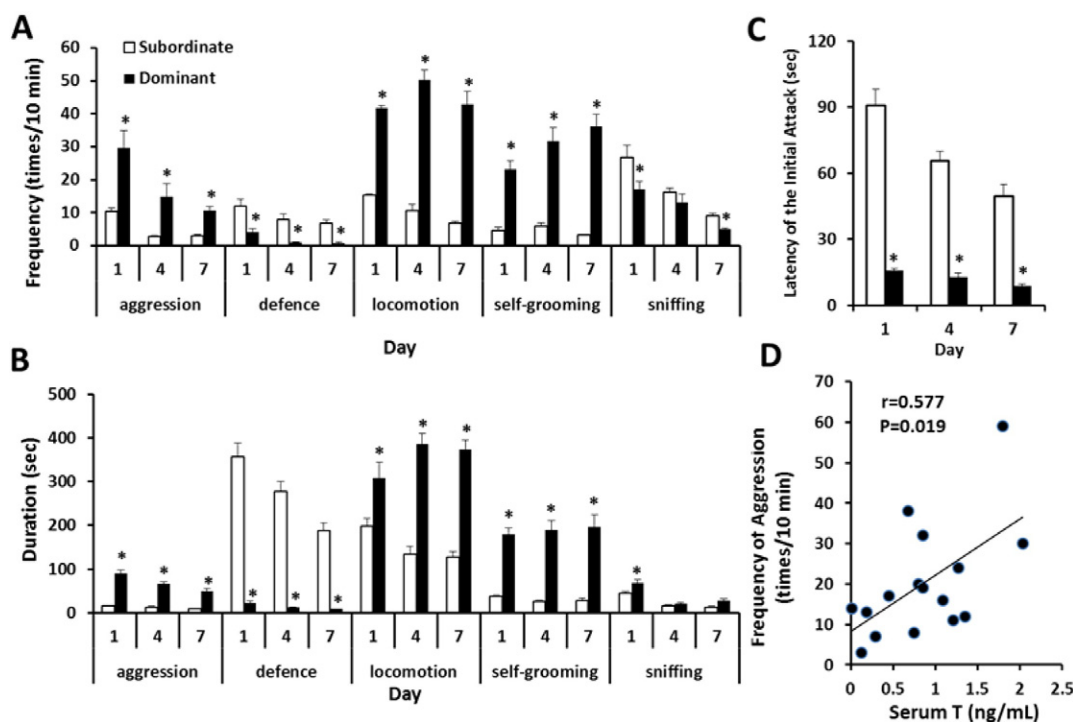


Fig. 1. Agonistic behaviors displayed in subordinate and dominant male Mongolian gerbils. Frequency (A) and duration (B) of agonistic behaviors displayed in male Mongolian gerbils. Dominant males had significantly shorter latency to attack than subordinates (C). Frequency of aggression was positively correlated with serum T in male Mongolian gerbils (D). Values were mean \pm SE. * $P < 0.05$.

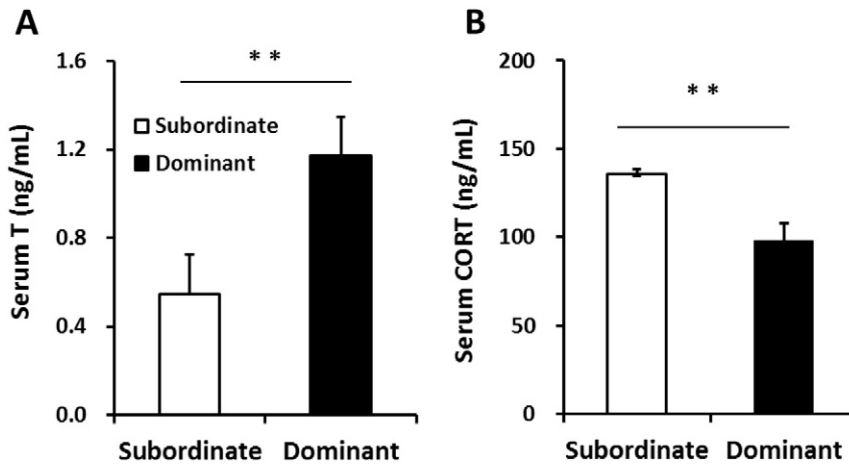


Fig. 2. Serum T (A) and CORT (B) concentrations in male Mongolian gerbils. Values were expressed by mean \pm SE. ** $P < 0.01$. T, testosterone; CORT, corticosterone.

dominant males was about 30% lower than that in their subordinate counterparts ($t = 3.634$, $df = 7$, $P = 0.008$, Fig. 2B).

3.3. Energy intake and RMR

No significant differences in GEI ($t = 1.258$, $df = 7$, $P = 0.249$, Fig. 3A), DEI ($t = 1.512$, $df = 7$, $P = 0.174$, Fig. 3A), digestibility ($t = 0.758$, $df = 7$, $P = 0.473$, Fig. 3B) and BMR ($t = 1.012$, $df = 7$, $P = 0.345$, Fig. 3C) were found between the subordinate and dominant gerbils. DEI ($r = 0.448$, $P = 0.092$) and RMR ($r = 0.433$, $P = 0.094$) were not correlated with serum T concentrations. DEI ($r = 0.132$,

$P = 0.626$) was not positively correlated with serum CORT concentrations but RMR ($r = 0.795$, $P = 0.017$) was positively correlated with serum CORT concentrations.

3.4. Body mass, organ mass and sperm quality and quantity

Body and organ masses were summarized in Table 1. There was no significant difference in the body mass of the subordinate and dominant gerbils ($t = 0.080$, $df = 7$, $P = 0.939$). The testis ($t = 2.003$, $df = 7$, $P = 0.085$) and seminal vesicle ($t = 2.002$, $df = 7$, $P = 0.085$) masses of the dominant gerbils (1.177 ± 0.059 g; 0.515 ± 0.077 g) had a trend but

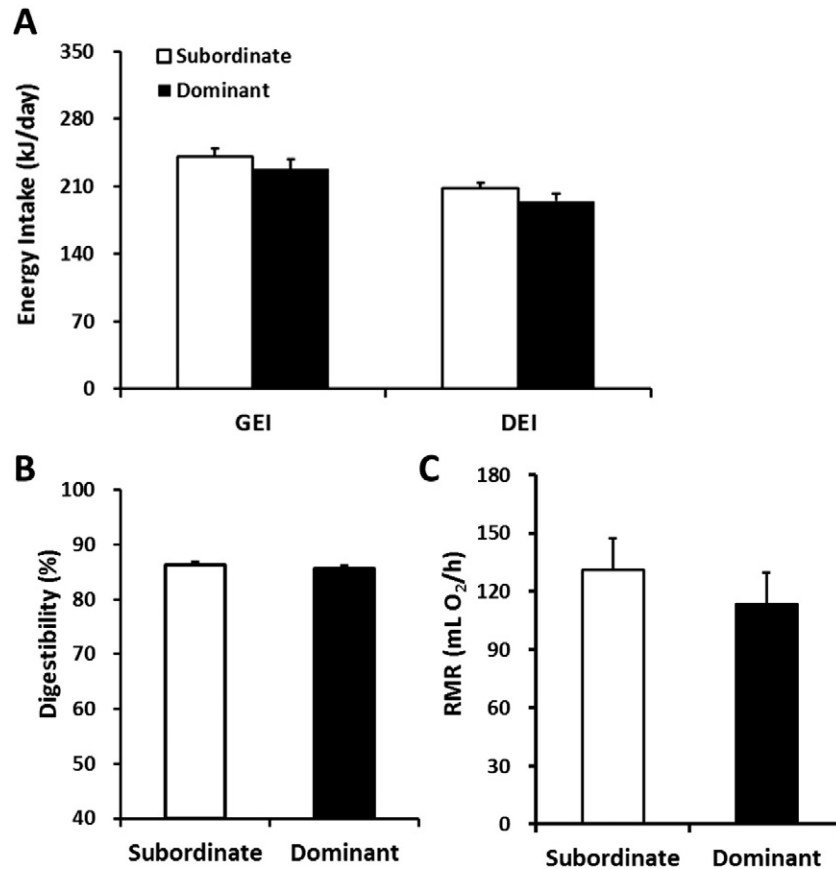


Fig. 3. Energy intake and RMR in male Mongolian gerbils. (A) Gross energy intake (GEI) and dry matter intake (DMI); (B) digestibility; (C) resting metabolic rate (RMR). Values were expressed by mean \pm SE.

Table 1

The masses in the body, epididymis, testis, seminal vesicles and sebaceous glands in dominant and subordinate male Mongolian gerbils.

	Body (g)	Epididymis (g)	Testis (g)	Seminal vesicles (g)	Sebaceous glands (g)
Subordinate	72.2 ± 1.8	0.260 ± 0.044	1.007 ± 0.120	0.312 ± 0.044	0.115 ± 0.020
Dominant	72.4 ± 3.1	0.330 ± 0.030	1.177 ± 0.059	0.515 ± 0.077	0.156 ± 0.023
<i>P</i>	0.939	0.227	0.085	0.085	0.172

Values were expressed by mean ± SE.

were not significantly different from those of the subordinate gerbils (1.007 ± 0.120 g; 0.312 ± 0.044 g). Epididymis ($t = 1.325$, $df = 7$, $P = 0.227$) and sebaceous gland ($t = 0.523$, $df = 7$, $P = 0.172$) masses were 1.3 times and 1.4 times in the dominant gerbils that of the subordinate gerbils.

Sperm evaluation was summarized in Fig. 4. The mean sperm concentration was significantly higher in the dominant than in the subordinate gerbils ($t = 7.829$, $df = 7$, $P < 0.001$, Fig. 4A), but no significant difference was detected in sperm motility between those gerbils ($t = 1.353$, $df = 7$, $P = 0.218$, Fig. 4B). However, the total percentage of abnormal sperm in the dominant gerbils was about 41% lower than that in the subordinate gerbils ($t = 2.114$, $df = 7$, $P = 0.072$, Fig. 4C). Serum T concentration tended to be positively correlated with sperm concentration ($r = 0.457$, $P = 0.075$, Fig. 4D).

4. Discussion

The present study showed that stable and overt dominant–subordinate relationship was quickly formed under chronic social encounters.

The dominant male Mongolian gerbils showed more aggression and initiated attack sooner throughout experiment, and also had more and better quality of sperm compared with the subordinate gerbils. But they had the same energy intake and RMR as subordinate gerbils. T concentration increased with the increase in aggression, quantity and quality of sperm in Mongolian gerbils. And relatively higher levels of serum CORT were associated with less sperm counts and poor-quality sperm in subordinate gerbils. Together, our data indicated that there was no trade-off between pre- and post-copulatory sexually selected traits but hormones orchestrated the relationship between these traits in the male Mongolian gerbils.

Parker's sperm competition model predicted that males face a trade-off between pre-copulatory and post- sexually selected traits as they had a fixed total energy budget for reproduction to divide between these traits [3]. The dominant males initiated attack sooner and had higher aggression, self-grooming and locomotion behavior in everyday social encounters, which suggested that they expended more energy in these physical activities than the subordinate males. But there was no significant difference in energy intake and RMR between those

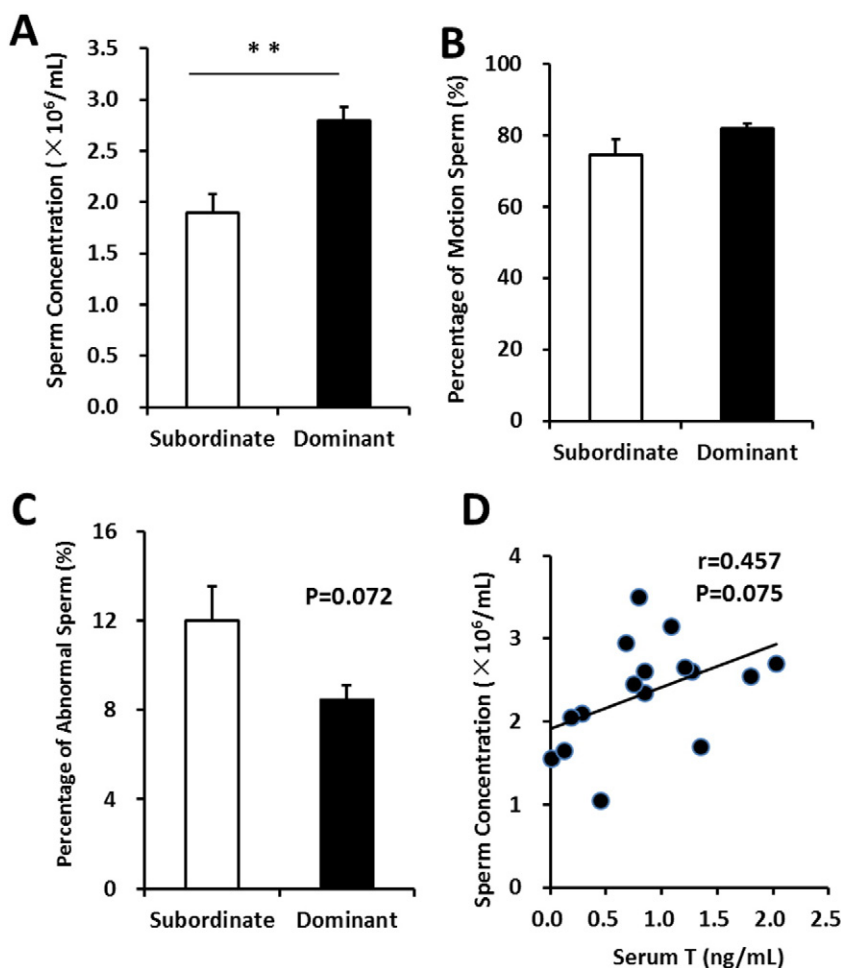


Fig. 4. Sperm concentration (A), percentage of motile (B) and abnormal (C) sperm in subordinate and dominant male Mongolian gerbils. Correlations of serum T with sperm concentration in male Mongolian gerbils (D). Values were expressed by mean ± SE. ** $P < 0.01$.

gerbils. Intriguingly, the dominant males also had higher quality and quantity of sperm than the subordinate males. Those data were in discrepancy with the Parker's sperm competition model and suggested that there was no trade-off between the pre- and post-copulatory sexually selected traits at the condition of adequate food resource in the laboratory. But Lüpold's findings that interspecific relationships between pre- and post-copulatory traits would gradually shift from strongly positive to strongly negative with increasing male–male contest competition [6] supported our data. Specifically, in the taxa where males are able to monopolize females, pre-copulatory sexual traits should be more important [6]. By contrast, if female monopolization is rarely, investments in pre- and post-copulatory male–male competitions would be equally important and generate positive covariation between them [6]. There are likely a number of explanations for those results. One possible explanation is that the dominant high-quality males can use resources more efficiently, and they have relatively more marginal benefits to invest in both pre- and post-copulatory sexual traits than subordinate males, generating positive covariation between these traits across males [29]. Another possible explanation is that investment in reproduction results in the trade-off with other life-history components, such as decreasing immune function or increasing reactive oxygen species production [30,31], which will decrease survival or future reproductive success. Additional studies are now required to determine whether there are trade-offs between life-history traits, such as reproduction and survival in the male Mongolian gerbils.

Testosterone supports reproductive processes such as the expression of secondary sexual characters, territorial aggression, courtship and sexual behaviors and sperm production at the beginning of the breeding season [11]. In the male Mongolian gerbils testes and accessory sex glands of the dominant males had a trend to be heavier than that of the subordinate males, and the T concentration of the dominant males was also higher. The relative more and better quality sperm by dominant male gerbils was found to nearly covary with serum T concentration. Circulating T concentrations were heritable, and males with naturally high T concentrations or exogenous T had higher social status, mate searching, mobility, and reproductive success than low-T males [32]. We found that the frequency and duration of aggression (social status) of gerbils increased with elevated T, which suggested that the dominant males with high circulating T concentrations were often more aggressive in competition for resources and potential mates [33, 34]. These results were supported by the previous study from semi-natural enclosures which showed that the dominant Mongolian gerbils had significantly heavier epididymis and testes, and those animals with higher T concentrations had more aggressive behaviors [35]. In the present study, we also found no significant correlations between the serum T concentrations and energy intake or RMR. The study in male house sparrows (*Passer domesticus*) housed in groups indicated that basal metabolic rate synchronously increased with T concentration [36]. In contrast, another study in singly-housed white-crowned sparrows (*Zonotrichia leucophrys gambelii*) indicated that the increases in serum T concentrations were associated with reductions in RMR [37]. These studies suggested that T enhanced both pre-copulatory (aggression, social status) and post-copulatory (ejaculate, testes) sexually selected traits, and the relationship between circulating T and energetics is dependent on social status or housing condition.

Agonistic behaviors and social status can act as important stressing agents. In our study, the subordinate male gerbils had higher concentrations of serum CORT than the dominant individuals, and they also had less sperm counts and poor-quality sperm. RMR was positively correlated with serum CORT concentrations, which was supported by the function of increased CORT secretion to mobilize energy to deal with the stressful situation [38]. The chronically-elevated CORT would underlie reproductive suppression in the social subordinates [38,39]. However, some other studies in bison bulls (*Bison bison*) showed that the dominant ones maintained high GC levels and copulatory success under unstable social status [40]. These results suggested that in stable social

groups, the socially subordinate individuals were usually subjected to social and psychosocial stress and reproductive depression because of the relatively high energy cost, while under unstable conditions the dominant ones paid a marked physiological cost for high social status and copulatory success. Therefore, stress hormones (GCs and CORT) affected reproductive fitness in terms of social status.

Empirical and theoretical studies show that the relationship between the pre- and post-copulatory sexual traits was mainly dependent on the marginal benefits gained by increasing investments in those sexual traits and the intensity of male–male contention [6,7]. But endocrine hormones (such as T and CORT) were other important mediators to orchestrate the relationship between these traits, obviously, which was greatly in need of further investigation.

In summary, our data revealed that the dominant male Mongolian gerbils had more aggressive behavior, had more and better quality of sperm compared with the subordinate males, but there were no differences in the energy intake and RMR between those gerbils, suggesting that there was no trade-off between the pre- and post-copulatory sexually selected traits of these animals. Our data also showed that the dominant male gerbils with high T and low CORT concentrations had a higher level of aggression, better quality and a larger quantity of sperm, while the subordinate individuals following a tactic with low T and high CORT concentrations had more defensive behavior, less sperm counts and poor-quality sperm, which suggested that hormones orchestrated the relationship between the pre- and post-copulatory sexually selected traits in the male Mongolian gerbils.

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