

Comparative analysis of sperm motility in liquid and seminal coagulum portions between Bornean orangutan (*Pongo pygmaeus*) and chimpanzee (*Pan troglodytes*)

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Abstract

Coagulum in the semen of some primates plays different roles, depending on the species. In the present study, we examined sperm motility in the coagulum and liquid portions of semen collected from captive individuals from two great ape species: two adult Bornean orangutans (*Pongo pygmaeus*) ($n = 27$) and three adult chimpanzees (*Pan troglodytes*) ($n = 14$). The results revealed that orangutan sperm remained motile for significantly longer in the coagulum than in the liquid portion (> 18 h). By contrast, chimpanzee sperm motility did not differ significantly over time between the two portions

of the semen, although motility was slightly higher in the liquid portion than in the coagulum. The evolution of the seminal coagulum is thought to be related to postcopulatory sperm competition; however, functions of seminal coagulum have not been completely elucidated. Our data from the orangutan semen suggest that in this species, seminal coagulum may strengthen own-sperm survival. This report is the first to provide evidence for this distinctive function of the seminal coagulum. This unique property of orangutan seminal coagulum might be attributable to their reproductive traits, e.g., difficulty in predicting ovulation due to a lack of genital swelling during estrus. The orangutan is a Critically Endangered species, and captive breeding, including artificial insemination (AI), is expected. However, worldwide, only one case of orangutan AI has been successful. Our findings may contribute to an understanding of their basic semen characteristics and help improve the AI method.

Keywords Sperm motility · Great ape · Male reproduction · Seminal coagulum · Ejaculated semen

Introduction

Primate ejaculate can be composed of three sequentially emitted portions: liquid, coagulum, and copulatory plug. The presence of the latter two portions depends on sexual- selection intensity and is correlated with the degree of female promiscuity and male–male competition (Dixson and Anderson 2002). Thus, the ejaculates of the polygynous gorilla and monogamous gibbon lack coagulum and plug because these species experience little to no sperm competition. In contrast, primates with multi-male/multi- female mating systems produce coagulum and plug. The texture of the coagulum and the presence of plug vary among species (Dixson and Anderson 2002). In some species (e.g., rodents, pigs, and snakes), it is considered that coagulum and plug act as physical barriers to subsequent ejaculate from other males (Martan and Shepherd 1976; Devine 1977; Voss 1979) and to prevent sperm loss from the female’s reproductive tract (Blandau 1945). Further, the coagulum gradually exudes a liquid fraction, and sperm are released from the coagulum into the cervix and uterus. In rhesus monkeys (*Macaca mulatta*), the coagulum can contain as much as 50–70% of the sperm in the ejaculate (Hoskins and Patterson 1967; Settlage and Hendrickx 1974); this might increase the possibility of fertilization by maintaining a continuous flow of sperm into the female reproductive tract. Dixson and Anderson (2002) hypothesized that the evolution of the seminal coagulum is related to postcopulatory sperm competition.

Orangutans are a semi-solitary species. Adult male orangutans exhibit intra-sexual dimorphism: flanged males have large bodies with developed secondary sexual characteristics, including prominent cheek pads, long fur, and a throat sack, whereas unflanged males have female-sized bodies and lack secondary sexual characteristics (Delgado and Van Schaik 2000). The orangutan females copulate with the flanged male during a given periovulatory period but can also opportunistically mate with additional unflanged males (van Schaik and van Hooff 1996; Dixson 2012; Tajima et al. 2018). Thus, orangutans have an intermediate mating system, which is reflected in the fact that their semen possesses coagulum but not a copulatory plug. However, studies on their semen characteristics are comparatively fewer than those of other great apes.

The two species (chimpanzee and bonobo) in the genus *Pan* produce seminal coagulum, probably because female promiscuity is highest in these species. In addition, they produce a copulatory plug of solidified semen. These two species are the only great apes, other than orangutan, with seminal coagulation. Here, we compared characteristics in the liquid and coagulum portions of the semen of orangutans and chimpanzees to add to our knowledge of orangutan semen. We focused particularly on the temporal variation in motile-sperm percentage in each portion, which is an important index in reproduction. In addition to their different mating styles, they have differences in genital swelling. Females of many primates, including chimpanzees, develop genital swelling during estrus to attract males (Wallis and Lemmon 1986). However, orangutan females develop genital swelling only during the gestation period and not as an external indicator of the periovulatory period (Galdikas 1981), which is unique to this genus. Based on these differences in mating style and female physiology, we predicted that orangutan semen would have distinctive characteristics, e.g., prolonged sperm motility to overcome comparatively unpredictable ovulation with few mating opportunities.

Methods

Animal management followed the Code of Ethics of the Japanese Association of Zoos and Aquariums (http://www.jaza.jp/jaza_pdf/library_jaza/atsukai.pdf). All procedures were approved by the Animal Welfare and Animal Care Committees of the Primate Research Institute (#2015–139) and the Wildlife Research Center (WRC-2018-KS003A). They were based on the Guidelines for Care and Use of Nonhuman Primates (Version 3, issued in 2010) from Kyoto University. Experiments on one chimpanzee kept at the Japan Monkey Centre were in collaboration with the zoo (#2,015,010), and all experiments were performed according to the Ethical Guidelines for the Conduct of Research on Animals by Zoos and Aquariums of the World Association of Zoos and Aquariums (Sato and Tomonaga 2010).

Semen was collected from two adult flanged Bornean orangutans, OU1 (Robin, ID: 0125 in the Great Ape Information Network; <http://www.shigen.nig.ac.jp/gain/>, 23 years old at the start of the study) and OU2 (Futoshi, ID: 0093, 28 years old), as well as three adult chimpanzees, CH1 (Akira, ID: 0306, ~34 years old), CH2 (Norihei, ID: 0297, 31 years old), and CH3 (Zamba, ID: 0543, 23 years old). Subjects were housed at the Zoorasia Yokohama Zoological Gardens (OU1), Chiba Zoological Park (OU2), Japan Monkey Centre (CH1), and Kumamoto Sanctuary of Kyoto University (CH2 and CH3). All apes, apart from CH1, were captive-bred. All apes were fed fresh fruits and vegetables; water was provided ad libitum.

Semen was collected without anesthesia when males became sexually aroused and showed sexual displays (OU1, $n = 16$; OU2, $n = 11$; CH1, $n = 7$, CH2, $n = 2$; CH3, $n = 5$). Collection was carried out in the morning and approximately once per month for all orangutans and one of the chimpanzees (OU1: July 2014 to May 2016, OU2: July 2014 to July 2015, and CH1: September 2015 to May 2016). For the remaining two chimpanzees (CH2 and CH3), sperm was collected every morning and/or early evening for 3 days (October 9 to 11, 2018). The decision to collect semen twice a day was based on a previous study that found no significant variations in motile-sperm percentage and sperm viability when semen collection was repeated hourly for 5 h (Marson et al. 1989).

Orangutans ejaculated directly into a 50-mL plastic tube (OU1) or into the keeper's hand (OU2). Semen from CH1 and CH2 was collected from the swept floor immediately after both individuals ejaculated after rubbing their penises against their legs. Semen from CH3 was collected using an artificial vagina (external cylinder; NFA260, internal rubber; NFA261, Fujihira Industry Co. Ltd., Tokyo).

In line with a previous research (Vandevoort et al. 1993), orangutan semen coagulated immediately post-ejaculation, and then the liquid portion was exuded approximately 30 min later (Fig. 1a). This liquid was decanted into a 15-mL plastic tube, and the remainder was treated as coagulum. The meager amount of liquid that continued to be exuded after 30 min was included in the coagulum portion. For chimpanzees, the white liquid and non-white coagulum were distinct at ejaculation (Fig. 1b, c); each portion was immediately collected in separate microtubes. Any liquid that adhered to the coagulum was treated as a part of it.

Seminal characteristics (volume, viability, sperm concentration, and percentage of motile sperm) were evaluated in the liquid portion immediately after collection in each facility. Sperm viability was assessed from 20 μ L of the sample, mixed with an equivalent volume of a live-dead exclusion stain (eosin-nigrosin stain). At least 250 sperm were counted in three or more fields and classified as viable (no stain uptake) or nonviable (partial or complete stain uptake). Sperm concentration and motile-sperm percentage were assessed by mixing 5 μ L of either liquid or coagulum with 95 μ L of P-1 Medium containing gentamicin (99,242; Irvine Scientific, CA, USA) for orangutans (Kinoshita et al. 2016) or 10 \times diluted Dulbecco's phosphate-buffered saline (048–29,805; Wako Pure Chemical Industries, Ltd., Osaka, Japan) for chimpanzees (Kusunoki et al. 2001). We evaluated it under the optimal solutions already known for each species. These solutions were used only for the dilution when evaluating samples for less than 2 min, and not for storage. Diluted samples (5 μ L) were placed in a Makler counting chamber to estimate the number of motile sperm. Regarding the motile-sperm percentage, researchers attempted to evaluate the temporal variation at hourly intervals in both semen portions, but the zoo research center's working hours precluded evaluation of some samples between 10 and 24 h post-collection, especially in OU2. The average value every 3 h was calculated. To estimate the differences between these species under the same condition, all samples were maintained in incubators set to 25 °C in the raw during the experiments because it is known that mammalian semen is best preserved when it is stored at around room temperature (Jishage and Suzuki 1993; Batista et al. 2011).

The Mann–Whitney *U* test (for orangutans) and Kruskal–Wallis test (for chimpanzees) were used to determine individual differences in seminal characteristics. One-way ANCOVA was performed to identify differences in motile-sperm percentage between coagulum and liquid semen over time; the analysis examined within-individual and between-individual patterns in each species. Between-species differences in each portion were also analyzed. All tests were performed in SPSS version 23 (IBM Japan Ltd., Tokyo, Japan). Significance was set at $P < 0.05$.

Results

Characteristics of ejaculated semen

We did not observe copulatory plugs in most CH1 and CH2 samples, although one CH2 sample contained a plug. In contrast, all CH3 ejaculate samples contained the plug.

Chimpanzees had lower liquid-semen volume than orangutans (Table 1). In addition, chimpanzee semen had an approximately two-fold higher sperm concentration than that of orangutans. However, motile-sperm percentage and sperm viability did not exhibit between-species differences. No significant individual differences were observed in semen characteristics ($P > 0.05$; Table 1), except for sperm concentration ($P < 0.05$, mean sperm concentration: OU1, $862.7 \times 10^6/\text{mL}$; OU2, $484.3 \times 10^6/\text{mL}$).

Temporal variations in motile-sperm percentage

We did not identify any significant individual differences in temporal variation of motile-sperm percentage among orangutans (ANCOVA: coagulum, $F = 2.430$, $df = 1$, $P = 0.123$; liquid, $F = 2.204$, $df = 1$, $P = 0.141$). In contrast, individual differences were present in the liquid portion of chimpanzee semen (coagulum, $F = 1.493$, $df = 2$, $P = 0.229$; liquid, $F = 11.105$, $df = 2$, $P < 0.001$). This result suggests an effect of the sampling method because semen obtained after masturbation (CH1 and CH2) differed from semen obtained via an artificial vagina (CH3) ($F = 14.304$, $df = 1$, $P < 0.001$).

For orangutans, sperm motility persisted significantly longer in the coagulum than in the liquid portion (ANCOVA: OU1, $F = 51.658$, $df = 1$, $P < 0.001$; OU2, $F = 6.938$, $df = 1$, $P < 0.05$). Analysis of average motility every 3 h revealed that sperm remained motile for over 18 h post-ejaculation in the coagulum (Fig. 2 and Supplementary Material Table S1). In contrast, chimpanzee coagulum and liquid semen did not differ significantly in temporal variation of motile-sperm percentage (ANCOVA: CH1, $F = 2.193$, $df = 1$, $P = 0.142$; CH2, $F = 0.042$, $df = 1$, $P = 0.839$; CH3, $F = 0.356$, $df = 1$, $P = 0.553$). In addition, the sperm motility was higher in the liquid portion than in the coagulum for CH3 only (Fig. 3 and Supplementary Material Table S1).

We observed between-species differences over time in the motile-sperm percentage for both coagulum and liquid semen (ANCOVA: coagulum, $F = 175.013$, $df = 1$, $P < 0.001$; liquid, $F = 4.349$, $df = 1$, $P < 0.05$). Orangutan sperm motility persisted longer in coagulum, whereas chimpanzee sperm motility persisted longer in the liquid portion.

Discussion

This study clarified previously unknown semen characteristics of Bornean orangutans, revealing differences between liquid semen and seminal coagulum, through a comparative analysis with chimpanzees. We showed that orangutan sperm in the coagulum portion were motile for longer

periods than that in the liquid portion. On the other hand, chimpanzee sperm did not exhibit this pattern. To the best of our knowledge, this is the first report describing this unique seminal characteristic in orangutans.

In this study, sperm concentration was much higher in chimpanzees than in orangutans. Among great apes, it is known that chimpanzees have both the largest gonads and the highest sperm concentration in ejaculate (Graham 1981). Previous research demonstrated that primates with multi-male, multi-female mating systems have heavier testes relative to body weight than polygynous or monogamous species (Harcourt et al. 1981; Dixson 1987). The evolutionary reason for this pattern is that postcopulatory sexual selection favors individuals with greater testicular mass, who are thus capable of producing more sperm (Birkhead and Møller 1998). Actually, it is well known that the testis size is much smaller in orangutans (e.g., 35.3 g) than in chimpanzees (e.g., 118.8 g) (Harcourt et al. 1981). Therefore, the sperm concentration results supported this trend and suggested that orangutan females are probably less promiscuous than chimpanzee females.

In chimpanzees, our results for sperm concentration and motile-sperm percentage immediately after collection corroborated other results for chimpanzee semen samples collected via masturbation (Marson et al. 1989, 1991). However, in orangutans, both variables were higher than reported previously for samples collected using an artificial vagina ($164 \pm 16.5 \times 10^6/\text{mL}$, $60 \pm 2.7\%$) (Vandevoort et al. 1993). Another study using rectal probe electroejaculation under anesthesia similarly found much lower mean sperm concentration and motile-sperm percentage ($61 \times 10^6/\text{mL}$ and 47%, respectively) (Warner et al. 1974). These between-study differences might be due to the stimulation of accessory glands in the reproductive tract and anesthesia use. The present study also identified individual differences in some characteristics. For instance, our study found that semen samples from masturbating chimpanzees lacked a copulatory plug, whereas it was present in samples from a chimpanzee that used an artificial vagina. Thus, differences in the sampling methods probably contributed to individual variation. Individual variation in ejaculation frequency during sampling and masturbation probably plays a role as well. Although some individual differences were observed, the tendency of the difference in motile-sperm percentage between the coagulum and the liquid portions was consistent within each species.

The seminal vesicles secrete a fluid, storing large amounts of sperm at ejaculation, and it contains various chemical components. Semenogelin proteins are the main structural component of seminal coagulum, and the rate of semenogelin evolution is correlated with the degree of female promiscuity and male-male competition (Jensen-Seaman and Li 2003; Kingan et al. 2003; Dorus et al. 2004; Carnahan and Jensen-Seaman 2008). In addition to its coagulating property, semenogelin is the precursor of sperm motility inhibitor (SPMI), which is secreted by the seminal vesicles (Robert and Gagnon 1996). Semenogelin and its degradation products are thought to affect sperm motility by acting on the sperm surface and entering them; however, the mechanism has not yet been elucidated (Robert and Gagnon 1999). Sperm immobilization occurs shortly after ejaculation. Lundwall (1998) considered that temporary sperm immobilization might preserve sperm energy. Further, considering the hypothesis shown in Fig. 1 of Kingan et al. (2003), the first male's semenogelin might not only provide a physical barrier by forming coagulum, but also inhibit sperm movement of a second male. However, the seminal coagulum could also be a reservoir to protect most of the sperm from the acidic vaginal environment by keeping it in a soft gelatinous lattice (García Granados et al. 2014). The presence of various physiologically active substances, including a high concentration of semenogelin, has been reported in seminal plasma; however, their physiological significance is still unknown.

According to our results, orangutan seminal coagulum contained many sperm and allowed gradual release of sperm. This suggests that, in orangutans, seminal coagulum might be important for the long-term stabilization of sperm motility post-ejaculation, which is different from chimpanzees. The uniquely long duration of sperm motility in orangutan seminal coagulum likely has adaptive significance.

Orangutans are the only semi-solitary species among the great apes. Unlike chimpanzees, their opportunities for copulation are considered to be low. One study noted that male and female orangutans maintained mating associations for 2 days only (Mitani 1985). Considering the low number of mating opportunities, orangutan coagulum may play less of a role in preventing entry of other males' sperm and more of a role in strengthening the individual's own-sperm survival. Fujii-Hanamoto et al. (2011) suggested that orangutans have higher fertilization efficiency than chimpanzees because comparisons of testicular microstructure have shown that the acrosomic system of orangutans is exceptionally well developed. The pressures of dispersed reproductive style and limited mating might select for unique traits not found in other apes. In addition, orangutan females develop genital swelling only during the gestation period and not as an external indicator of the periovulatory period (Galdikas 1981). This trait is unique to this genus because females of many primates, including chimpanzees, develop genital swelling during estrus to attract males (Wallis and Lemmon 1986). The male trait of seminal coagulum might be attributable to this difficulty in predicting ovulation.

Recently, the risk of extinction has become highly relevant for the orangutan. The orangutan has been designated a Critically Endangered species in the 2016 International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Ancrenaz et al. 2016). It is, therefore, essential to save the animals in zoos. However, captive orangutans are aging, and the population has been decreasing. Therefore, the application of artificial insemination (AI) is required. In great apes, the coagulum is often removed, and only the liquid portion of semen is used when AI is conducted (Gould 1982). To our knowledge, there is still only one example of successful AI in orangutans in the world (Forde 2014). Although our sample size was limited because much training was required to collect semen from animals without anesthesia, the results on semen obtained in a relatively natural state indicate that the coagulum portion of orangutan semen may play an important role in the long-term stabilization of sperm motility after ejaculation. This finding should not only contribute to the body of knowledge about orangutan reproduction but also significantly improve artificial insemination methods.

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References

- Ancrenaz M, Gumal M, Marshall AJ et al (2016) The IUCN Red List of Threatened. Species. <https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T17975A17966347.en>
- Batista M, Niño T, Santana M et al (2011) Influence of the preservation temperature (37, 20, 4, −196 °C) and the mixing of semen over sperm quality of majorera bucks. *Reprod Domest Anim* 46:281–288. <https://doi.org/10.1111/j.1439-0531.2010.01659.x>
- Birkhead TR, Møller AP (1998) Sperm competition and sexual selection. Academic Press, London
- Blandau RJ (1945) On the factors involved in sperm transport through the cervix uteri of the albino rat. *Am J Anat* 77:253–272. <https://doi.org/10.1002/aja.1000770205>
- Carnahan SJ, Jensen-Seaman MI (2008) Hominoid seminal protein evolution and ancestral mating behavior. *Am J Primatol* 70:939–948. <https://doi.org/10.1002/ajp.20585>
- Delgado RA, Van Schaik CP (2000) The behavioral ecology and conservation of the orangutan (*Pongo pygmaeus*): A tale of two islands. *Evol Anthropol* 9:201–218. [https://doi.org/10.1002/1520-6505\(2000\)9:5%3c201::AID-EVAN2%3e3.0.CO;2-Y](https://doi.org/10.1002/1520-6505(2000)9:5%3c201::AID-EVAN2%3e3.0.CO;2-Y)
- Devine MC (1977) Copulatory plugs, restricted mating opportunities and reproductive competition among male garter snakes. *Nature* 267:345–346. <https://doi.org/10.1038/267345a0>
- Dixson AF (1987) Observations on the evolution of the genitalia and copulatory behaviour in male primates. *J Zool* 213:423–443. <https://doi.org/10.1111/j.1469-7998.1987.tb03718.x>
- Dixson AF (2012) Primate sexuality: Comparative studies of the prosimians, monkeys, apes, and humans, 2nd edn. Oxford University Press, New York
- Dixson AL, Anderson MJ (2002) Sexual selection, seminal coagulation and copulatory plug formation in primates. *Folia Primatol* 73:63–69. <https://doi.org/10.1159/000064784>
- Dorus S, Evans PD, Wyckoff GJ et al (2004) Rate of molecular evolution of the seminal protein gene SEMG2 correlates with levels of female promiscuity. *Nat Genet* 36:1326–1329. <https://doi.org/10.1038/ng1471>
- Forde K (2014) First orangutan born through artificial insemination. <http://america.aljazeera.com/articles/2014/6/23/orangutans-assistedreproduction.html>. (Accessed 15 June 2020)
- Fujii-Hanamoto H, Matsubayashi K, Nakano M et al (2011) A comparative study on testicular microstructure and relative sperm production in gorillas, chimpanzees, and orangutans. *Am J Primatol* 73:570–577. <https://doi.org/10.1002/ajp.20930>
- Galdikas BMF (1981) Orangutan reproduction in the wild. In: Graham CE (ed) Reproductive biology of the great apes. Academic Press, New York, pp 281–300
- García Granados MD, Hernández López LE, Córdoba Aguilar A et al (2014) Effect of photoperiod on characteristics of semen obtained by electroejaculation in stump-tailed macaques (*Macaca arctoides*). *Primates* 55:393–401. <https://doi.org/10.1007/s10329-014-0414-6>
- Gould KG (1982) Ovulation detection and artificial insemination. *Am J Primatol* 3:15–25. <https://doi.org/10.1002/ajp.1350030507>
- Graham CE (1981) Reproductive biology of the great apes: Comparative and biomedical perspectives. Academic Press, New York
- Harcourt AH, Harvey PH, Larson SG, Short RV (1981) Testis weight, body weight and breeding system in primates. *Nature* 293:55–57. <https://doi.org/10.1038/293055a0>
- Hoskins DD, Patterson DL (1967) Prevention of coagulum formation with recovery of motile spermatozoa from rhesus monkey semen. *J Reprod Fertil* 13:337–340. <https://doi.org/10.1530/jrf.0.0130337>
- Jensen-Seaman MI, Li WH (2003) Evolution of the hominoid semenogelin genes, the major proteins of ejaculated semen. *J Mol Evol* 57:261–270. <https://doi.org/10.1007/s00239-003-2474-x>
- Jishage K, Suzuki H (1993) Maintenance of the fertilizing ability in capacitated mouse spermatozoa. *J Reprod Dev* 39:363–367. <https://doi.org/10.1262/jrd.39.363>
- Kingan SB, Tatar M, Rand DM (2003) Reduced polymorphism in the chimpanzee semen coagulating protein, semenogelin I. *J Mol Evol* 57:159–169. <https://doi.org/10.1007/s00239-002-2463-0>
- Kinoshita K, Kuze K, Miyakawa E et al (2016) Liquid storage of captive Bornean orangutan (*Pongo pygmaeus*) sperm collected without anesthesia. Society and the American Society of Primatologists, Chicago
- Kusunoki H, Daimaru H, Minami S et al (2001) Birth of a chimpanzee (*Pan troglodytes*) after artificial insemination with cryopreserved epididymal spermatozoa collected postmortem. *Zoo Biol* 20:135–143. <https://doi.org/10.1002/Zoo.1015>
- Lundwall A (1998) The cotton-top tamarin carries an extended semenogelin I gene but no semenogelin II gene. *Eur J Biochem* 255:45–51. <https://doi.org/10.1046/j.1432-1327.1998.2550045.x>
- Marson J, Gervais D, Meuris S et al (1989) Influence of ejaculation frequency on semen characteristics in chimpanzees (*Pan troglodytes*). *J Reprod Fertil* 85:43–50. <https://doi.org/10.1530/jrf.0.0850043>
- Marson J, Meuris S, Cooper RW, Jouannet P (1991) Puberty in the male chimpanzee: Progressive maturation of semen characteristics. *Biol Reprod* 44:448–455. <https://doi.org/10.1095/biolreprod.44.3.448>
- Martan J, Shepherd BA (1976) The role of copulatory plugs in reproduction in the guinea pig. *J Exp Zool* 196:79–84. <https://doi.org/10.1002/jez.1401960108>
- Mitani JC (1985) Mating behaviour of male orangutans in the Kutai Game Reserve, Indonesia. *Anim Behav* 33:392–402. [https://doi.org/10.1016/S0003-3472\(85\)80063-4](https://doi.org/10.1016/S0003-3472(85)80063-4)

- Robert M, Gagnon C (1996) Purification and characterization of the active precursor of a human sperm motility inhibitor secreted by the seminal vesicles: Identity with semenogelin. *Biol Reprod* 55:813–821. <https://doi.org/10.1095/biolreprod55.4.813>
- Robert M, Gagnon C (1999) Semenogelin I: a coagulum forming, multifunctional seminal vesicle protein. *Cell Mol Life Sci* 55:944–960. <https://doi.org/10.1007/s000180050346>
- Sato Y, Tomonaga M (2010) WAZA (world association of zoos and aquariums) ethical guidelines for the conduct of research on animals by zoos and aquariums. *Jpn J Anim Psychol* 60:139–146. <https://doi.org/10.2502/janip.60.2.4>
- Settlage DS, Hendrickx AG (1974) Observations on coagulum characteristics of the rhesus monkey electroejaculate. *Biol Reprod* 11:619–623. <https://doi.org/10.1095/biolreprod11.5.619>
- Tajima T, Malim TP, Inoue E (2018) Reproductive success of two male morphs in a free-ranging population of Bornean orangutans. *Primates* 59:127–133. <https://doi.org/10.1007/s10329-017-0648-1>
- van Schaik CP, van Hooft J (1996) Toward an understanding of the orangutan's social system. In: Marchant LF, Nishida T (eds) *Great ape societies*. Cambridge University Press, Cambridge, pp 3–15
- Vandevoort CA, Neville LE, Tollner TL, Field LP (1993) Noninvasive semen collection from an adult orangutan. *Zoo Biol* 12:257–265. <https://doi.org/10.1002/zoo.1430120303>
- Voss R (1979) Male accessory glands and the evolution of copulatory plugs in rodents. *Occas Pap Museum Zool Univ Mich* 689:1–27
- Wallis J, Lemmon WB (1986) Social behavior and genital swelling in pregnant chimpanzees (*Pan troglodytes*). *Am J Primatol* 10:171–183. <https://doi.org/10.1002/ajp.1350100207>
- Warner H, Martin DE, Keeling ME (1974) Electroejaculation of the great apes. *Ann Biomed Eng* 2:419–432. <https://doi.org/10.1007/BF02368098>

| | Orangutans | Chimpanzees |
|---|-------------------------------------|--|
| Volume (mL) | 1.4 ± 0.7; 0.2–3.0, P=0.178 | 0.09 ± 0.06; 0.02–0.24, P=0.936 |
| Sperm concentration (× 10 ⁶ /mL) | 708.5 ± 443.5; 92.8–1792.2, P=0.030 | 1,761.9 ± 1,110.8; 206.2–3167.8, P=0.559 |
| Motile sperm (%) | 85.1 ± 6.8; 65.3–93.3, P=0.645 | 79.2 ± 11.7; 62.4–96.8, P=0.140 |
| Viability (%) | 82.4 ± 6.6; 65.2–91.7, P=1.000 | 83.6 ± 7.1; 75.1–96.5, P=0.177 |

Mean ± SD; range, P value of the Mann–Whitney *U* test for orangutans and Kruskal–Wallis test for chimpanzees in the individual differences

Table 1 Characteristics of liquid semen from two orangutans and three chimpanzees analyzed immediately after collection

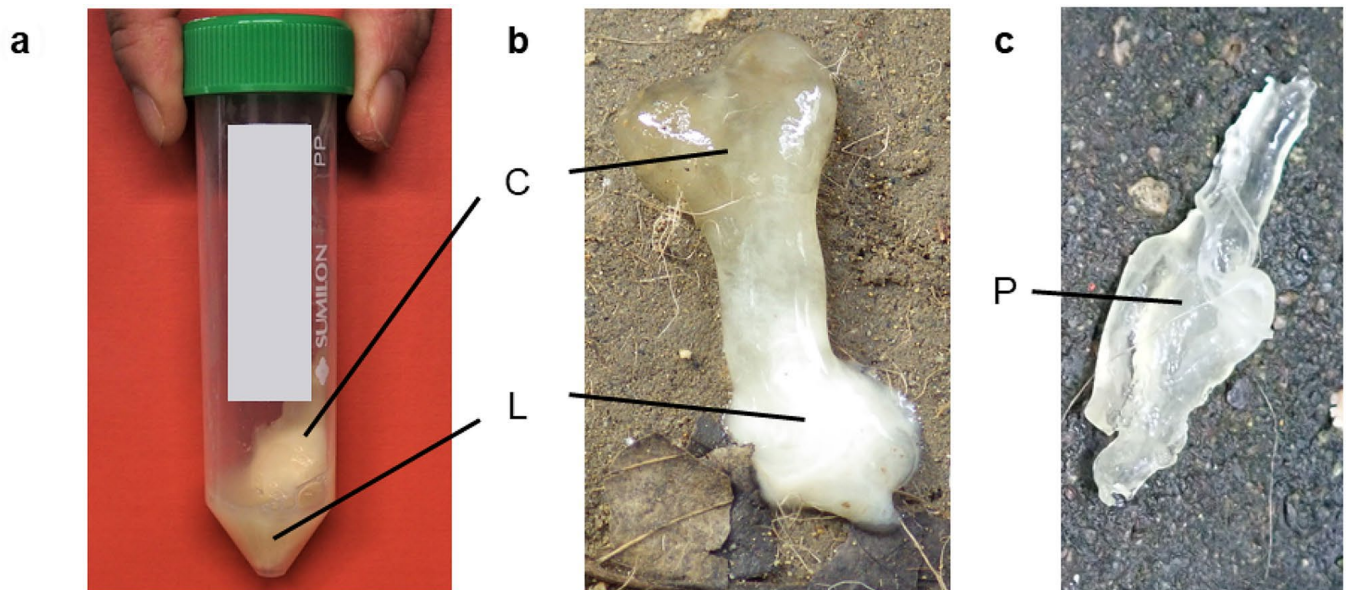
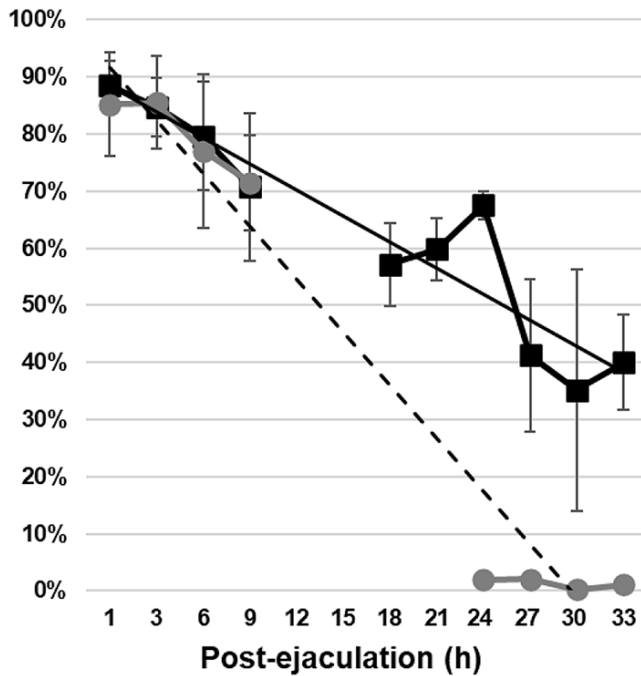


Fig. 1 Ejaculated semen of Bornean orangutan (*Pongo pygmaeus*) and chimpanzee (*Pan troglodytes*). a Orangutan seminal coagulum and liquid portions. b Chimpanzee seminal coagulum and liquid portions. c Chimpanzee copulatory plug. The seminal coagulum, liquid portions, and copulatory plug are labeled “C,” “L,” and “P,” respectively

(OU1)



(OU2)

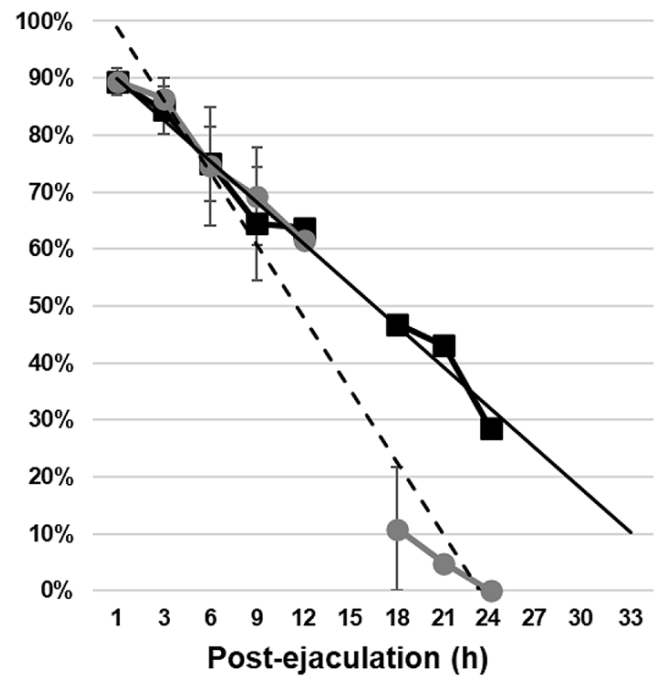
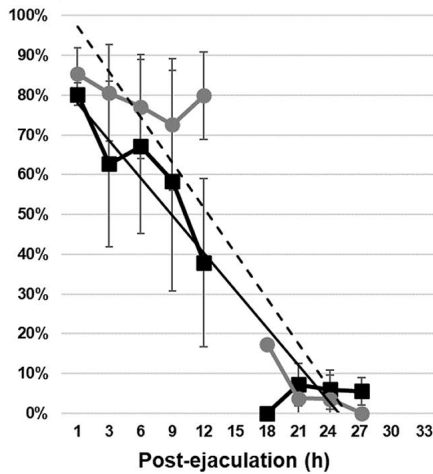
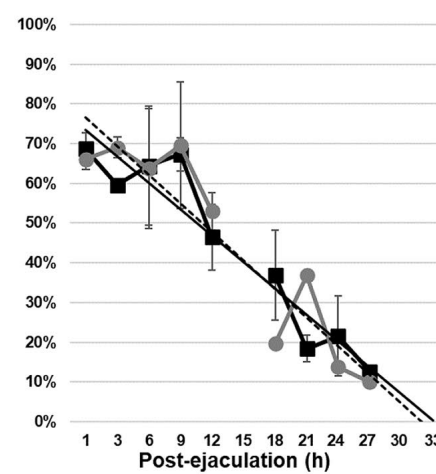


Fig. 2 Average sperm motility at 3-h intervals after ejaculation, in liquid semen and coagulum from two orangutans. Black and grey lines show motile-sperm percentage in seminal coagulum and liquid semen, respectively. Continuous and dotted lines show linear approximations of sperm motility in the coagulum and liquid semen, respectively. Error bars indicate standard deviation. For detailed results for each individual, refer to Supplementary Material Table S1

(CH1)



(CH2)



(CH3)

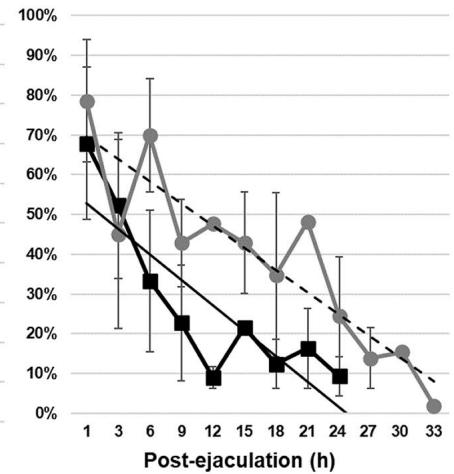


Fig. 3 Average sperm motility at 3-h intervals after ejaculation, in liquid semen and coagulum from three chimpanzees. Black and grey lines show the motile-sperm percentage in seminal coagulum and liquid semen, respectively. Continuous and dotted lines are the linear approximations of sperm motility in the coagulum and liquid semen, respectively. Error bars indicate standard deviation. For detailed results for each individual, refer to Supplementary Material Table S1

