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Disruption of gamete fusion alters the sperm-egg ratio at gamete interaction

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Abstract

Background The mechanisms enabling sperm to locate unfertilized eggs within the fallopian tubes remain a subject of debate in reproductive biology. Previous studies using polytocous mammals observed a 1:1 sperm-egg ratio within the ampulla at the time of fertilization. From these observations, it is hypothesized that this mechanism could be linked to sperm-egg fusion, such that unfertilized eggs may attract sperm until fusion occurs, whereupon the attraction ceases.

Methods To test this, fertile male mice were mated with infertile homozygous *Cd9Null* females, whose eggs cannot fuse with sperm, leading to the accumulation of supernumerary sperm in the perivitelline space. Fertile heterozygous *Cd9Het* females, were used as controls.

Results The results revealed that both *Cd9Null* and *Cd9Het* females ovulated similar numbers of eggs (6.53 ± 0.61 vs. 5.50 ± 0.53 eggs/ampulla). The majority of eggs produced by *Cd9Het* females were fertilized by one single sperm, without any additional sperm found bound to the zona or within the perivitelline space. In contrast, most of the eggs ovulated by *Cd9Null* females either showed an accumulation of supernumerary sperm within in the perivitelline space or showed no sperm bound to the zona nor present within the perivitelline space.

Conclusions These findings indicate that genetic ablation of Cd9 leads to an imbalance in the 1:1 sperm-egg ratio observed within the ampulla. This information may set the foundation for future studies with the aim to identify the specific mechanisms that sperm use to locate unfertilized eggs and whether they become ineffective when gamete fusion is prevented.

Background

In mammalian reproduction, a large quantity of sperm enter the female reproductive tract, yet few manage to reach the oviduct and find an oocyte. Sperm in the oviduct bind to the oviductal epithelium of the isthmus, forming a sperm storage reservoir [1–3]. Within this reservoir, sperm undergo capacitation—a process that enables sperm to fertilize the oocyte [4, 5]. Capacitation occurs asynchronously, and only up to 10% of spermatozoa are capacitated, reducing the number of capacitated sperm in the ampulla to approximately 10–20 cells [6–8]. Given the minute size of the gametes (~ 120 µm) relative to the oviductal dimensions, the likelihood of incidental arrival of sperm at the oocyte is minimal. This leads to the hypothesis of the presence of guidance mechanisms that direct the sperm from the isthmus through the ampulla and the cumulus oophorus, before sperm bind the extracellular zona pellucida surrounding the egg [9, 10].

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Sperm of non-mammalian species such as echinoderms [11], insects, and fish [12, 13], use specific signaling mechanisms to locate unfertilized eggs. Although it remains uncertain whether mammalian sperm employ similar signaling mechanisms for egg localization, investigations in mice, rats, and pigs demonstrate that individual spermatozoa can successfully localize and fertilize each egg within the ovarian ampullae [14, 15]. During normal mating, most eggs within the cumulus oophorus of polytocous mammals are fertilized within a relatively brief period of time [16]. Indeed, effective localization of unfertilized eggs is important for successful fertilization, as delayed fertilization can negatively impact preimplantation embryonic development [17, 18].

Sperm infrequently reverse their direction of motility once interacting with the zona pellucida, therefore, it is suspected that molecular mechanisms that have yet-to-be-identified facilitate the guidance of sperm towards unfertilized oocytes, to ensure that each egg interacts with at least one sperm. Also, numerous pioneer studies in polytocous mammalian species (e.g., mice, rats, rabbits) report a 1:1 sperm-egg ratio within the ampulla at the time of fertilization [7, 8, 19–23]. These observations may be explained by random effect — due to tens of sperm being present within the ampulla [24], one single sperm may find one unfertilized egg by random chance. Alternatively, a signaling mechanism may mediate the attraction of one sperm to one unfertilized egg; such a mechanism may cease once the egg has fused with the sperm. At present, it remains uncertain whether the '1:1' sperm-to-egg ratio is influenced by gamete fusion or by random effect. If unfertilized eggs possess a mechanism to attract sperm that ceases upon gamete fusion, then the remaining sperm would redirect to locate unfertilized eggs that are still capable of sperm attraction. This model predicts that preventing gamete fusion may lead to an accumulation of an excess number of sperm gathering around a small number of eggs, resulting in the majority of eggs not interacting with any sperm. After binding and crossing the zona pellucida, sperm adhere to the egg plasma membrane, also known as the oolemma. After successful adhesion, sperm fuse with the oolemma. CD9, is a tetraspanin protein expressed in the oolemma and was the first one identified as necessary for gamete adhesion prior to fusion [25]. Females lacking CD9 exhibit significantly reduced fertility or infertility due to impaired sperm adhesion to the oolemma, resulting in supernumerary sperm accumulating within the egg's perivitelline space [26]. In this study, we use $Cd9^{Null}$ mice to investigate sperm-egg ratios following spontaneous mating in mice and we explore the role of gamete fusion in monospermic interactions with ovulated oocytes.

Methods

Genotyping

Transgenic $Cd9^{Null}$ mice were genotyped using PCR with the following cycle conditions: [95 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min] × 30 cycles, followed by 72 °C for 7 min, then storage at 4 °C for >30 min. This was performed using primers from previous studies [26].

Light microscopy

Samples were mounted in PBS, and images of oocytes and embryos were acquired using a confocal microscope LSM 800 (Zeiss) with 10X or 20X objectives, at room temperature. LSM 800 images were processed with ZEN 3.2 software (Zeiss), exported as 300 dpi resolution TIFF files and compiled using Adobe Illustrator 25.0. Maximum intensity projections of confocal optical sections were generated and combined with DIC images of oocytes, embryos .

Mating and gamete collection

Female mice used in the experiment were allowed to undergo their natural ovulation cycle without artificial stimulation. Homozygous and heterozygous *Cd9* KO female mice (with an outbred genetic background) were co-mated overnight with sexually mature ICR-CD1 males, aged 12–14 weeks, confirmed to be fertile based on their ability to sire offspring. Eight independent mating experiments were performed. After mating, females that displayed a copulatory plug were euthanized and oocytes were collected from the ampullae. After images were acquired, the oocytes of the homozygous Cd9 KO ($Cd9^{Null}$) females were evaluated for the presence of sperm within the perivitelline space and the oocytes collected from the of heterozygous *Cd9* KO ($Cd9^{Het}$) females were evaluated to count the number of one- and two-cell embryos.

Gamete or embryo quantifications and statistics

Sperm bound to oocytes or located within the perivitelline space, as well as the number of fertilized eggs and one- and two-cell stage embryos, were quantified using maximum intensity projections acquired through confocal microscopy (LSM800, Zeiss). Presence of sperm within the perivitelline space or the formation of embryos from gamete fusion were detected and analyzed using the ZEN 3.2 software (Blue Edition, Zeiss), and descriptive statistics were used to analyze number of sperm/oocytes or sperm/embryo using One-Way ANOVA, followed by the Tukey's Honestly Significant Difference (HSD) post-hoc tests. Sperm counts ($n=5$ experiments) were shown as boxplots with error bars indicating data points within the 10th and 90th percentiles and a horizontal line representing the median. The middle two quartiles were shown as boxplots, and bold points were used to mark

outliers. All statistical analyses were conducted using R Studio (v. 3.6.3).

Results

Studies in mice, rats, and rabbits report a 1:1 sperm-egg ratio within the ampulla at the time of fertilization [7, 8, 19–23]. These observations may be explained as a random effect — such that, due to the presence of hundreds of sperm within the ampulla, a single sperm may find one unfertilized egg by random chance. Alternatively, a yet-to be identified signaling mechanism may mediate the attraction of one sperm to one unfertilized egg, which would, in turn, facilitate in the redirection of sperm away from the fertilized eggs and encourage sperm interaction with the remaining unfertilized eggs. One could argue that this putative mechanism may be dependent on sperm-egg fusion, such that unfertilized eggs attract sperm until gamete fusion, whereupon this mechanism ceases in function. Therefore, the remaining sperm would find other unfertilized eggs. This model raises the prediction that preventing gamete fusion may result in the gathering of supernumerary sperm to a small number of unfertilized eggs, resulting in the majority of unfertilized eggs without sperm.

To test this hypothesis, fertile male mice were mated with *Cd9*^{Null} females, whose eggs cannot fuse with sperm, accumulating supernumerary sperm within the perivitelline space [26]. Fertile *Cd9*^{Het} females were used as the controls. Males mated normally with naturally ovulated female mice; 16 h post mating, we collected ampullae from female mice showing a clear copulatory plug and uterine horns filled with sperm — which are clear indicators of successful copulation. Unfertilized and fertilized eggs were collected and stained with WGA 633 and Hoechst to detect zona pellucida and sperm, respectively.

Under natural ovulation, *Cd9*^{Null} and *Cd9*^{Het} females ovulated a comparable number of MII eggs (6.53±0.61 vs. 5.50±0.53 eggs/ampulla)(Fig. 1A). To quantify the eggs presenting a 'one-egg to one-sperm' ratio, for the *Cd9*^{Null}, we counted the number of eggs presenting one single sperm in the perivitelline space; and for the *Cd9*^{Het}, we counted the number of fertilized eggs (2-cell embryos) presenting no sperm in the perivitelline space (Fig. 1A-C). The majority of *Cd9*^{Het} eggs (78.54±2.98%; s.e.m) were shown to be crossed by one single sperm, which is consistent with previous studies [7, 8, 19–23, 27], while the remaining eggs were either shown to have not been crossed by sperm (10.15±5.13%; s.e.m) or to have been crossed by multiple sperm at 2–3 sperm/egg (11.31±2.89%; s.e.m). By contrast, the majority of *Cd9*^{Null} eggs (54.42±7.81%; s.e.m) were shown to have been crossed by no sperm, while the remaining eggs were either shown to have one single sperm within the perivitelline space (10.15±5.11%; s.e.m) or to have accumulated a supernumerary quantity of sperm within the perivitelline space at 2–14 sperm/egg (34.79±7.05%; s.e.m) (Fig. 1C and D).

Therefore, it is reasonable to conclude that the 1:1 sperm/egg ratio observed in the ampullae of fertile females is dependent on gamete fusion. This evidence suggests the existence of mechanisms for sperm to locate unfertilized eggs within the ampulla.

Discussion

This study shows that *Cd9*^{Null} oocytes collected from naturally ovulated females mated with fertile males exhibit either an absence of sperm or an excessive accumulation of sperm in the perivitelline space. This imbalance in the sperm-egg ratio is attributed to the lack of gamete fusion. This evidence provides additional support for the existence of a localized attraction mechanism that directs sperm towards unfertilized eggs within the ampulla.

Several studies conducted on mammals, including humans [28–30], initially supported the idea of an undefined chemical attraction mechanism originating from the egg's microenvironment [31, 32] (cumulus cells or the egg itself [10]), which could be replicated in vitro using egg-conditioned media [28]. Early research focused on

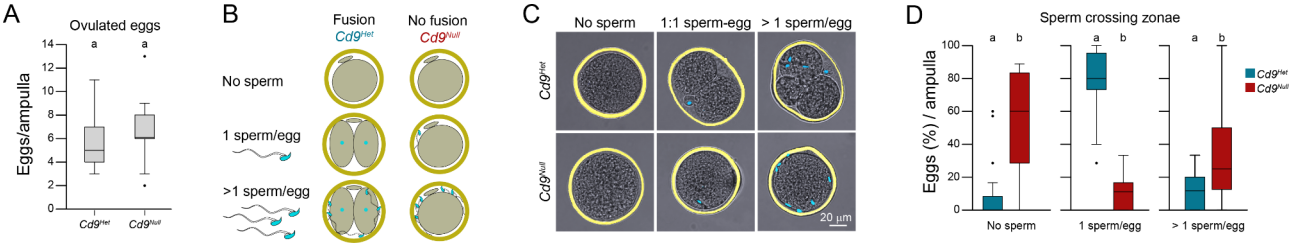

Fig. 1 Sperm-egg interactions in *Cd9*^{Null} and *Cd9*^{Het} females. (**A**) Quantification of naturally ovulated MII oocyte from *Cd9*^{Null} and *Cd9*^{Het} females ($n = 16$). Boxplots represent the median (vertical line) number of ovulated oocytes, with data points within the 10th and 90th percentiles shown as error bars. Boxes encompass the middle two quartiles, and dots indicate outliers. Superscript letters denote statistical significance ($P < 0.05$) as determined by One-way ANOVA followed by Tukey's HSD (honestly significant difference) post-hoc test. (**B**) Schematics showing different possible scenarios for sperm-egg ratio in the presence or absence of gamete fusion. (**C**) Maximum intensity projections of confocal optical sections to a single plane combined with DIC images of MII oocytes, fertilized eggs or embryos collected after in vivo mating, presenting no sperm, 1 sperm, or more than one sperm per oocyte. Yellow, WGA-633; cyan, sperm nuclei stained with Hoechst. (**D**) Same as in A, for observations shown in (**C**)

the follicular fluids' ability to attract sperm, revealing that follicular fluids could induce sperm accumulation in approximately 50% of cases in vitro [33]. Subsequent studies in mice and rabbits used a chemotactic Zigmond chamber [34], designed to establish chemical gradients, to test if motile sperm could respond to even a 1% concentration change of putative chemo-attractant candidates [34]. These experiments identified subpopulations of sperm in mice and rabbits that reacted to follicular or oviductal fluids [29, 30], showing significantly enhanced sperm motility parameters such as curvilinear velocity, total distance traveled per unit time, linear velocity, and linearity [29, 30].

More studies in mice and humans demonstrated that ovulated eggs or cumulus cells could also attract sperm in vitro. Crude extracts from mouse ovulated eggs induced significant changes in sperm dynamic parameters, including curvilinear velocity, linear velocity, linearity, progressive motility, and directionality [32]. In humans, using the Zigmond chamber, media conditioned with MII human eggs or their surrounding cumulus cells attracted around 8% of human sperm [28]. These findings align with previous reports showing a low number of human sperm responsive to chemo-attractive stimuli [35–38].

Unfortunately, no genetic follow-up studies have confirmed the existence of signaling mechanisms mediating attraction to unfertilized eggs (or repulsion from fertilized ones), leaving their identity and role in fertilization still unknown. One could hypothesize the presence of a molecule responsible for this short-distance attraction; its absence would result in a loss of sperm attraction to the egg, potentially leading to female infertility (sperm unable to locate eggs in the ampulla) or severe subfertility (sperm may randomly find a few eggs but miss the majority).

In addition, a few studies suggest that a gradient of progesterone, ranging from pico- to micro- molar concentrations, extend from the periphery of the cumulus mass towards the ovulated egg and facilitates the attraction of sperm towards unfertilized eggs by activating the sperm-specific flagellar and Ca^{2+}-selective channel CatSper [39–41]. This model would raise the prediction that all $Cd9^{Null}$ eggs should be located and crossed by sperm within the ampulla. However, our observations revealed that only a minority of $Cd9^{Null}$ oocytes showed sperm within the perivitelline space, suggesting that a progesterone gradient may not be sufficient to ensure effective sperm attraction and distribution to all oocytes within the ampulla.

Another hypothesis posits that the observed 1:1 sperm-egg ratio may not be influenced by a chemotactic gradient, but, rather, by a post-fertilization interference to sperm binding capacity to the zona pellucida, which would occur hours after fertilization [42, 43]. Upon binding to the intact ZP2 protein in the zona [44, 45], sperm cross the zona and fuse with the oolemma. Following fusion, cortical granules release ovastacin, a metallo-endoprotease that cleaves ZP2 [46], rendering the zona inaccessible to further sperm binding (complete block to sperm binding [47]). Thus, sperm encountering a fertilized egg with cleaved ZP2 would move on to find an unfertilized egg. Although it is plausible that such a mechanism may be sufficient to ensure a 1:1 sperm-egg ratio in the ampulla, it may not be as effective, as complete block to sperm binding typically takes hours after gamete fusion [47]. In this instance, the remaining unbound sperm would continue to be active and move throughout the ampulla attempting to find eggs randomly, including fertilized eggs that have already achieved gamete fusion.

By identifying and utilizing molecules mediating sperm attraction (proven necessary in gene-edited animals), it will be possible to improve sperm selection methods. Instead of solely relying on morphological features [48], embryologists could select sperm based on their ability to be attracted to unfertilized eggs. This approach could be especially beneficial during intracytoplasmic sperm injection (ICSI), where a single sperm is directly injected into an egg. By gaining deeper insights into sperm functionality, we could potentially enhance the chances of successful pregnancies and healthy births of embryos conceived by ICSI.

Conclusions

The findings of this study indicate that the genetic disruption of gamete fusion affects the typical 1:1 sperm-egg ratio. This highlights the role of gamete fusion in maintaining this even ratio across the majority of oocytes within the ampulla. Future research will explore molecules released by unfertilized eggs that potentially facilitate attraction, as well as molecules from fertilized eggs that may induce sperm repulsion or encourage redirection to unfertilized eggs.

Abbreviations

ANOVA	Analysis of variance
DIC	Differential interference contrast
eCG	Equine chorionic gonadotropin
hCG	Human chorionic gonadotropin
HSD	Honestly significant difference
IACUC	Institutional animal care and use committee
KO	Knockout
LSM	Laser scanning microscope
MII	Metaphase II
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
s.e.m	Standard error of the mean
TU	University of tulsa
WGA	Wheat germ agglutinin

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Authors Contribution
ES and MA designed the study. ES performed mating experiments. ES and MA carried out sample collections, imaging and data analyses and interpretation. MA and ES wrote the manuscript.

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Data availability
All the data shown in this manuscript are included within the article.

Declarations

Ethics approval and IACUC Protocols
Experiments involving normal or *Cd9Null* mutant mice were performed in compliance with the guidelines of the Animal Care and Use Committee of the University of Tulsa, following approved protocols TU-0050 and TU-0050R1.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Hunter RH, Léglise PC. Tubal surgery in the rabbit: fertilization and polyspermy after resection of the isthmus. Am J Anat. 1971;132:45–52.
2. Hunter RH, Léglise PC. Polyspermic fertilization following tubal surgery in pigs, with particular reference to the rôle of the isthmus. J Reprod Fertil. 1971;24:233–46.
3. Smith TT, Yanagimachi R. The viability of hamster spermatozoa stored in the isthmus of the oviduct: the importance of sperm-epithelium contact for sperm survival. Biol Reprod. 1990;42:450–7.
4. Puga Molina LC, Luque GM, Balestrini PA, Marín-Briggiler CI, Romarowski A, Buffone MG. Molecular basis of human sperm capacitation. Front Cell Dev Biol. 2018;6:72.
5. Hino T, Muro Y, Tamura-Nakano M, Okabe M, Tateno H, Yanagimachi R. The Behavior and Acrosomal Status of Mouse Spermatozoa in Vitro, and within the Oviduct during fertilization after Natural mating. Biol Reprod. 2016;95:50.
6. Cohen-Dayag A, Tur-Kaspa I, Dor J, Mashiach S, Eisenbach M. Sperm capacitation in humans is transient and correlates with chemotactic responsiveness to follicular factors. Proc Natl Acad Sci U S A. 1995;92:11039–43.
7. Cummins JM, Yanagimachi R. Sperm-egg ratios and the site of the acrosome reaction during in vivo fertilization in the hamster. Gamete Res. 1982;5:239–56.
8. Yanagimachi R, Mahi CA. The sperm acrosome reaction and fertilization in the guinea-pig: a study in vivo. J Reprod Fertil. 1976;46:49–54.
9. Miller DJ. Sperm in the mammalian female Reproductive Tract: surfing through the Tract to try to beat the odds. Annu Rev Anim Biosci. 2024;12:301–19.
10. Eisenbach M, Giojalas LC. Sperm guidance in mammals - an unpaved road to the egg. Nat Rev Mol Cell Biol. 2006;7:276–85.
11. Seifert R, Flick M, Bönigk W, Alvarez L, Trötschel C, Poetsch A, et al. The CatSper channel controls chemosensation in sea urchin sperm. EMBO J. 2015;34:379–92.
12. Yanagimachi R, Cherr G, Matsubara T, Andoh T, Harumi T, Vines C, et al. Sperm attractant in the micropyle region of fish and insect eggs. Biol Reprod. 2013;88:47.
13. Yanagimachi R, Harumi T, Matsubara H, Yan W, Yuan S, Hirohashi N, et al. Chemical and physical guidance of fish spermatozoa into the egg through the micropyle. Biol Reprod. 2017;96:780–99.
14. Avella MA, Baibakov BA, Jimenez-Movilla M, Sadusky AB, Dean J. ZP2 peptide beads select human sperm in vitro, decoy mouse sperm in vivo, and provide reversible contraception. Sci Transl Med. 2016;8:336ra60.
15. Hunter RH. Sperm:egg ratios and putative molecular signals to modulate gamete interactions in polytocous mammals. Mol Reprod Dev. 1993;35:324–7.
16. Yanagimachi R. Mysteries and unsolved problems of mammalian fertilization and related topics. Biol Reprod. 2022;106:644–75.
17. Hammond J. The fertilisation of rabbit ova in relation to time: a Method of Controlling the Litter Size, the duration of pregnancy and the weight of the Young at Birth. J Exp Biol. 1934;11:140–61.
18. Chang MC, Pincus G. Physiology of fertilization in mammals. Physiol Rev. 1951;31:1–26.
19. Bavister BD. Fertilization of hamster eggs in vitro at sperm:egg ratios close to unity. J Exp Zool. 1979;210:259–64.
20. Moricard R, Bossu J. Arrival of fertilizing sperm at the follicular cell of the secondary oocyte; a study of the rat. Fertil Steril. 1951;2:260–6.
21. Overstreet JW, Cooper GW. Sperm transport in the reproductive tract of the female rabbit: I. The rapid transit phase of transport. Biol Reprod. 1978;19:101–14.
22. Overstreet JW, Cooper GW. Sperm transport in the reproductive tract of the female rabbit: II. The sustained phase of transport. Biol Reprod. 1978;19:115–32.
23. Shalgi R, Kraicer PF. Timing of sperm transport, sperm penetration and cleavage in the rat. J Exp Zool. 1978;204:353–60.
24. Croxatto HB. Physiology of gamete and embryo transport through the fallopian tube. Reprod Biomed Online. 2002;4:160–9.
25. Bhakta HH, Refai FH, Avella MA. The molecular mechanisms mediating mammalian fertilization. Development. 2019;146:dev176966.
26. Le Naour F, Rubinstein E, Jasmin C, Prenant M, Boucheix C. Severely reduced female fertility in CD9-deficient mice. Science. 2000;287:319–21.
27. Cummins JM. Hyperactivated motility patterns of ram spermatozoa recovered from the oviducts of mated ewes. Gamete Res. 1982;6:53–63.
28. Sun F, Bahat A, Gakamsky A, Girsh E, Katz N, Giojalas LC, et al. Human sperm chemotaxis: both the oocyte and its surrounding cumulus cells secrete sperm chemoattractants. Hum Reprod. 2005;20:761–7.
29. Fabro G, Rovasio RA, Civalero S, Frenkel A, Caplan SR, Eisenbach M, et al. Chemotaxis of Capacitated rabbit spermatozoa to follicular fluid revealed by a Novel directionality-based Assay1. Biol Reprod. 2002;67:1565–71.
30. Oliveira RG, Tomasi L, Rovasio RA, Giojalas LC. Increased velocity and induction of chemotactic response in mouse spermatozoa by follicular and oviductal fluids. Reproduction. 1999;115:23–7.
31. Guidobaldi HA, Teves ME, Uñates DR, Giojalas LC. Sperm transport and retention at the fertilization site is orchestrated by a chemical guidance and oviduct movement. Reprod Camb Engl. 2012;143:587–96.
32. Giojalas LC, Rovasio RA. Mouse spermatozoa modify their motility parameters and chemotactic response to factors from the oocyte microenvironment. Int J Androl. 1998;21:201–6.
33. Ralt D, Goldenberg M, Fetterolf P, Thompson D, Dor J, Mashiach S, et al. Sperm attraction to a follicular factor(s) correlates with human egg fertilizability. Proc Natl Acad Sci U S A. 1991;88:2840–4.
34. Zigmond SH. Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. J Cell Biol. 1977;75:606–16.
35. Ralt D, Manor M, Cohen-Dayag A, Tur-Kaspa I, Ben-Shlomo I, Makler A, et al. Chemotaxis and chemokinesis of human spermatozoa to follicular factors. Biol Reprod. 1994;50:774–85.

36. Cohen-Dayag A, Ralt D, Tur-Kaspa I, Manor M, Makler A, Dor J, et al. Sequential acquisition of chemotactic responsiveness by human spermatozoa. Biol Reprod. 1994;50:786–90.
37. Eisenbach M, Tur-Kaspa I. Do human eggs attract spermatozoa? BioEssays. News Rev Mol Cell Dev Biol. 1999;21:203–10.
38. Jaiswal BS, Tur-Kaspa I, Dor J, Mashiach S, Eisenbach M. Human sperm chemotaxis: is progesterone a chemoattractant? Biol Reprod. 1999;60:1314–9.
39. Strünker T, Goodwin N, Brenker C, Kashikar ND, Weyand I, Seifert R, et al. The CatSper channel mediates progesterone-induced Ca2 + influx in human sperm. Nature. 2011;471:382–6.
40. Lishko PV, Botchkina IL, Kirichok Y. Progesterone activates the principal Ca2 + channel of human sperm. Nature. 2011;471:387–91.
41. Sumigama S, Mansell S, Miller M, Lishko PV, Cherr GN, Meyers SA, et al. Progesterone accelerates the completion of sperm capacitation and activates CatSper Channel in Spermatozoa from the Rhesus Macaque. Biol Reprod. 2015;93:130.
42. Nishio S, Emori C, Wiseman B, Fahrenkamp D, Dioguardi E, Zamora-Caballero S, et al. ZP2 cleavage blocks polyspermy by modulating the architecture of the egg coat. Cell. 2024;187:1440–e145924.
43. Gahlay G, Gauthier L, Baibakov B, Epifano O, Dean J. Gamete recognition in mice depends on the cleavage status of an egg's zona pellucida protein. Science. 2010;329:216–9.
44. Avella MA, Baibakov B, Dean J. A single domain of the ZP2 zona pellucida protein mediates gamete recognition in mice and humans. J Cell Biol. 2014;205:801–9.
45. Tokuhiro K, Dean J. Glycan-independent Gamete Recognition Triggers Egg Zinc Sparks and ZP2 cleavage to prevent polyspermy. Dev Cell. 2018;46:627–e6405.
46. Burkart AD, Xiong B, Baibakov B, Jiménez-Movilla M, Dean J. Ovastacin, a cortical granule protease, cleaves ZP2 in the zona pellucida to prevent polyspermy. J Cell Biol. 2012;197:37–44.
47. Inoue M, Wolf DP. Sperm binding characteristics of the murine zona pellucida. Biol Reprod. 1975;13:340–6.
48. Check JH, Adelson HG, Schubert BR, Bollendorf A. Evaluation of sperm morphology using Kruger's strict criteria. Arch Androl. 1992;28:15–7.

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