HIROSHIMA UNIVERSITY

“Unlock Secrets of Gender within Sperm”

~Sperm Sexing Method for Animal Production~

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Multiplication of Animal

The process of multiplication is called as "Reproduction"

- **Reproduction** is the biological process in which a child (next generation) is born from parents".
- The ability of reproduction is called as Fertility.

Reproduction system

Asexual
(Parthenogenesis)
- ✓ Microorganisms, lower animals
- ✓ Protozoans, Sponges, Coelenterates,
- ✓ Certain worms and Tunicates

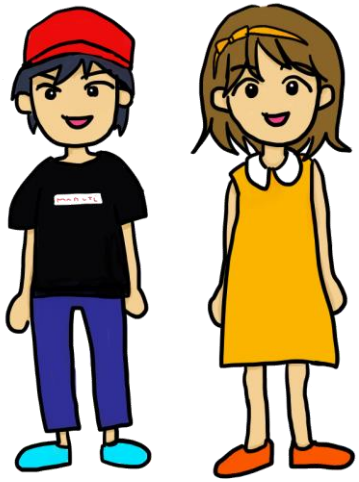
Sexual
(Male & Female)
Higher animals

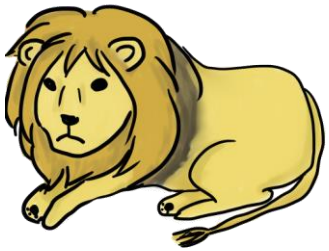
Sex determination

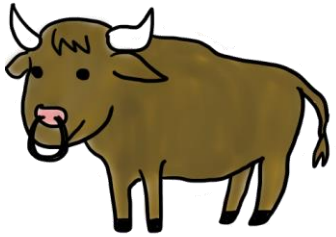
Reptiles

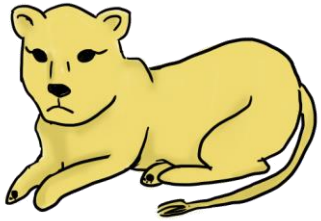
Temperature at hatching decides their sex

Mammals









The sex chromosomes decides their sex

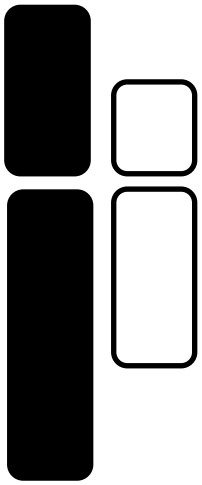
Mammalian sex chromosomes

X chromosome

No. of bp
15,000 (human)

No. of genes
1,000 (human)

Functional genes
- Androgen receptor
- Glucose-6 - dehydrogenase
- etc…



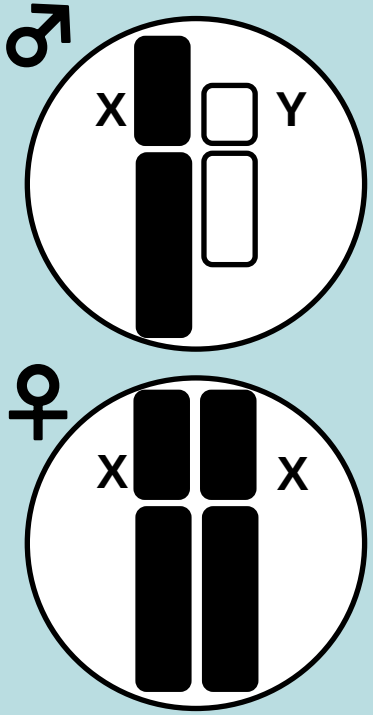
Y chromosome

No. of bp
5,000 (human)

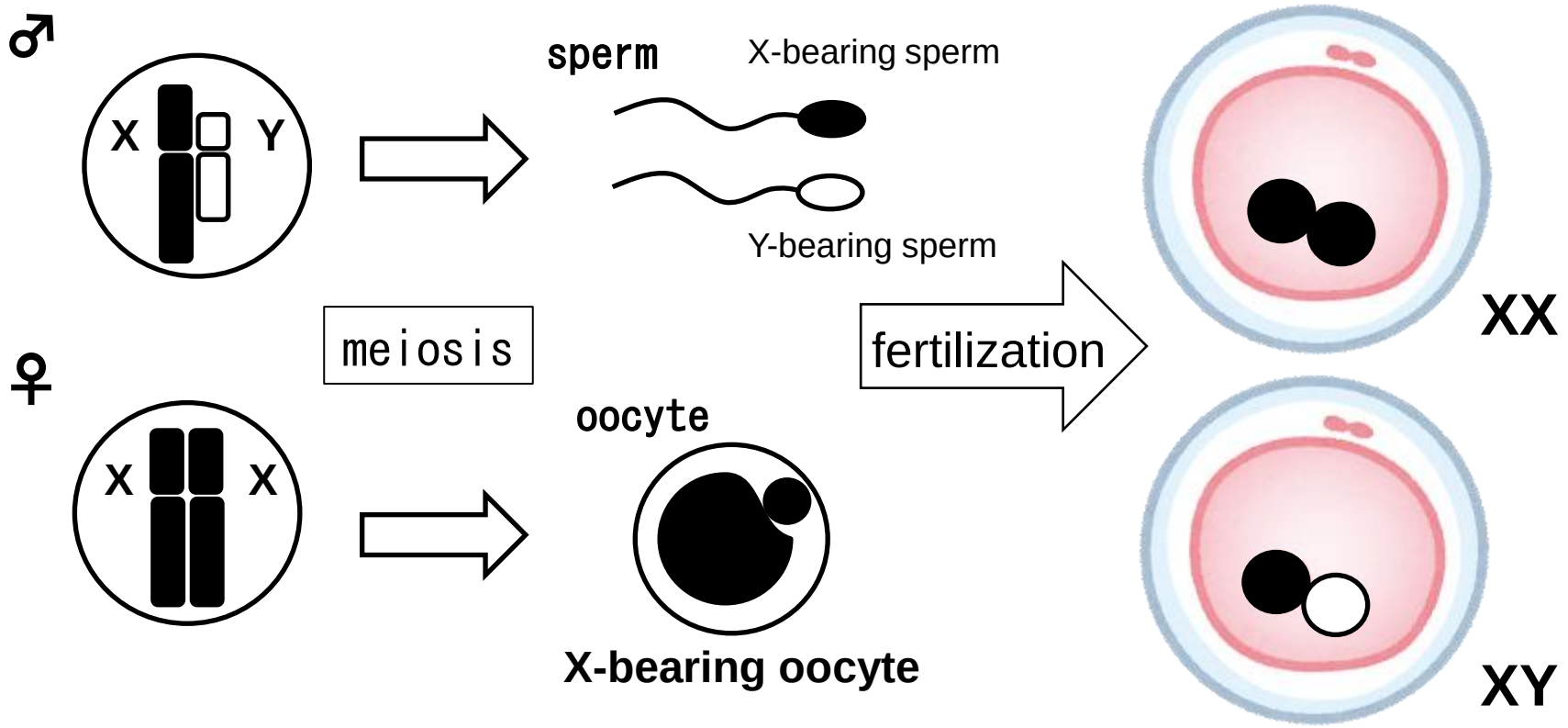
No. of genes
78 (human)

Functional genes
SRY, etc...

Hetero(XY)→male(♂)
Homo (XX)→female(♀)

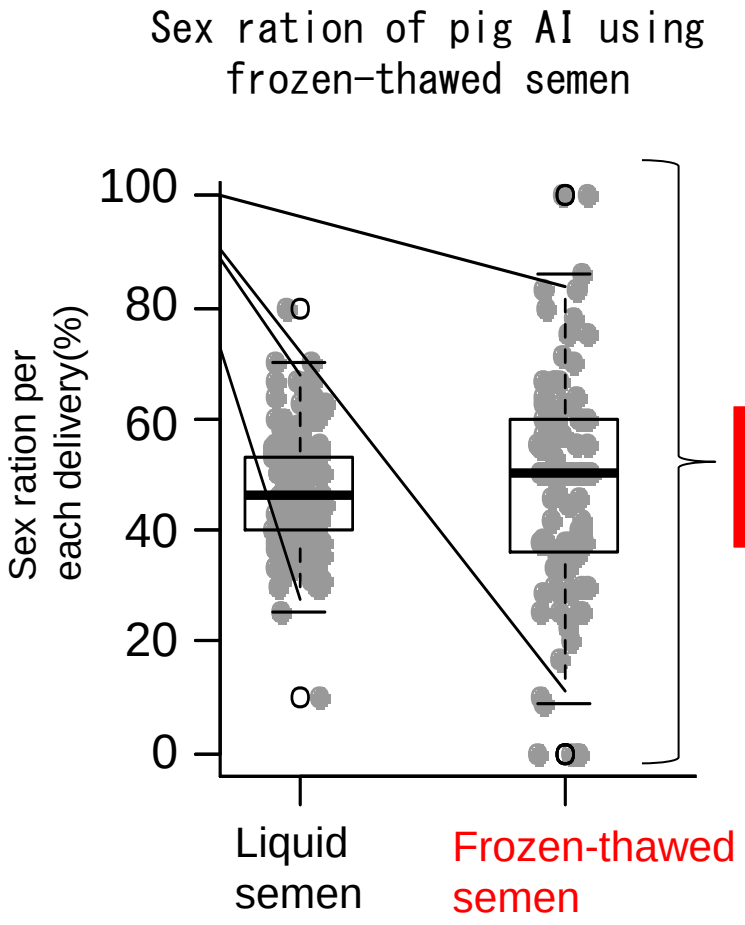


Gametes generation and Fertilization



Each type of sex chromosome, X or Y in sperm decides sex of embryo

Our unpublished data of pig artificial insemination using frozen-thawed semen



Academic Question

estimating that

1. Sperm have potential different functions between X-sperm and Y-sperm.
2. The different functions are masked under the physiological condition to keep the 50:50 male and female ration.
3. The unphysiological environments appear the potential differences to change the sex ration.

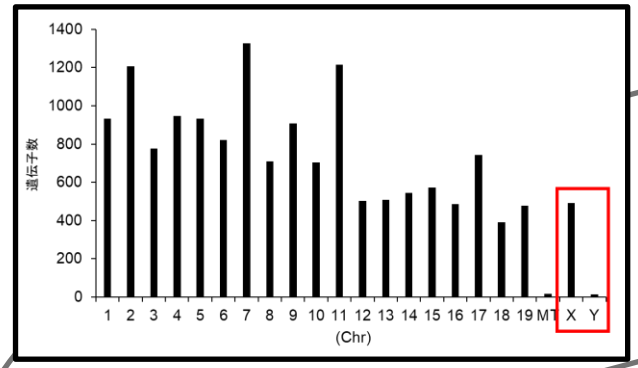
Possibility of technological development

Finding the potential differences between X-sperm and Y sperm and setting the limited environments to appear the differences would

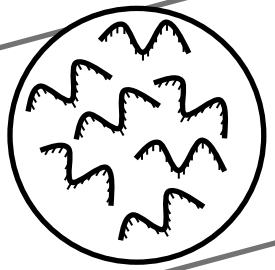
“enable to develop the simple sperm sexing method”.

Strategy to find potential different functions between X-sperm and Y-sperm

Transcriptome analysis

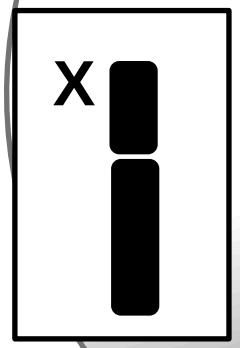


Total RNAs



sperm

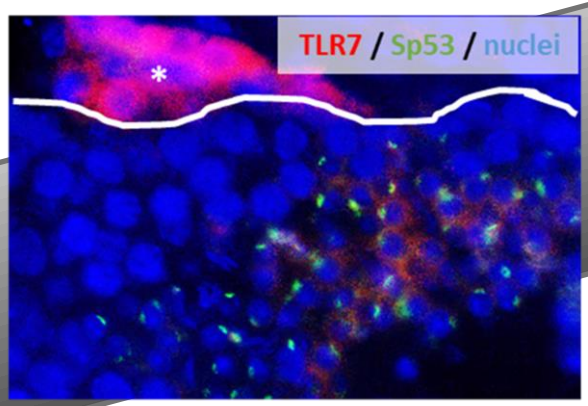
Select genes encoded by X-chromosome



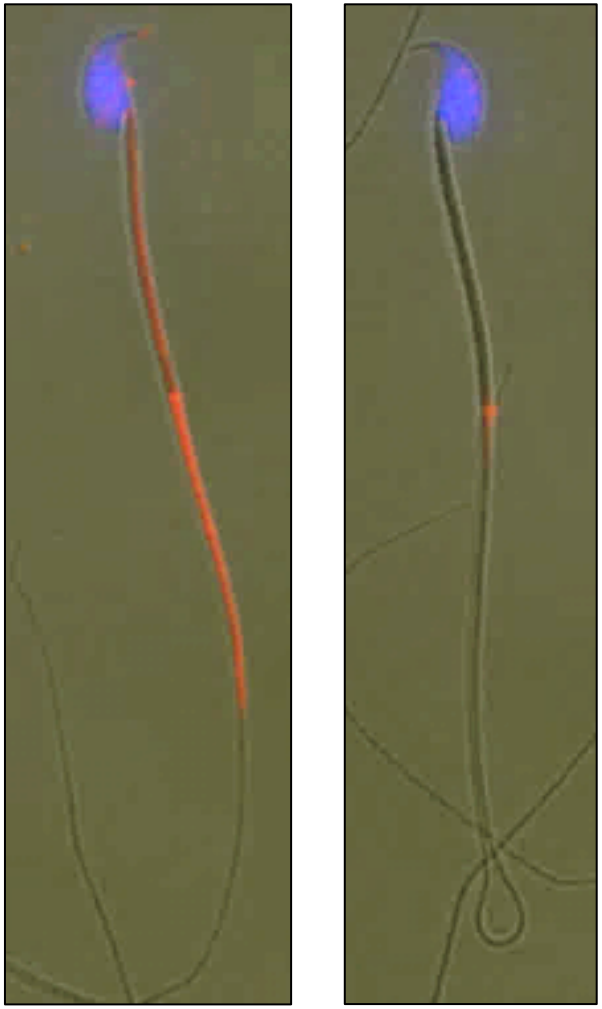
Select genes encoding receptor

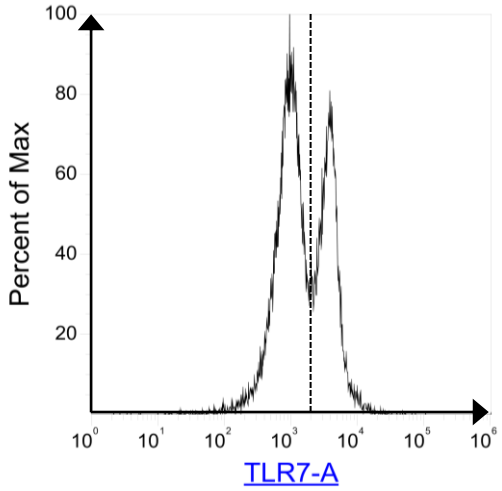
Short name	FPKM
Pgrmc1	2.30
P2ry10	1.63
Il2rg	1.59
Tlr8	0.43
Ar	0.37
Tlr13	0.36
Il13ra1	0.34
Htr2c	0.31
Gpr174	0.28
Cysltr1	0.26
Tlr7	0.25
Gria3	0.20
Gpr34	0.19
Gabra3	0.19
Gabre	0.15
Gpr64	0.13
Il13ra2	0.12
Eda2r	0.12

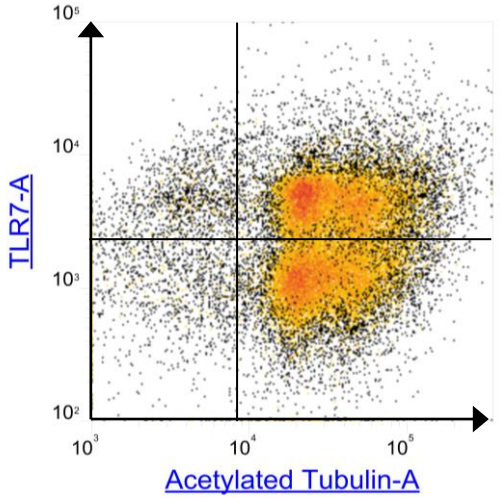
Candidate proteins expressed in the number of spermatids

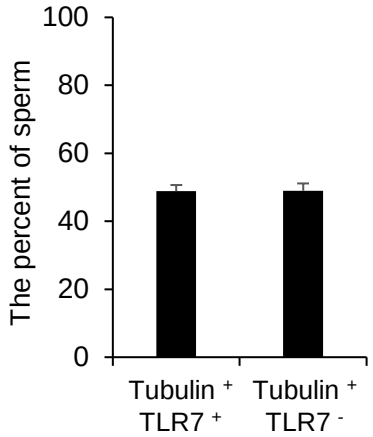


TLR7 was selectively expressed in X-sperm



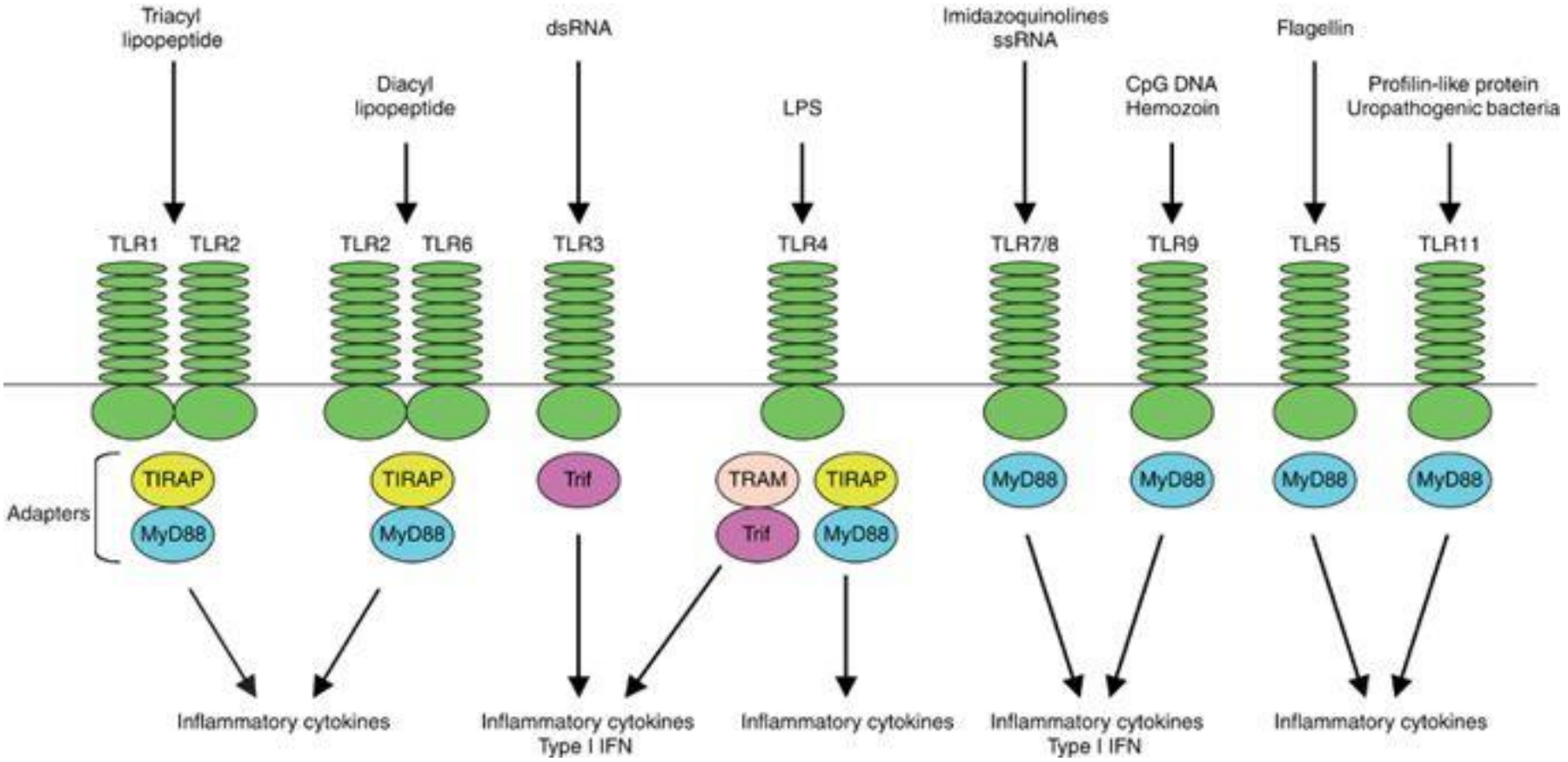






TLR7 is expressed in only X-sperm

What is TLR7? ~TLR7 is a member of toll like receptor family~



Kawai and Akira, Cell Death & Differentiation volume 13, pages816–825(2006)

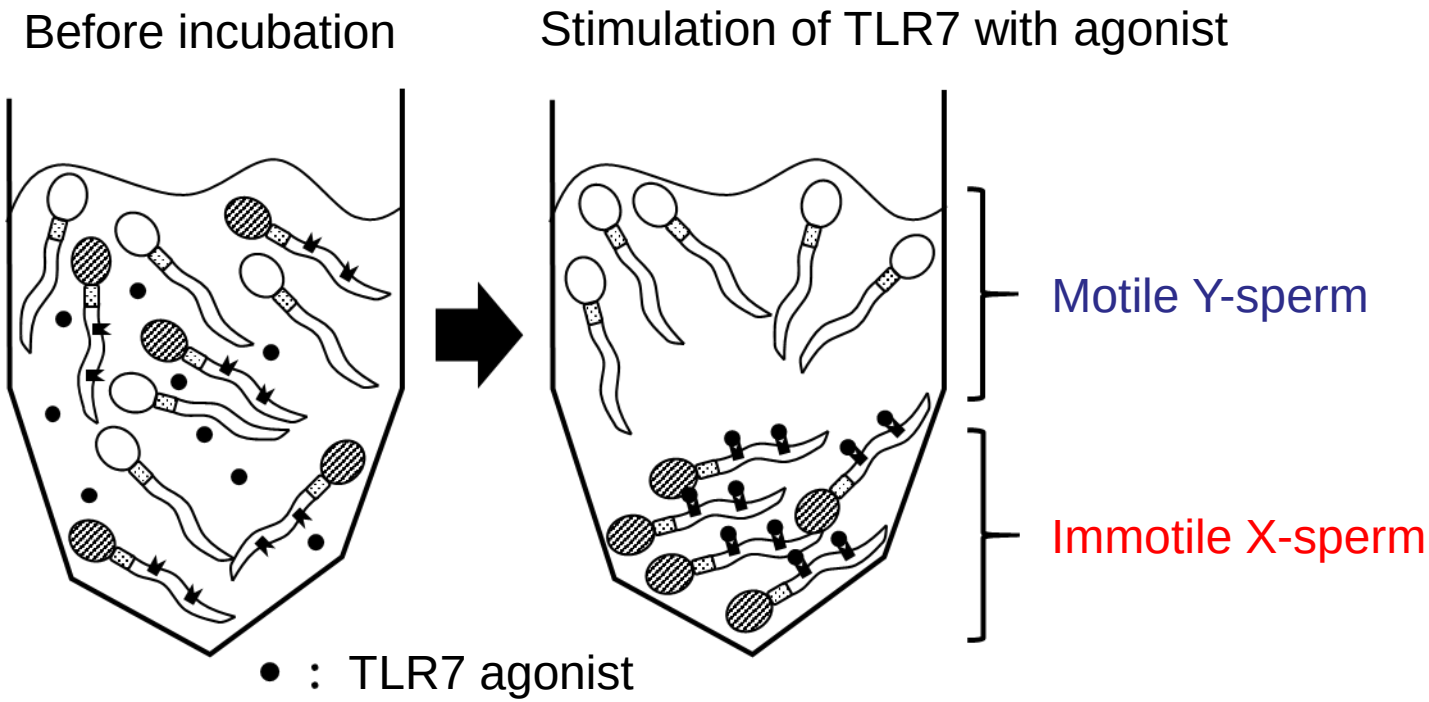
TLR7 is receptor to recognize RNA virus.

The stimulation of TLR7 suppresses ATP production in RNA virus infected cells

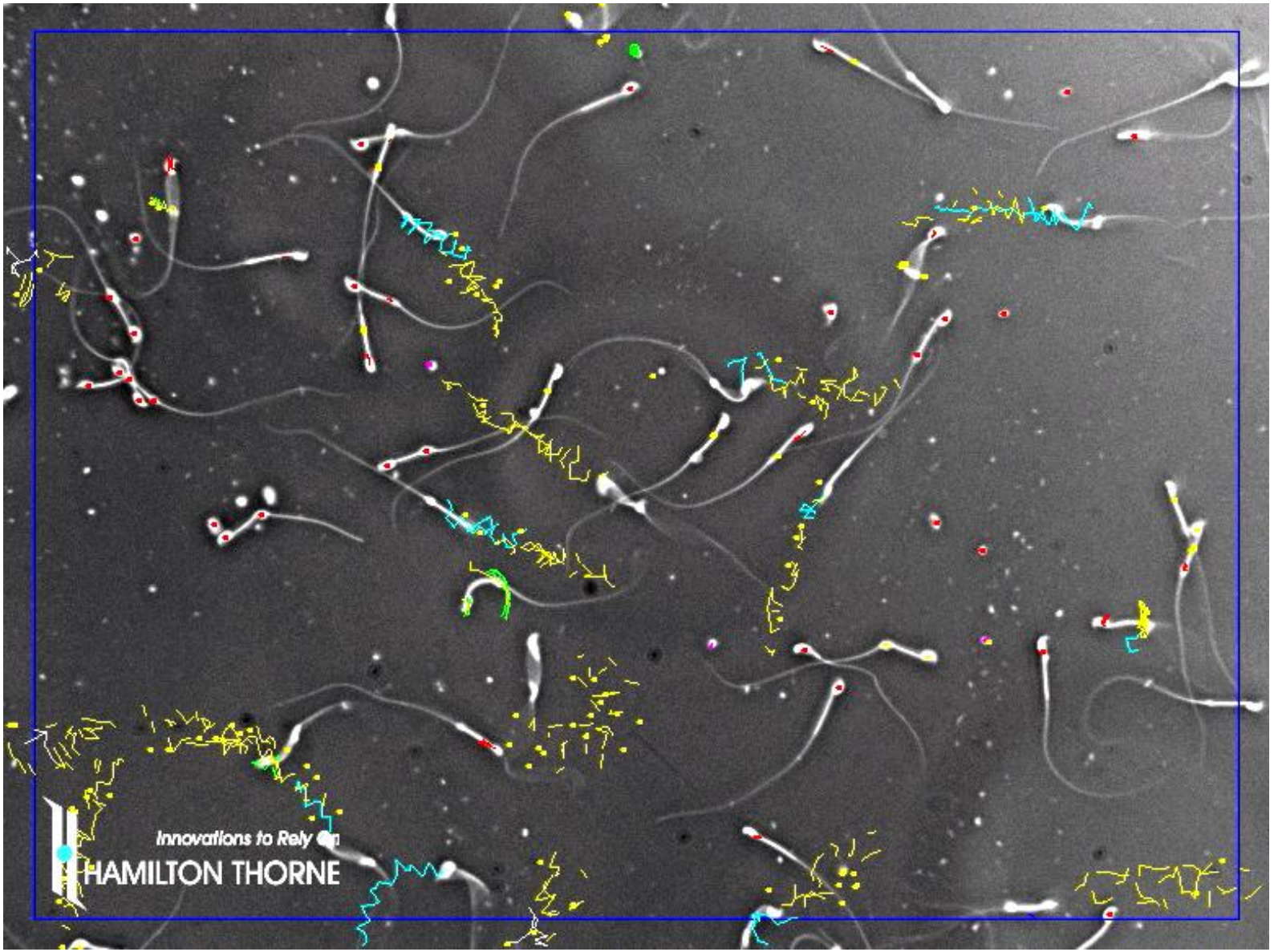
TLR7 was expressed in X-sperm
+ known information
① TLR7 suppresses ATP production
② Sperm motility is dependent on ATP
③ Sperm swims up against gravity

Evidence-based hypothesis

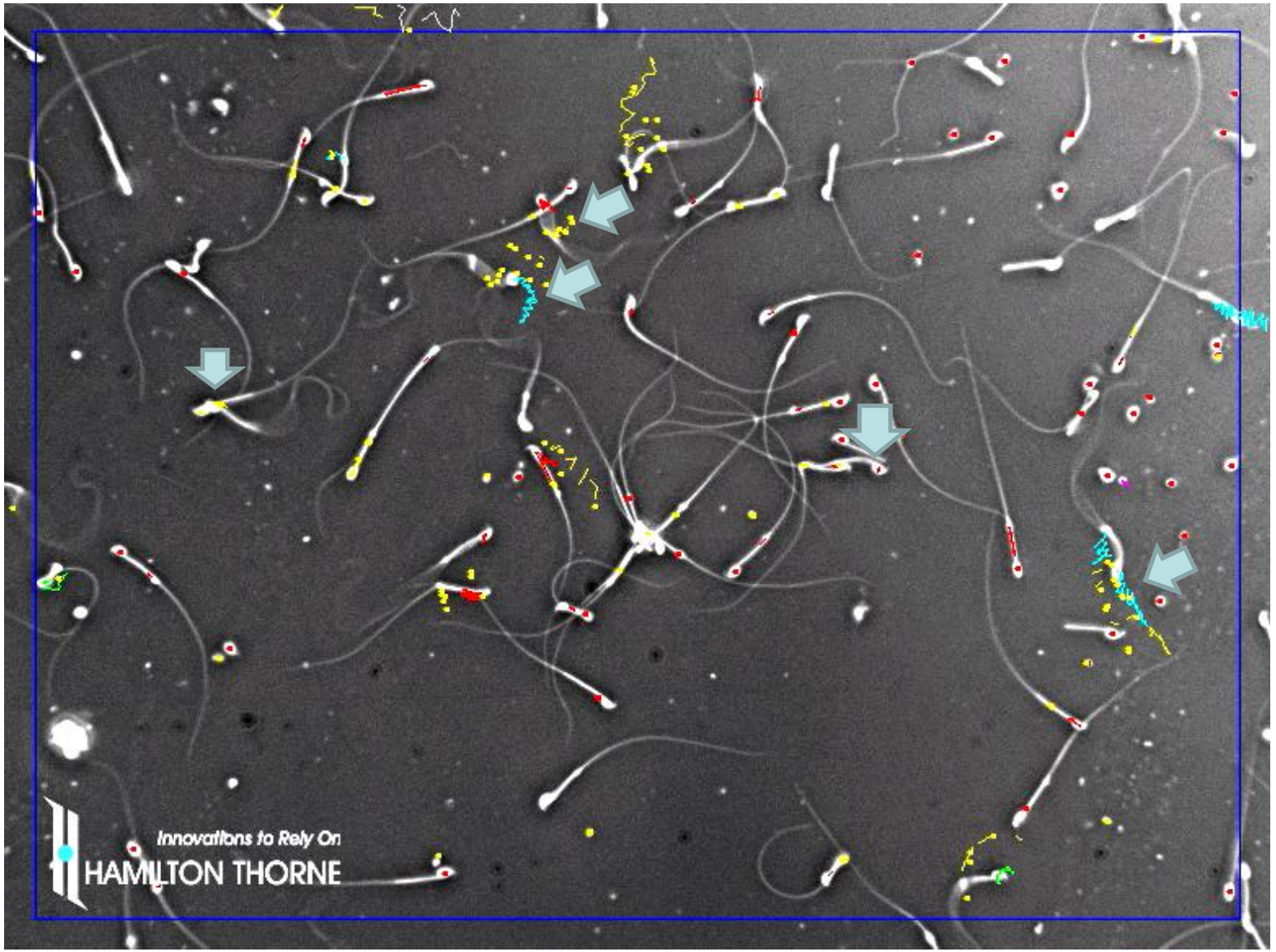
Stimulation of TLR7 causes X-sperm to stop motile and the immotile sperm would be precipitated.



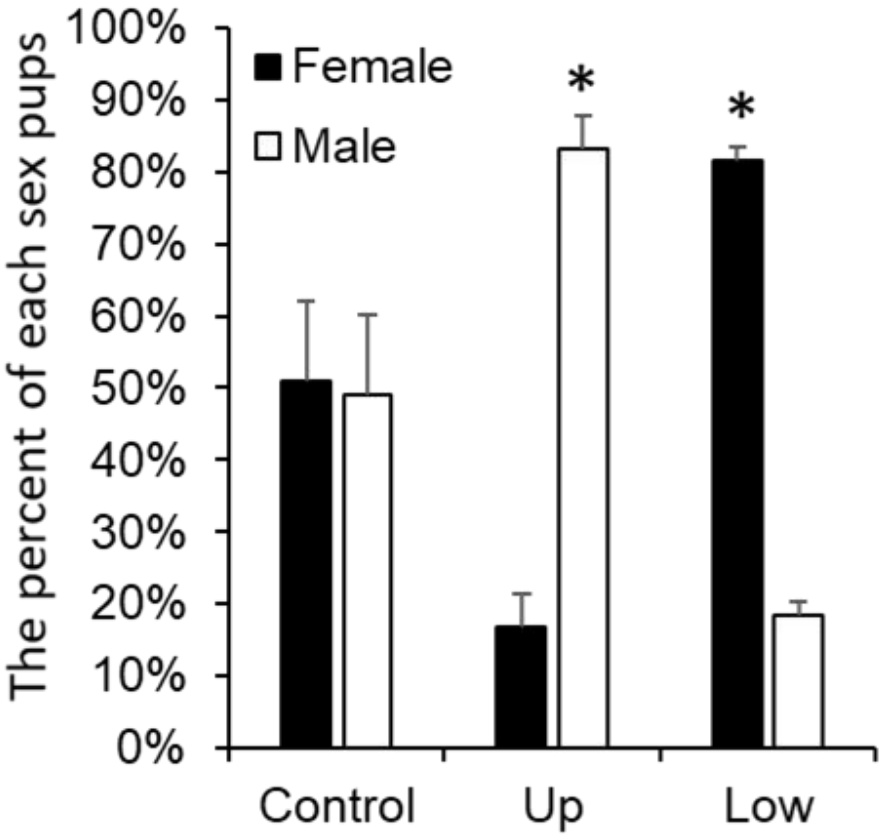
All of live sperm show high motility in upper layer



Live sperm stop their motility after the treatment with TLR7 agonist



IVF using separated sperm by the treatment with TLR7 agonist



- Pocking the sex of a baby one step closer as scientists separate X and Y-chromosome-carrying sperm: The implications would be colossal, *"Newsweek", Aug 13th, 2019*
- Sperm separation method may allow gender selection in IVF, *"The Guardian", Aug 13th, 2019*
- Changing sperm speed can influence offspring's sex, mouse study suggests, *"Science", Aug 13th, 2019*
- Scientists unlock secretes of gender within sperm for first time in major breakthrough, "*The Telegraph*", *Aug 13th, 2019*
- Scientists find way to distinguish male sperm from female, which may allow couples to choose IVF boy or girl, *"INDEPENDENT" Aug 13th, 2019*
- Japanese scientists find simple way to choose sex by separating sperm, *"CHINADAILY.com.cn", Aug 14th, 2019*
- Sperm can "EASILY" be separated into male and female as scientists warn the "concerning" breakthrough could lead to a gel for at-home sex selection, *"Daily Mail", Aug 14th, 2019*

Hiroshima University scientists behind new sex selection method get $2.7M grant to help India's smallholder farmers grow dairy herds

By Hiroshima University Department of Public Relations

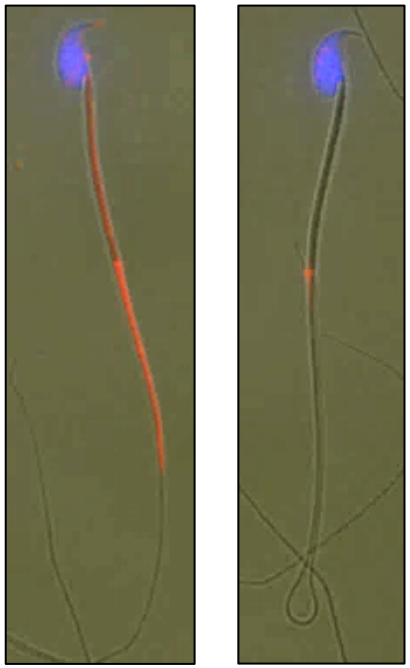
> Hiroshima University scientists will work on a low-cost sex selection system for bovines using their new technology to help grow dairy herds and boost the incomes of India's smallholder farmers.

A farmer in India milking his cow herd. Hiroshima University researchers will work on a low-cost bovine sex selection model for smallholder farmers in India using a new sperm separation technique they developed. The system aimed to simplify the artificial insemination process is seen to improve the income of smallholder farming families by increasing the numbers of their dairy cattle that can boost local dairy production while reducing economic losses caused by the birth of surplus bulls. (Pixabay)

Hiroshima University scientists who developed a new sex selection method received a $2.7 million grant from the Bill & Melinda Gates Foundation to work on a simplified and low-cost artificial insemination system for bovines that could help improve living standards of India's smallholder dairy farmers.

Develop own basic research into practical research

Our scientific findings



Stimulation of TLR7 expressed in X-sperm reduces their motility

Social issues in daily industry (farms)

FAO of the United Nations released a study on the impact of dairy development on poverty reduction.

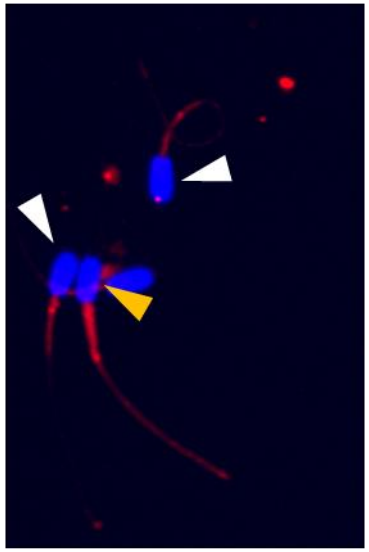
- Milk is produced from cows (female cattle).
- Increasing dairy production requires increasing the number of cows (female cattle)

The simple sperm sexing method is seen to improve the income of smallholder farming families by increasing the numbers of their dairy cattle that can boost local dairy production.

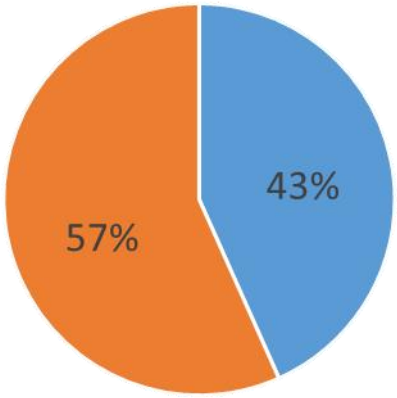
We are working on a low-cost bovine sex selection technique for smallholder farmers in India using a new sperm separation technique we developed.

In livestock animals, TLR7 is only detected in X-sperm

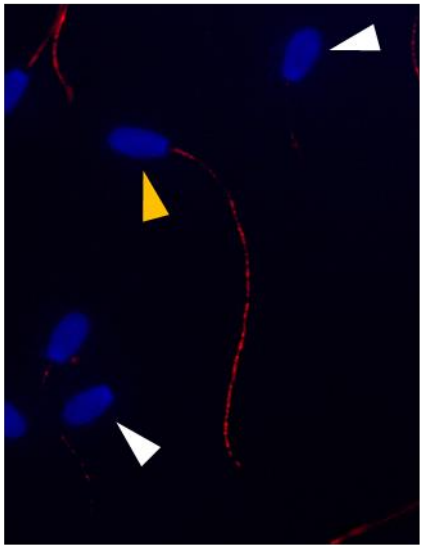
Boar sperm



% (TLR7 positive)

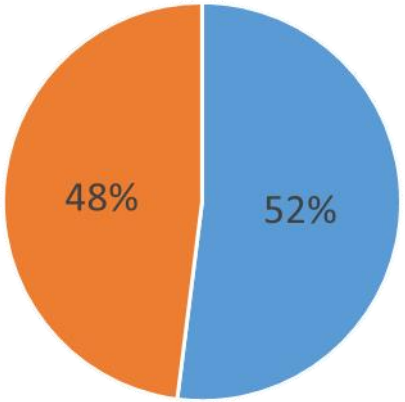


Bull sperm



TLR7 / nucleu

% (TLR7 positive)



The half of sperm are TLR7 positive sperm in bull and boar sperm

nature protocols

PROTOCOL

https://doi.org/10.1038/s41596-020-0348-y

Check for updates

A simple sperm-sexing method that activates TLR7/8 on X sperm for the efficient production of sexed mouse or cattle embryos

Takashi Umehara[1], Natsumi Tsujita[1], Zhendong Zhu[1], Moeka Ikedo[2] and Masayuki Shimada[1]✉

The preferred sex of livestock differs among breeders; for example, dairy farmers prefer female calves for the production of milk, whereas cattle meat producers often prefer males. Sexing of laboratory animals is also beneficial in some research fields, including reproductive biology and metabolic studies. Most sexing methods separate X sperm and Y sperm with a cell sorter. Here, we describe a system in which treatment with the TLR7/8 ligand (R848) separates X sperm from Y sperm. Because this protocol does not require any special equipment or professional skills, it can be easily applied in laboratories where in vitro fertilization (IVF) is performed. The sperm are treated with 0.03 μM R848 in 1 mL of modified human tubal fluid (mHTF) medium (mouse sperm) or 3 mL of mHTF medium (bull sperm) for 60 min, and then the upper layer (400 μL in mouse sperm or 1 mL in bull sperm) and the precipitate are separately collected. After each sample is washed by centrifugation, the sperm are suspended in ligand-free IVF medium and can then be used for IVF. More than 90% of the embryos made with upper-layer sperm are XY in both mice and cattle, and >80% of the embryos made with precipitated sperm are XX in both species. Separation of X sperm and Y sperm for IVF can be completed within 2 h.

Introduction

Choosing the sex of mammalian offspring is important in both industry and science[1,2]. In agriculture, production characteristics are often sex linked, and there are economic benefits if producers can predetermine sex. For example, dairy farmers prefer female calves for the production of milk, whereas cattle meat producers often prefer males because the price for male cattle is generally higher than that for heifers, because steers have superior growth[3]. Thus, sexing is an important tool for the efficient management and production of livestock.

Choosing sex is also beneficial in a research context when conducting experiments on mammals. Reproductive researchers generally focus on either the male or female reproductive system. Researchers focusing on metabolism and behavior mostly use males, because female phenotypes are easily changed by hormonal fluctuations of the estrous cycle. The gene-targeting CRISPR–Cas9 technique is frequently used in basic research fields[4]. However, to assess the functions of genes in each organ and/or at a specific developmental stage, Cre/LoxP technology is now the more beneficial tool[5]. The production efficiency of *loxP* homozygotes (*lox*/*lox*) with *Cre* is 25% because heterozygous (*lox*/+) females/males with *Cre* are mated with male/female homozygotes (*lox*/*lox*) without *Cre*. Thus, organ-specific gene-targeted mice of each sex are generated in only 12.5% of cases. Therefore, 8 to 9 pups are required to obtain one experimental animal of the correct sex. Implementation of an easy and efficient sexing method for laboratory use would benefit both the livestock industry and the scientific community.

Alternative methods

In mammals, sex is determined by the sex chromosomes, the X chromosome and the Y chromosome. Handyside et al.[6] developed a method to determine the sex of human preimplantation embryos by detecting the Y chromosome with a polymerase chain reaction (PCR). Bradbury et al.[7] applied this method to mice, determining embryo sex and transferring embryos of the desired sex. This method has now been used in multiple species, including cow[8], ewe[9] and sow[10]. However, this method is not useful for broad-scale applications in livestock or the scientific field because of the

[1]The Research Center for Animal Science, Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan. [2]Oita Prefectural Agriculture, Forestry and Fisheries Research Center, Bungo-Ono, Japan. ✉e-mail: mashimad@hiroshima-u.ac.jp

Take home message

~ science and technology solve social issues and achieve a smart society ~

<Novel findings>

TLR7 was expressed in X-sperm

+ known information

① TLR7 suppresses ATP production

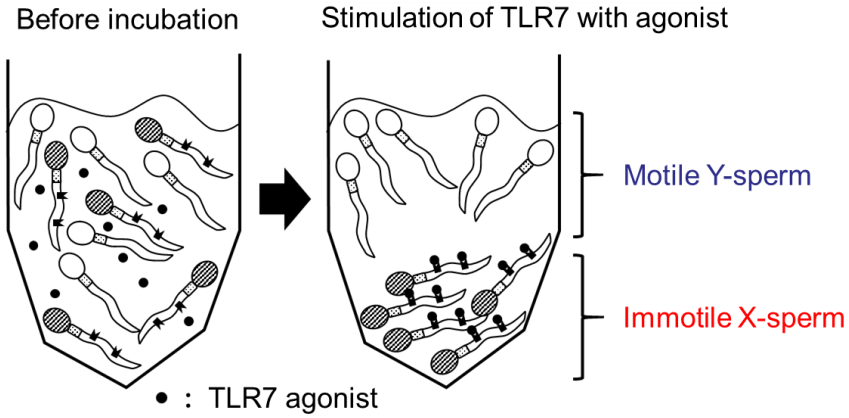
② Sperm motility is dependent on ATP

③ Sperm swims up against gravity

We firstly proved

The functional difference between X- and Y-sperm.

Furthermore, based on the difference in the responsible ability of X and Y sperm to TLR7 agonist, we clarified the conditions for precipitating only X sperm.



This scientific achievement not only enables sustainable dairy production,

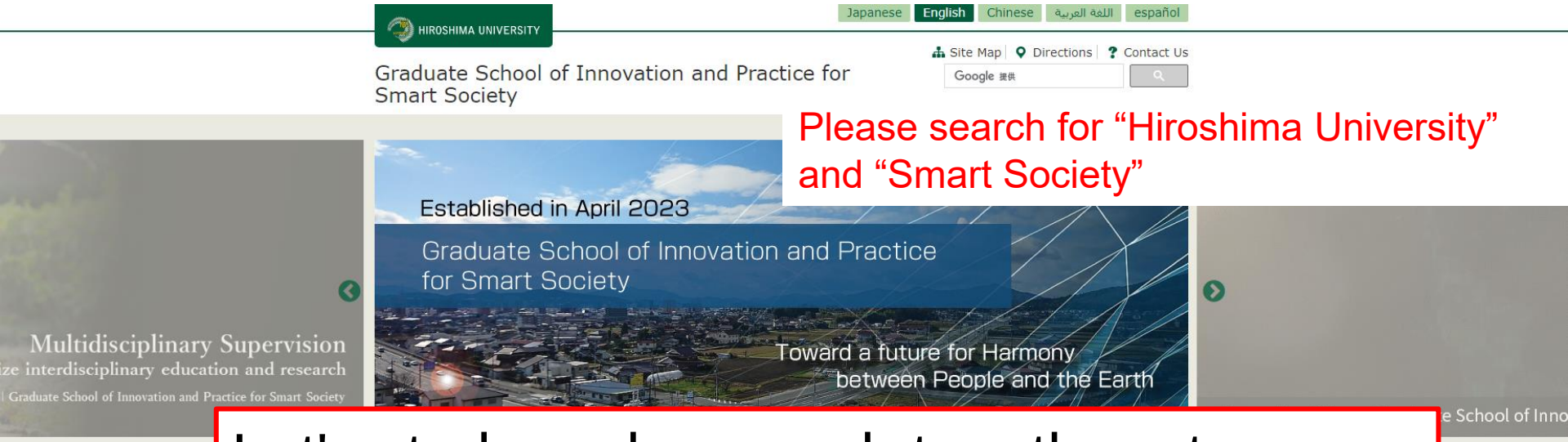
but also contributes to raising the income of small-scale dairy farmers in developing countries.

HIROSHIMA UNIVERSITY

Graduate School of Innovation and Practice for Smart Society

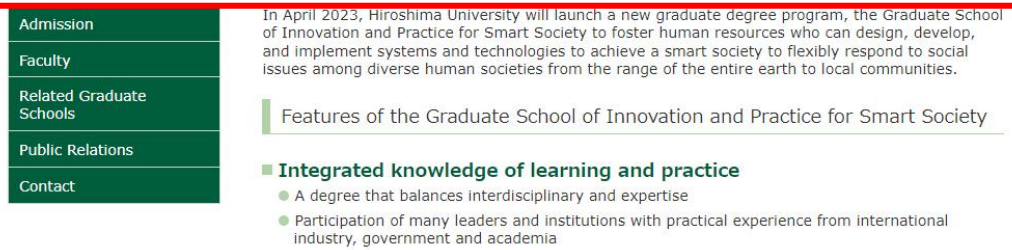
(Established in April 2023)

In April 2023, Hiroshima University will launch a new graduate degree program, Graduate School of Innovation and Practice for Smart Society to foster human resources who can design and develop systems and technologies to achieve a smart society to flexibly respond to social issues among diverse human societies from the range of the entire earth to local communities.



Let's study and research together at Hiroshima University,

to solve the world's social issues through science and technology,
and realize a smart society.



https://www.hiroshima-u.ac.jp/en/smart_society