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A brief history of sex determination

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ABSTRACT

A fundamental biological question that has puzzled, but also fascinated mankind since antiquity is the one pertaining to the differences between sexes. Ancient cultures and mythologies poetically intended to explain the origin of the two sexes; philosophy offered insightful albeit occasionally paradoxical perceptions about men and women; and society as a whole put forward numerous intuitive observations about the traits that distinguish the two sexes. However, it was only through meticulous scientific research that began in the 16th century, and gradual technical improvements that followed over the next centuries, that the study of sex determination bore fruit. Here, we present a brief history of sex determination studies from ancient times until today, by selectively interviewing some of the milestones in the field. We complete our review by outlining some yet unanswered questions and proposing future experimental directions.

1. Origin myth and philosophy: a social perspective of sexes

Differences between the sexes have been a matter of intense debate since well before any scientific era. In fact, narratives about the origins of sex can be traced back to 2000 years B.C. (Before Christ) from ancient Oriental legends known in Mesopotamia, which probably influenced many cultures, including the ancient Israelites, the Greeks, or the Hindu (Krappe, 1936).

One of the most famous is surely the Bible's mythic story of Adam and Eve (Genesis 2: 21–24). More precisely, God (Yahweh) created Adam in his image; then, he created Eve from one of Adam's ribs as a companion. There is however another, less well known, interpretation of the Genesis text. Adam was first referred as a non-gendered man (Genesis 2:7), then as both “man and woman” (Genesis 5:1,2), and finally as a male, once Eve was created (Genesis 2:23–24). Hence, according to a pre-Christian rabbinical tradition, Adam was in fact a hermaphrodite who was able to procreate by himself (reviewed in (Mittwoch, 2000)); however, God thought that Adam should not be single and therefore decided to give him a companion. He thus created a woman from one of Adam's sides (Freedman and Myers, 2000).

The notion of a divided individual is also described in Plato's *Symposium* (c. 385-370 B.C.): a group of eminent men attend a banquet, where they are asked to give a speech in praise of love. Aristophanes, one of the participants, says that one must understand human nature in order to understand the origins of love; this is because Primeval people were spherical creatures, with four hands, four legs and two faces looking at opposite directions (Fig. 1). Due to their strength, they represented a potential threat to the gods, and hence, Zeus decided to cut them into two. Yet, as soon as they were separated, they sought to reunite and embrace (Jowett, 1970).

Since antiquity, each era and culture has developed theories related to how sex determination can be influenced: from bandaging a woman's foot or a man's testis, to eating specific food (McCartney, 1922). In ancient times, male children were generally much more welcome than female, as they were more likely to calm down the wrath of the Gods; they were also considered to be a solid support for the elderly. In fact, this preference for producing male offspring was the main incentive in the search of understanding how sex is determined in order to be able to control it (McCartney, 1922). The concept of sex determination was actually a matter of debate for many ancient Greek philosophers. The pre-Socratic philosopher Parmenides (born c. 515 B.C.) hypothesized that the child's sex was determined by its position in the womb: males on the right side, females on the left. Anaxagoras (born c. 500 B.C.) believed that the father determined the sex of a child: males were formed from the semen of the right testis, while females were formed from the left. Later on, Empedocles (c. 490-430 B.C.) suggested that organisms are made of the four elements –fire (hot), water (cold), air (moist), and earth (dry)– and that males are hotter than females (Lesky, 1951).

Around the same time, Aristotle (384-322 B.C.) criticized the theories of Parmenides and Anaxagoras. He had evidence that children of both sexes can be present on the same side of the uterus, and that men

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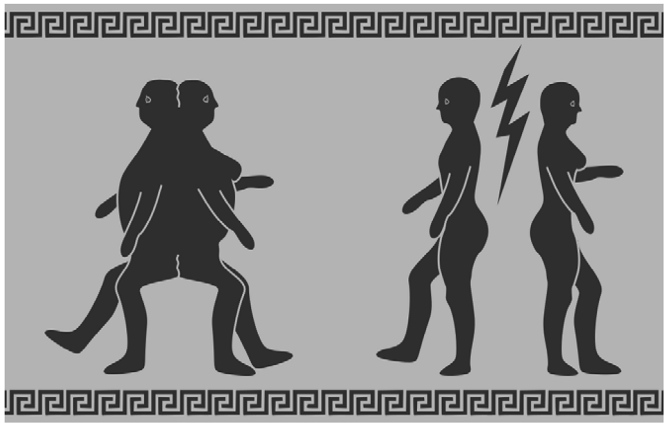

Fig. 1. Primeval people. In Plato's *Symposium* (c. 385-370 B.C.), Primeval human were round, with four legs, four arms and two faces. The primeval human had three sexes: male-male, male-female, and female-female. As their strength could represent a threat, the gods split the humans. Thereafter, the two halves search for one another to reunite into one, and this search is what we know as love.

with only one testicle could have both male and female offspring (Aristotle and Peck, 1942; Mittwoch, 2000) – an observation that contradicted Anaxagoras' belief that male and female embryos form from semen of the right or left testis, respectively. He also formulated a theory for sex determination, according to which males have an abundance of the superior element, fire, whereas females have an abundance of water and are therefore rather cold (Lesky, 1951). In his work "*On the Generation of Animals*", he mentions that males are stronger animals, which, because of their heat, convert food into a perfect concoction, the concentrated semen. Females on the other hand are weaker animals, which, due to the lack of heat, are not able to concoct semen. Instead, they produce an abundant quantity of blood, the catamenia (*i.e.* the menstrual blood), which is the material substance of the embryo. Through copulation, the male discharges his semen in the female and the embryo results from the mixture of semen with catamenia. If the male masters the concoction of his semen, the embryo forms as a male; if he fails to concoct because of a heat deficiency, the embryo develops as a female.

Although we may now smile at Aristotle's account of sex determination, he probably had the closest perspective to reality. He raised the point of both male and female contribution to the development of an embryo, as well the role of male semen in the determination of a child's sex. He also proposed that the sex of an embryo is established during development after a period of ambiguity, by the formation of organs that differ between male and female. Finally, based on the observation that a eunuchs' body traits transform from masculine to more feminine, he suggested that testes are responsible for the masculinization (Aristotle and Peck, 1942).

2. Transitioning from myths to science: first insights into the biology of sex differences

Remarkably, the theories of heat, laterality, or food involvement in sex determination survived for nearly two thousand years (Mittwoch, 2000), and were put to question only when systematic scientific research began. The common view in the mid-17th century was that in mammals, male semen mixed in the uterus with the hypothetical female semen and that this mixture turned into an egg and eventually a fetus.

In 1651, renowned physician and scientist William Harvey published his work "*Exercitationes anatomicae de generatione animalium*" on the generation of animals. Harvey provided a comparative description of the reproductive organs from several animals and stated that "all animals whatsoever, even viviparous creatures, man himself, are all engendered from an egg" (Harvey and Whitteridge, 1981) – hence his aphorism *ex ovo omnia* (from the egg, all).

Soon after this, in 1672, Dutch anatomist Reinier de Graaf provided the first evidence for the existence of mammalian eggs (Cole, 1930). Although he did extensive studies on testicular morphology (Loriaux and Loriaux, 2016), de Graaf focused his attention on the female reproductive tract; in fact, he was one of the first to dissect a human ovary. At the time, the female reproductive organs in chickens were called ovaries, as they produced the eggs; in mammals though, they were called "female testes", since they were not considered to be the equivalent of a chicken's ovary. Nonetheless, de Graaf observed structures within the "female testis", which were very similar to those in the chicken's ovary (de Graaf, 1671). He found small vesicles full of liquid, which he thought corresponded to the albuminous fluid of the avian egg. He proposed that the vesicles, or eggs, contained an element that was necessary for fetal development. Following this, de Graaf studied the female reproductive tract of rabbits; through serial dissections at various intervals after coition, he was able to follow the "*ova*" from the ovary down to the uterus. Of note, what he was actually looking at were follicles full of fluid, and not the actual oocyte. He concluded that all animals originate from an egg, which exists before coitus in the female testis; thus, female testes were called ovaries (Loriaux and Loriaux, 2016).

The discovery and use of the microscope was a defining turning point in the study of reproduction. Antonie Van Leeuwenhoek, a Dutch businessman and draper, but also a gifted lens maker, used his handcrafted microscopes in the 1670s, and became the first man to observe sperm cells in human and dog fluid, which he referred to as "animalcules" (from the Latin word *animalculum*, which means "tiny animal") (Fig. 2) (van Leeuwenhoek, 1677). He claimed that these animalcules play an important role in the formation of embryos – a notion that ran counter to the theory of spontaneous generation that was popular at that time (Ruestow, 1983).

Between 1780 and 1785, Lazzaro Spallanzani performed in vitro

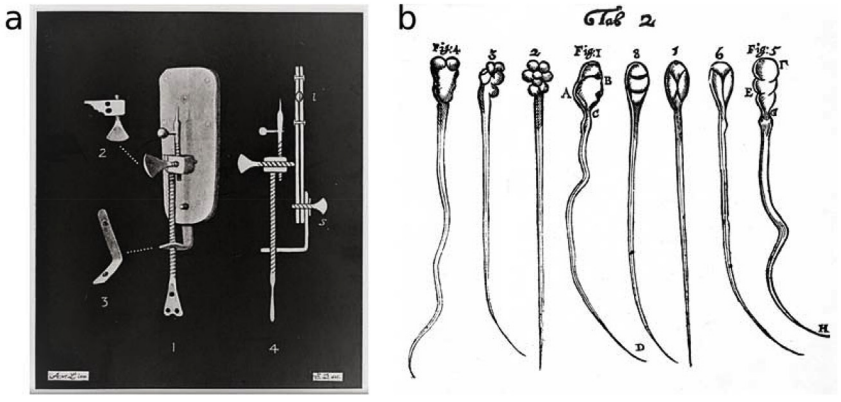

Fig. 2. Discovery of spermatozoa by Antonie van Leeuwenhoek. (a) Scheme of Antonie van Leeuwenhoek's microscope. 1 shows the whole instrument from the back, 2 and 3 show detailed parts, 4 shows a longitudinal section of the instrument, and l shows the location of the lens (Dobell and van Leeuwenhoek, 1960). (b) Van Leeuwenhoek's drawings of spermatozoa from rabbit (1–4) and dog (5–8), in 1677 (source: Wellcome Library, London).