Compliments of the Author

SPERM FORMATION IN THE DOMESTIC ANIMALS WITH CONSIDERATION OF THE STERILITY AND FERTILITY IN MULES

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"Sperm Formation in the Domestic Animals with Consideration of the Sterility and Fertility in Mules"

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I. INTRODUCTION—Stimulated by the appearance of Wodsedalek's paper (January 1916 Biol. Bull.) on the spermatogenesis of the horse, I conceived the idea of making a similar study of the germ cells of the common mule, with a view of ascertaining the cause of sterility in this valuable beast of burden. The first great difficulty which confronts the cytologist in undertaking such a laborious problem, is in obtaining the desired material. In the spring of 1915 this matter was taken up with the president of the Ohio Valley Veterinary Medical Association, Dr. Walker France, who considered the problem of sufficient importance to justify him in rendering assistance. More than a week was spent in "Fording" over the hills of Pike and Gibson counties with the doctor in one of his mad wholesale slaughters of the future progeny of horse colts and the useless passion of as many mules. To the amusement of the farmers, the testes were greedily snatched from the doctor's knife, cut into small bits and placed in Fleming, Bouin, alcohol, acetic and picroacetic fixations, which are extensively used by the cytologist.

At that time I was working on the plasmosome or achromatic nucleolus, of one of the true bugs, and was not in position to turn my personal attention to the newly collected material, until the plasmosome paper was in the hands of the publishers. In the meantime, Mrs. Goldsmith, while working at the Indiana University Biological Station, prepared and studied a vast amount of this material. After three months' work, it was found that all animals, both horse and mule, from which the material had been taken, were too young, as none of them contained mature cells. This material was then stored for future use and arrangements made with Dr. France and other veterinarians, to assist in the collection of material from older animals. However, in January 1916 all plans were abandoned when a paper on "The cause of Sterility in the Mule" by J. E. Wodsedalek, of Idaho University, made its appearance in the *Biological Bulletin*. Since our observations on the earlier germ cells of both the horse and mule agreed with those of Wodsedalek, and further since the problem was

given up in recognition of his valuable work, the speaker wishes to present this paper only as a brief treatise on some of the old and new phases of this subject, and not as a cytological contribution. It should be recognized, however, that many months' time has been spent on the problem and much first hand knowledge obtained.

In presenting a paper such as this, it seems wise to sacrifice technical details for a general and more comprehensive discussion. I desire, then, to briefly discuss the *general* methods of sperm formation and to refer the cases of various domestic animals to this general type. It is quite a long and complicated process to follow the history of a cell or group of cells from a non-differentiated germ layer of a tiny embryo, to a mature sperm of the adult, indeed, much more taxing than to listen to even a non-technical paper on the subject.

II. DIFFERENTIATION OF THE GERM CELLS—The maxim of Virchow 1855 "omnis cellula e-cellula" (all cells come from cells), after being tested from all angles for more than three score years by the world's most painstaking investigators, has come to be universally accepted. Since, according to this theory all the cells of the body must have originated from a single cell, and further since this single cell can be traced back to the ovaries and testes of the parents, it is obvious that there are two types of cells. These are referred to as somatic and germ cells. The former, which constitute the body tissue, live through a life cycle and then die, while the latter have an immortal life. According to this conception the individual is composed of millions of sub-branches from one single off-shoot of the germ cell. This might be illustrated by a vine which sends up annual shoots. The plants themselves die, but underneath the surface the roots continue to send up new sprouts each year. The branches of the vines, which spring up and then die, correspond with the somatic cells of each individual, while the underground roots are homologous with the never dying germ cells of the ovaries and testes. Thus each individual germ cell constitutes only a small link in the chain of life flowing from the ovaries of Eve to the final destruction of the human race. It is interesting to note that from the standpoint of somatic cells, the

father is older than the son, while if the continuity of the germ cells be considered, the son is one generation older than his father.

III. A TYPICAL ANIMAL CELL (Fig. 1.)— The germ cells are usually much larger than the somatic cells and are thus used as a basis for general study. All cells are made up of a nucleus enveloped by a more or less viscous substance, the cytoplasm. The substance inside of the nucleus is spoken of as nucleoplasm or karyoplasm. In the nucleus there is usually found a heavy network (chromatin network) and a dense mass of chromatin, the chromatin-nucleolus. There may also be found a finer network of achromatic substance, (linin-network) and sometimes a spherical mass, the plasmosome, of the same material (Goldsmith '16). The nucleus together with the cytoplasm constitutes the protoplasm.

IV. THE METHOD OF CELL DIVISION—After the cell has reached a certain stage of growth, the chromatin-network begins to condense at various points in the nucleus—usually near or in contact with the nuclear membrane (Fig. 5). These aggregations become more and more definite and individualized until they each assume a compact and characteristic form, (prophase stage, Fig. 6.) These newly formed bodies are called chromosomes and are uniformly characteristic of the species in which they are found. They vary from two in a thread worm to 1,600 in a ceratin radiolaria. A small body (the centrosome, (Fig. 7) from which radiates cytoplasmic lines (astral rays Fig. 7), now appears on either side of the nucleus. The nuclear wall is then destroyed as prominent fibers (spindle fibers Fig. 7) connect these two points as centers and force the chromosomes into a median position, thus presenting a typical spindle (metaphase condition Figs. 19 and 7). The chromosomes now divide lengthwise and one-half of each passes to opposite poles of the spindle. While the chromosomes are on their way to the poles (anaphase stage Fig. 8) the entire cell begins to divide. This continues until after the chromosomes have reached the spindle (telophase Fig. 9), when the two daughter cells are completely separated. The chromosomes of each daughter cell now reassume their early woolly appearance and gradually pass into the network condition, while the nuclear membrane and the other parts of the cell are being reconstructed in preparation for the next divisions.

V. THE "GREEN" GERM CELLS—(spermatogonia). The processes in cell division are essentially the same whether somatic or germinal. However, since the former cells are smaller, the component parts are more compact and not so readily differentiated.

From a very early embryonic condition until near the time the animal is capable of reproduction, the large cells which were set aside for this purpose, gradually increase in number by the above method of cell division. (See plate "Green Germ Cells.")

When the animal is near maturity (soon to be capable of reproducing), a number of these cells seem to lose their division energy. They then enter a quiescent or rather a ripening stage in preparation for the great function for which they were ordained, namely, that of reproduction. We who live "microscopic lives" speak of this stage as the *growth*, or *maturation* period of the germ cell. The speaker has one form under investigation in which he is able to predict by the appearance of the cells those which have about lost their division energy and are ready to undergo maturation.

VI. THE RIPENING OF THE GERM CELLS— (*The maturation period*). A. *The growth stage*. After an early germ cell has lost its division energy and is ready for ripening, the two daughter cells resulting from the last division fail to reconstruct as usual to form another division spindle. The nuclear wall is usually reformed but the nucleus itself is filled with a dense fibrillar but somewhat granular network (Fig. 2). Among this homogeneous network there is usually (indeed, always in the domestic animals) found one or more compact bodies resembling the chromosomes (Fig. 2 to 11). In fact, we have demonstrated conclusively that these bodies have persisted as distinct individuals from the chromosomes of the earlier divisions, all others having been spun out to make this new network. These dense bodies never break up as do the other chromosomes, but continue as independent elements while the cell is ripening. They are called the "sex-chromosomes" or better "female producing chromosomes" since they are, at least, associated with sex-production.

As the cell and nucleus continue to grow, some very interesting changes take place in the fibrillar network. Typically it forms into as many long strands as there were chromosomes in the earlier stages not considering the sex-chromosomes (Fig. 3). The ends of these dense threads are now mysteriously drawn to one region, usually against the wall of the nucleus. Since they seem to be floating in a nuclear sap the median parts of each thread swing out into the nucleus, forming a bundle of very clear loops (Fig. 4). The cytologist calls this the bouquet stage of the ripening cell. The female producing chromosome is usually in the thickest part of this bundle. As these loops decrease in size, presenting a very compact condition (synizesis) they are ofttimes observed to pair (synapsis). Since each loop represents a chromosome, the num-

ber of these bodies is now evidently reduced one-half, excluding the mysterious sex-chromosome. This reduction in number is very significant, since it is necessary in order to keep the number constant for each species of animal. It will readily be seen now that when a father cell and a mother cell, each with *half the normal number of chromosomes,* unite, that the daughter cell will possess the normal number of the parents—the daughter the number of the mother and the son that of the father. Should this fortunate process not happen, and the mother and father pass to the child their normal number instead of one-half, our cells would soon be bursting with chromosomes and thus future production would be impossible.

The compact mass of chromatic fibers now break up and are again faintly distributed through the nucleus (Fig. 5). As the cell still continues to grow, this faint chromatin material collects into as many aggregations as there were pairs of fibers (Fig. 6). These masses now condense and form the chromosomes for the first of the two ripening divisions (Figs. 7, 8 and 9.)

B. *The two ripening divisions.* The germ cells now undergo two divisions, differing somewhat from those discussed earlier. The number of chromosomes in these divisions is one half, plus, the sex-chromosomes which had no mate in the growth period. This female producing body still (in most forms and in all domestic animals) refuses to associate with the other chromosomes but takes its position at one side of the spindle, but in ad-

vance of its neighbors (Figs. 7 and 8). All the common chromosomes divide as usual and the respective halves pass into the daughter cells. Since the sex-chromosome passes undivided to one cell we have two different types of cells produced. It will be noted later that the one with the extra body (Figs. 8, 10 and 11) will produce a female while the one without this important chromosome (Figs. 9, etc.) will give a male. In some forms (not in domestic animals) the sex-chromosome divides in the first but passes bodily to the daughter cell in the second division, the resulting process being the same.

These two types of cells now divide once more, all chromosomes taking part in the process, and then pass into another state of reconstruction. These reconstructing cells are now called "spermatids" (Fig. 10) and are transformed directly into sperms (Fig. 11). This process of transformation is very complicated and has been worked out to the most minute details. The most important point to consider here is the fact that all the chromosomes, except the female-producers, again pass into the fibrillar condition. In some forms the sex-chromosomes can be followed step by step into the head of the mature spermatozoön.

VII. A TYPICAL RIPE MALE GERM CELL. A mature spermatozoön (Fig. 11) consists of a head, neck or middle piece, and a tail. At the forward point of the head there is a small sharp body, the *acrosome,* by means of which the sperm bores its way into the egg (ovum Fig. 12). The chromatin material is

Explanation of Plate

The small active cells at the top of the plate are known as spermatogonial (in the male) and oögonial (in the female) cells. They represent a few of the many divisions which must take place before a single germ cell of a tiny embryo can become the large reproducing testis or ovary of the adult animal.

Figures 2 to 6 inclusive and the corresponding figures to the right show some of the more important steps in the growth periods of the ripening germ cells.

At the stages shown in Figures 4 and 5, the germ cells of the mule and other hybrids first begin to show indications of abnormality which results in the final destruction of the cell.

Fig. 7. First ripening division of the male germ cell. (First spermatocyte division.)

Fig. 19. Corresponding division in the ovary. (First oocyte division.)

Figs. 8 and 9. Second ripening division. (Second spermatocyte division.)

Fig. 18. Second oocyte.

Fig. 10. Spermatids (first two with and the second two without the sex-chromosome).

Fig. 11. Mature spermatozoa.

Fig. 16. The single large mature egg.

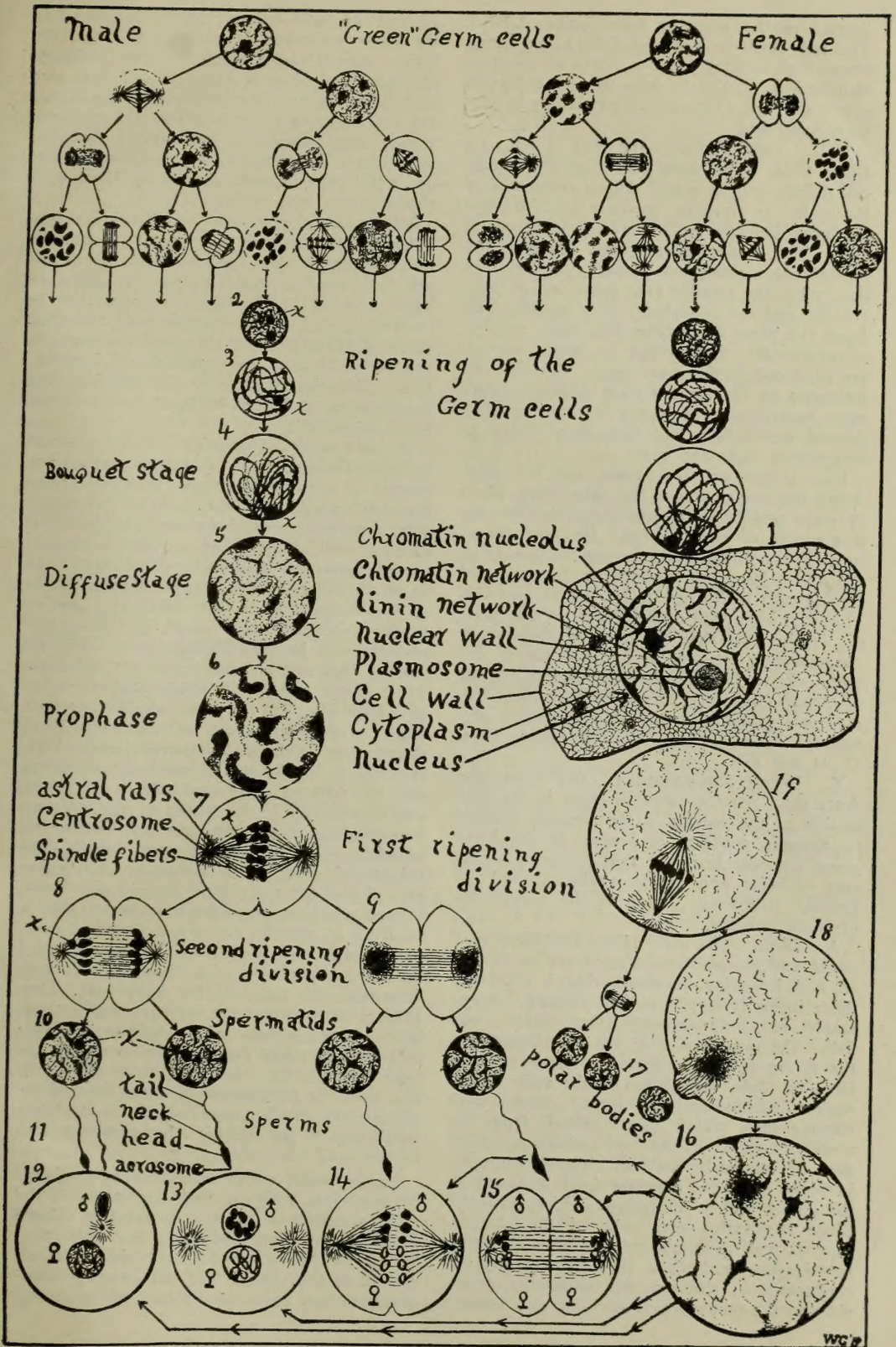Fig. 17. The three small eggs (the polar bodies) which never mature.

Figs. 12, 13, 14, 15, represent the possibility of the single egg (Fig. 16) being fertilized by any one of the four "brother" spermatozoa shown in Fig. 11. Since the first two contain the sex-chromosome, a union of either of these with the egg would result in female offspring (Figs. 12 and 13), while the other two would produce males (Figs. 14 and 15).

Figs. 12, 13, 14 and 15 also show that "the reduced number of chromosomes brought in by each parent cell (Figs. 12 and 13) unite to form the normal number for the offspring (Fig. 15)," and that "the chromosomes from each parent (Fig. 13) are equally distributed in every offspring (Fig. 15)."

Fig. 1. A typical animal cell.

♂ =Male.

♀ =Female.

Male "Green" Germ cells Female

Ripening of the Germ cells

Bouquet stage

Diffuse Stage

Chromatin nucleolus
Chromatin network
Linin network
Nuclear wall
Plasmosome
Cell wall
Cytoplasm
Nucleus

Prophase

astral rays
Centrosome
Spindle fibers

First ripening division

Second ripening division

Spermatids

Sperms

tail
neck
head
acrosome

polar bodies

located in the more or less elongated head. The tail which is surrounded by a thin membrane (tail envelope) is an organ of locomotion and is usually broken off as the sperm enters the egg (Fig. 12), its function having been completed.

VIII. THE RIPENING OF THE FEMALE GERM CELL. The development of the female cell differs only in minor details from that of the male. In ripening, however, each division produces daughter cells extremely different in size. The larger ones (Figs. 18 and 16) continue to mature and prepare for fertilization, while the smaller (the polar bodies, Fig. 17) die and are absorbed. It is readily seen from the plate that, from the same number of green-cells, four times as many sperms are produced as there are eggs. This is very fortunate as the sperms must undergo much more hazardous experiences in reaching their desired destination, and thus more liable to destruction than the eggs.

IX. UNION OF THE SPERM AND THE EGG. After the sperm enters the egg (Figs. 12 to 15), the middle piece, which carries "the active center of cell division," *the centrosome,* turns in the direction of the egg nucleus (Fig. 12) and a joint spindle between the male and female cells is formed (Figs. 13, 14 and 15). The most important consideration in this process is the fact that *the reduced number of chromosomes brought in by each parent cell now unite to form the normal number for the offspring* (Fig. 15). Furthermore, *the chromosomes from each parent are equally distributed in every daughter cell* (study Figs. 13, 14 and 15).

X. SPERM FORMATION IN THE DOMESTIC ANIMALS. All the essential steps in the formation of the sperms in the common domestic animals, conform in general to the foregoing consideration. It thus becomes necessary to refer only to the variations and especially interesting points in the germ cells of these animals.

A. *Birds.* The cells of the birds are very small, and the constituent parts are so crowded and irregular in shape, that a study of these forms is very laborious. Guyer, for example, has only recently (Oct., 1916) published the results of over ten years' study on the sperm formation in the common rooster. Here he finds, in additicn to the mass of irregular chromosomes, a larged curved sex-chromosome, which stands aloof from its neighbors while on the first maturation spindle. "A similarly constant element, differing in form from the common fowl is found in the guinea and in the guinea chicken hybrid." Since the early germ cells of the chicken have sixteen ordinary chromosomes plus this sex-element, and since this element neither conjugates in the ripening process nor divides in the first ripening division, one daughter cell has eight ordinary chromosomes, while the other possesses, in addition to these, the large curved sex-element. The ordinary chromosomes of each daughter cell now pair and fuse, leaving four in one and four plus the sex determiner in the other. All chromosomes again divide in the second division, and thus it is brought about that two classes of cells are formed containing 4 and 5 chromosomes respectively.

In the hybrid pheasants and hybrid guinea-chickens the normal development continues up to synapsis when the chromatin fibers are unable to unravel from the synaptic knot (see Figs. 4 and 5). The mass of chromatin remains attached to the nuclear wall and then either disintegrates, or abnormal spermatozoa are formed which are incapable of fertilization. This clearly accounts for the fact that the breeding of hybrid birds of various kinds is an impossibility.

B. *Mammals.* One of the first mammals to be studied was man. Although perhaps not classed as a domestic animal, the "close relationship" would warrant a brief reference. Man represents one of the most problematical cases of cell study. The number of somatic chromosomes has been variously counted from 24 to 38 (Fleming 24, Farner, Moore, Walker 32, Wieman 33 to 38), while the number in the testes are given as 47 by Winiwarter and 22 by Guyer and Montgomery. The first worker obtained his data from a white man, while Guyer and Montgomery worked on negro material. In the case of Winiwarter's work on the germ cells of the white man, the numbers in the maturation divisions (Figs. 7, 8 and 9) are 23 and 24, while Guyer shows that the corresponding numbers in the negro are 10 and 12. If the works of the last two investigators stand the test of future researches, we will have a very definite and substantial criterion by which we can segregate the white and black races. No doubt, it soon will be a very simple matter to take a small bit of muscle, connective tissue or even nasal epithelium from any individual, count the chromosomes in the dividing cells and determine the race of that individual. Hoy (1916) suggests that the variation in the number of chromosomes in the different individuals studied might represent intermediate hybrids between the white and black races. In this case, the percentage of negro blood flowing in any man's veins, would be in direct proportion to the number of chromosomes in the cells of his body.

Furthermore, Guyer finds in man two sex-chromosomes which do not associate with the other elements on the first maturation spindle (Fig. 7) but pass bodily to one pole, thus giving 10 chromosomes in one daughter cell and 12 in the other. The ten common chromosomes now unite two by two, giving second

division cells (Figs. 8 and 9) and thus mature sperms (Fig. 11) with 5 (5 double) and 7 (5 double plus the two sex-chromosomes) chromosomes respectively. Assuming the woman (negro) to have 24 chromosomes—that is 12 after they have been reduced ready for fertilization—we can readily predict the sex of the offspring of each cell. If the sperm carrying the twelve chromosomes (Fig. 11—first two sperms) should come in contact with a human egg (Fig. 16), a new cell (Figs. 12 and 13) and later an embryo having 24 chromosomes would be formed. This would of course be a female, since the male has only twenty-two chromosomes, while the union of a sperm having only 10 chromosomes (Fig. 11 —last two sperms) and an egg would result in an embryo of 22 chromosomes—a male.

Wodsedalek (1913) has clearly demonstrated the presence of eighteen rod-shaped chromosomes in the male pig and 20 in the female. Two of the chromosomes in the male cells usually stand apart from the other. They remain very conspicuous throughout the ripening stages and in the first division stand quite aloof from their neighbors, passing undivided to the "female daughter cell." In the second ripening division they divide as other chromosomes, giving eight for one cell and 10 (8 common and 2 sex-chromosomes) for the other. The common chromosomes fuse at this point as in man, to form half the number of double elements, but this has no effect upon the final result. If the cell with eight meets a female cell, all of which have 10 after ripening, the result would be a male pig, while if the cell with the two female producing chromosomes meets a female cell the result would be a pig with 20 chromosomes, which is to say, a female. It is interesting to note that in the pig and many other forms the mature spermatozoa are of two distinct sizes, representing the two sexes, —the female-producing being the larger, due to the presence of the two extra chromosomes.

Except from the standpoint of the number of chromosomes, the method of sperm formation in the horse is almost identical with that of the pig. In the horse there is only one female determining chromosome, which behaves as the two in the pig. The number in the green cells of the stallion is 37, representing two types of sperms with 18 (9 double) and 19 (9 double, 1 single) chromosomes, respectively. According to these figures, the mare which has never been examined, should possess 38 chromosomes.

Further consideration might be given to the general methods of sperm formations in other mammals, as the cells of the cat, squirrel, rabbit, white mouse, bull, dog, guinea-pig, rat, opossum and bat have been, or are being studied. The above discussion, however, will suffice to show the general process involved in the sperm formation of the normal animals.

XI. CAUSE OF STERILITY OF THE MULE. In conclusion I wish to call attention to a case of cell study which is attracting the attention of many classes of people, from the secluded cytologist to the most practical farmer. The cause of the sterility in the common mule is especially interesting since it is a well known fact that this animal possesses the necessary organs and a passion similar to that of the horse or ass. A study of the early cells of the testis of this animal shows normal cells with 50 ordinary chromosomes and one sex-determiner. It will be remembered that the horse has only 36 plus the extra chromosome. "This suggests that the number in the ass is about 65, thus making a difference of about 28 chromosomes between the parents of the hybrid." With such marked difference in the number of chromosomes in the parents, one would expect a catastrophe somewhere along the line of cellular development. Indeed, the germ cells do meet with such a disaster while in the process of ripening. (Figs. 4, 5, etc.)

We have shown in other forms that the maternal and paternal chromosomes are not directly associated in the somatic and early germ cells, but lie side by side, divide and carry on their other necessary functions independently, though in the same cell. This clearly accounts for the real existence of the mule. Should these chromosomes not act independently, the conflict would occur at fertilization and thus the mule would be an impossibility. This independent action further permits the existence of the desired characteristics of both horse and ass in one intermediate individual, but does not explain the possibility of an offspring from this hybrid.

While considering the typical ripening stage, it was noted that there is a pairing of homologous chromosomes from father and mother. "In case of the mule, however, because the ovum and sperm contributed such unequal numbers of chromosomes, there are many without a homologue with which to mate, and even in case of homologues the physiological incompatibility of the two plasms renders the pairing difficult and incomplete, or prevents it entirely." This disturbance is sufficient to cause a destruction of the ripening germ cell. Wodsedalek (1916) insists that, "Most of the cells disintegrate during the prophase, especially during the period of synapsis (Figs. 4, 5 and 6.) Others meet their fate in the metaphase of the early anaphase stage (Fig. 7). *The remaining few that survive the anaphase succumb soon after, and no secondary spermatocytes* (Figs. 8 and 9) *nor spermatids* (Fig. 10) *and consequently no*

spermatozoa (Fig. 11) *are formed and the hybrid remains sterile.*

"*There are no authentic cases on record showing that fertility ever occurs in this hybrid.*"

Shortly after Wodsedalek uttered these sweeping conclusions, Lloyd-Jones of Iowa State College cites (Jour. of Heredity., Nov., 1916) a number of cases from literature in which female mules and "hinnies" were unquestionably the mothers of colts. The most authentic case cited was that of a hinny owned by Mr. J. M. Bryant, of Quincy, Ind. This hinny was reported as having been in foal three times. The first two colts were deformed and died, but the last pregnancy resulted in normal twins. Another interesting case is reported by Dr. D. W. Sullivan, of Weed, Cal. This animal is out of a standard bred mare by a mammoth jack. She was put to a black Percheron stallion and produced a male "grade mule."

I have been unable to find any record either authentic or otherwise of a reproducing male mule. It is true that the test is less likely to be made with the male mule, as practically all are castrated when yearlings and they do not reach sexual maturity until about two years old.
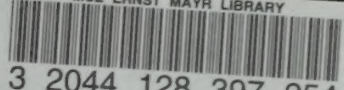
When such contradictory cases as those just cited are brought to light, the cytologist can only become more conservative and seek an explanation for the exceptions. In the first place, we could easily assume with Lloyd-Jones that the maturation of germ cells of the female were sufficiently different to permit an occasional complete development of an ova, but never of a spermatozoön. To one who has made a comparative study of the development of the germ cells of the two sexes, this assumption does not seem justifiable, especially in the present case, where the number of chromosomes of the parents differs so greatly. In fact, it seems just as possible for a male mule to reproduce as a female, the chances of course, being greater for the latter to be put to test.

In a study of hybrid pheasants it was found that most maturing cells were destroyed about the time of the first maturation division (Fig. 7). However, some continued their normal development through the second division (Figs. 8 and 9), and still others formed abnormal sperms (Fig. 11, fourth sperm). Since the same condition, though less marked, was found in the hybrid pigeons, and the mule, it seems evident that the point of destruction depends entirely upon chance. This being the situation, it seems quite plausible to assume that in rare instances a chromosomial arrangement might be brought about by which a certain number of maternal and paternal chromosomes are paired, those having no mate either continuing unpaired or being extruded from the cell. Much more light will be thrown upon this subject when material for the study of the germ cells of the ass can be obtained. Regardless of the accuracy of these or any other technical assumptions, we are forced to agree if the above cases are to be accepted, that the production of offspring by the common mule is indeed a reality.

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