THE RELATION OF THE SEX OF OFFSPRING TO THE TIME OF COITUS DURING THE OESTROUS CYCLE.*

BY

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THERE are many who hold the opinion that in the case of the human as well as in other animals, the length of time which elapses between the extrusion of the ovum from the ovary and its fertilization by the sperm is a decisive factor in determining the sex of the resulting individual. This hypothesis was elaborated at length by Thury (1863, 1864), who maintained that an ovum when first extruded was female in respect of its potentialities, but that at some point during its passage along the genital tract it became male. Fertilization immediately after ovulation yielded a female, fertilization some time later yielded a male, and fertilization at points between these times resulted in the production of intersexual forms. Thury, applying this hypothesis to the problems of practical animal breeding, was able to claim that, in the case of cattle, service during the earlier phases of oestrus yielded a preponderance of heifer calves, whereas service towards the end of oestrus resulted in the production of an excess of males. Thury's hypothesis was accepted and endorsed by Baust (1871), Guiard (1903), and Bell (1914). But it is reasonable to question the validity of these observations, since it is now known that it is a matter of considerable difficulty to recognize the exact stage of oestrus exhibited by an animal such as the cow. Moreover, the exact relationship of oestrus and ovulation in these forms is not clear even yet.

It is the case, however, that recent work has revealed considerable evidence which seems to show that sex-reversal of the egg before fertilization does occur in certain forms. The experiments of Hertwig (1906, 1912), of Kushakevitch (1910), and of Witschi (1914) have shown, for example, that delayed fertilization and also the exposure of the ova of the frog to high temperature before fertilization lead to a profound disturbance of the sex ratio among the resulting generation. If the male frog was permitted to fertilize half of the eggs of the female and was then removed, to be replaced after an interval to fertilize the remaining eggs, after the lapse of eighty-nine hours none but male offspring were obtained from the second half. It was established that this result was not due to a selective fertilization, to a sexually selective mortality among the embryos, or to the abnormal extrusion of the X-chromosome from the ovum during its maturation. The correct interpretation of the results would seem to be that some 50 per cent. of the eggs were fertilized by X-chromosome-bearing spermatozoa to become genetic females (XX in chromosome constitution), but that the conditions of the experimentation were such as to transform them into individuals with the characterization of the male, the sex-chromosome constitution of the zygote being overridden by the effects of delayed fertilization upon the metabolism of the egg before fertilization. King (1912) has shown that desiccation of the toad's egg yields exactly opposite results. She obtained 87 per cent. of females in an experiment in which the mortality among individuals of unknown sex was insignificant. The interpretation of her results would appear to be similar.

It has been found by Whitman (1919) and Riddle (1912, 1916) that the mating of pigeons and ringdoves belonging to two widely different zoological families results in the production of male offspring only, and that female offspring alone were obtained from the eggs of doves which had been forced to lay excessively and at an abnormally rapid rate. In birds the female is the digametic sex. Riddle was able to show that eggs which yield males can be distinguished from those which yield females, that maleness is associated with eggs of smaller size, higher water content, and less stored energy, and that the production of none but males or of none but females is associated with the antecedent production of eggs of one or of the other kind. He submits

that delayed fertilization leads to an increased metabolic rate in the egg, and that a high metabolic rate leads directly to the development of a male-type physiological state and characterization. Wide crossings involve the pooling of hereditary factors which in their action lead to the establishment of one or other kind of metabolic level, male or female, in the zygote. Extending his conclusions to the case of cattle, Riddle suggests that the greater production of males from eggs which have remained unfertilized for a period of hours is almost certainly correlated with an increased water content which the eggs obtain before fertilization. He grants that it is not established that the mammalian ovum does indeed take up water from the fluid it encounters in the reproductive passages, but points out that this is so in the case of every amphibian, reptile, and bird so far examined. However, it still remains to be shown what applies to the amphibian applies also to the case of the mammal.

Russell (1891) had collected data relating to the sex ratio of calves according to the time of service during the oestrous period, and this investigation was continued and extended by Pearl and Parshley (1913), who published the following data.

TABLE I.

Time of Coitus.	Total Offspring.	Males.	Females.	Sex Ratio.
Early in oestrus... ..	248	123	125	98.4
Middle of oestrus	125	67	58	115.5
Late in oestrus	107	65	42	154.8
	480	255	225	113.3

From these figures the authors concluded that the time of service had an appreciable influence upon the sex ratio. In a later paper by Pearl (1917), however, additional data were considered, and the conclusion reached that there was no definite or permanent relation between the time in the heat period at which the cow was served and the sex of the offspring. The commonly held opinion of the breeder and the suggestiveness of the earlier data of Pearl and Parshley were to be regarded as unsoundly based on insufficient statistics, and the relation of time of service and sex as purely accidental and not significant. It is impracticable for private breeders accurately to record the beginning of oestrus: the onset of activity of the cow towards the bull cannot be regarded as a trustworthy criterion of the onset of oestrus.

In the case of the rat, if the methods of Long and Evans (1922) and of Slonaker (1924) are employed to detect the onset and progress of the oestrous cycle, it is eminently possible to ensure that coitus shall occur at any particular point during this cycle. In the rat the vagina is moist and of a pinkish colour during the di-oestrous pause of the oestrous cycle. During the phase of pro-oestrus, oestrus, and met-oestrus, on the other hand, the vagina is dry and whitish, and the vaginal lips are more or less swollen. Long and Evans have shown that through the systematic examination of vaginal smears it is possible to follow exactly the course of the oestrous cycle. For purposes of description it was found to be convenient to divide the cycle into stages. In stage 1 the vaginal smear consists solely of medium, uniform sized epithelial cells, whilst the lips of the vagina are somewhat swollen. This stage lasts for ten to fourteen hours. In stage 2 relatively few cornified cells are present in the smear. The vagina is patent and its lips are swollen and of a characteristic purple colour. The onset of this stage is sudden, the change from stage 1 being complete in two to three hours. This stage lasts twelve to thirteen hours, and it, together with the next stage, corresponds to true oestrus. In stage 3 the smear consists of large numbers of cornified cells, many of them cohering to form large clumps. The swelling of the vaginal lips is receding. This stage lasts twelve hours. True oestrus, therefore, occupies some twenty-four to thirty-six hours. In stage 4 leucocytes make their appearance in the smear, which now consists of cornified cells and leucocytes. The lips of the vagina are no

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longer swollen. This stage lasts four to eight hours. In dioestrus, lasting fifty to sixty hours, the smear consists of epithelial cells and leucocytes.

Slonaker, using the revolving cage, was able to show that the peak of physical activity of the rat coincided with the cornified cell stage of Long and Evans, and Cooley and Slonaker, employing these methods of estimating the exact stages of the oestrous cycle in the albino rat at which coitus was noted, carried out experiments designed to examine the relation of the time of service during the oestrous cycle and the sex ratio. They found that their results showed no appreciable difference in the sex ratio among offspring of matings during early and late oestrus. A ratio of 114.6 males per 100 females resulted from matings during early oestrus, one of 110.5:100 from those during late oestrus. The difference, though suggestive, is not significant, when the numbers of litters considered are taken into account.

In the human it is now generally accepted that ovulation occurs fourteen to eighteen days after the first appearance of the menses. At the time when most of the data concerning the relation between the time of coitus during the catamenial period and the sex of the child were secured, little or nothing was known concerning the correlation of menstruation and ovulation, and so many of them are not really useful.

The observation that the Jews exhibit a high secondary sex ratio has been explained on the ground that coitus is forbidden during the first week after menstruation, and that the ova are, therefore, overripe and potentially male when fertilized. But Pearl and Salaman (1913) failed to find any evidence that in the human the time of fertilization of the egg relative to the menstrual period had any influence upon the sex ratio among the offspring. There are data, however, which seem to suggest that such a correlation does exist. Both Siegel (1916) and Fürst (1886), for example, found that impregnations during the earlier days of the intermenstrual period yielded an excess of males, whereas later impregnation gave a preponderance of females, though Pryll (1916), on the other hand, did not observe this difference. Their data are tabulated below. Freeborn's (1916) figures show an excess of females in the case of impregnation during the first half of the intermenstrual period, an excess of males from those during the second half. Bolaffio (1922), on the other hand, noted exactly opposite results.

TABLE II.

No of Cases.	1-9 Days after Beginning of Menses.			10-14 Days after Beginning of Menses.			15-22 Days after Beginning of Menses.			Authority.
	♂♂	♀♀	Sex Ratio.	♂♂	♀♀	Sex Ratio.	♂♂	♀♀	Sex Ratio.	
80	37	7	528:100	4	9	44:100	3	20	15:100	Siegel.
193	40	18	222:100	23	28	82:100	27	33	82:100	Fürst.
581	136	111	122:100	69	62	111:100	71	67	106:100	Pryll.

The evidence is distinctly conflicting. Ewart (1922) has pointed out that one source of error common to all the above observations is their neglect of the fact that male foetuses at birth are some five to six days older than female foetuses. Siegel (1921) acknowledges the validity of this criticism, and an examination of his records showed that the average length of gestation of males was 272.6 days, whereas that of females was 267.5 days.

The subject is of such interest that it was thought to be desirable to undertake further experimentation. The albino rat was chosen as experimental material; six males from one and the same litter were used. They belonged to a strain which habitually had given an unexceptional average sex ratio (105.4:100 for a four-year period). One hundred females were used; they all belonged to the same strain as the males, being all related and line bred. The experiment was carried out during the period May to September. The experimental animals were maintained under uniform conditions in the colony house, and the diet was the standard diet given to all the general stock.

The progress of the oestrous cycle was recorded by the method of Long and Evans. A drop of saline was placed on a microscopic slide; a metal spatula, moistened with saline, was inserted 1.5 cm. into the vagina, and the vaginal wall gently scraped. The spatula was then stirred in the drop of saline on the slide and this examined under a low-power microscope.

There are certain matters which have to be borne in mind in experimentation such as this. The older the animal the longer is the oestrous cycle, but oestrus proper is of longer duration in younger females. This usually begins in the late afternoon and continues throughout the night and the earlier part of the following morning. Service near the end of oestrus proper is effected only with difficulty in many instances, the female being at this time unreceptive. Further, the female will on occasion accept the male three to five hours before the oestrous cycle begins, so that it is very necessary to recognize the particular phase of the cycle by means of vaginal smears before coitus is allowed. By service early in oestrus is meant coitus during the first three hours of true oestrus; by later service, coitus during the last three hours of true oestrus, when cornified cells fill the microscopic field.

Fruitful coitus can be recognized by the presence of sperm in the post-coital vaginal smear, by the presence of the bouchon vaginale, and by the increase in weight of the female. She gradually becomes heavier and heavier up to the fiftieth day, and thereafter the further increase in weight is rapid in true pregnancy. Pseudo-pregnancy is not uncommon, however, more particularly in the case of coitus during late oestrus, and is to be recognized by the reappearance of oestrus fifteen days after service.

Allowances have to be made for a possible swing of the sex ratio with the chronological order of the pregnancy. In the case of the human there is clearly a relation between the age of the mother and the number of the pregnancy. Primiparae are usually 20 to 30 years old; second, third, and subsequent pregnancies occur in women over 30. Parkes (1924), for example, has shown that two-thirds of primiparous pregnancies occur in women under 28 years of age, second and third pregnancies occur mainly between the ages 23 and 32, and fourth pregnancies occur between 23 and 37. It is recognized that the sex ratio swings with the chronological order of the pregnancy. It is higher in earlier pregnancies (Newcomb, 1904; Ahlfeld, 1872; Winkel, 1903; Hecker, 1874; Little, 1919). This swing may be related to the number of the pregnancy, to the increased age of the mother, or to both of these factors. Cooley and Slonaker (1925) and King (1915) have shown that this rule of a decreasing sex ratio with increase in the mother's age applies also to the rat, and Copeman and Parsons (1904) have shown that it holds also in the case of the mouse. In the present experiments it was decided, therefore, to consider only the third litter of each of the females. There is a progressive lengthening of the gestation period with succeeding pregnancies in the case of the rat. The second is some eight hours longer than the first, the third some six to twenty-four hours longer than the second. Coitus early in oestrus is associated with a somewhat longer gestation period than coitus late in oestrus, as would be expected, since it involves a longer interval between coitus and fertilization. The results of this experimentation may be tabulated as follows:

TABLE III.

No. of ♀♀	Time of Coitus.	Total Litters.	Pseudo-Pregnancy.	Total Offspring.	Average Litter.	♂♂	♀♀	Sex Ratio.
50	First three hours of oestrus proper	35	15	189	5.4±0.13	98	91	51.84±3.37
50	Last three hours of oestrus proper	28	22	159	5.7±0.12	77	82	48.43±3.37
		63	37	348	5.53	175	173	

The conclusions which emerge from this study—concerned, it is true, with numbers that are much too meagre to be of any real significance—are entirely negative. It must be

remembered that it is wellnigh impossible for any one investigator to conduct experimentation on a sufficiently large scale, and that it is therefore desirable that experiments of this kind should be multiplied so that out of a considerable series adequate data may be secured. So far as they go, the present figures tend to support the contention that the time of service in relation to the oestrous cycle is a factor of no importance in the matter of the determination of sex in the mammal.

Summary.

In an experiment designed to examine the sex ratio among individuals resulting from coitus during the first three hours and during the last three hours of oestrus respectively in the rat, no difference was noted between these two groups.

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DISCUSSION.

Dr. JAMES YOUNG (Edinburgh) thought that it was impossible to discuss Dr. Crew's paper until he had had time to read it and digest it. He looked forward to a further series of investigations by Dr. Crew of an even more complete nature.

The PRESIDENT (Dr. Haig Ferguson) complimented Dr. Crew on his most interesting paper, but was sorry that it should be thought that the male was the product of stale eggs.

Dr. CREW, in reply, thought that obstetricians would not be allowed to plead ignorance of this very important subject much longer. It was not the egg but the sperm which determined the sex, but any abnormality affecting the egg would upset the sex-determining mechanism.

UTERINE FIBROIDS AFTER THE MENOPAUSE.*

BY

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UTERINE fibroids may give rise to symptoms over a wide range of age, but in practice we find that a very large proportion of patients consult us for the first time between the ages of 45 and 50, and if we advise an operation the first inquiry of the patient is the possibility of an early menopause with the probability of cessation of symptoms. Naturally every patient wishes to avoid an operation, and every right-minded surgeon wishes to save her this ordeal, but he is expected to advocate the best method of treatment, and, in giving this opinion, he must not allow his judgement to be unduly biased by sentiment. In deciding for or against operation two important considerations must be borne in mind: first, the fact that with uterine fibroids the menopause is frequently delayed; and secondly, the possibility of malignant and degenerative changes occurring in the fibroids after the menopause. It is the second consideration with which I wish specially to deal.

The more experience I obtain in my work the more impressed am I with the serious complications which may occur in connexion with these tumours after the menopause. It is impossible to calculate the percentage of fibroids after the menopause which undergo serious changes, since we only see those patients in whom the fibroid is giving trouble; but, even if the percentage is small, we must give due weight to the fact that the removal of fibroids in a comparatively healthy woman before the age of 50 presents a very slight risk, whereas the same operation in a patient over 60, or still more over 70 years of age, for a fibroid which has undergone degenerative changes from which the patient's general health has suffered, is a very much more serious matter. My own belief is that a patient with a fibroid of moderate or large size will act more wisely in getting rid of it while in a good state of health than in running the risk of serious trouble developing at a later stage of life.

During the last six and a half years I have operated upon 62 patients for definite symptoms due to uterine fibroids after the menopause, and should have operated upon three others if their general condition would have permitted it; it is upon these 65 cases that I base this paper. During this same period I have been consulted by several patients in whom the fibroid was so small and the symptoms so slight that I have advised against operation; these cases are not considered. The majority of these 65 patients were between the ages of 50 and 60, but 8 were between 60 and 70 years of age, and 3 were over 70. In several instances patients complained of two symptoms, either of which was sufficiently severe to call for operation, so in discussing these symptoms separately these patients will appear in both sections.

General Ill Health: Patient not Fit for Operation.

There were three patients whose general health precluded the possibility of operation. One was 60 years of age, with recent haemorrhage from the tumour, but she had had a recent stroke, and even if the tumour proved to be malignant I did not feel justified in subjecting her to any operation. The second was also 60 years of age, and had severe pain in the lower abdomen, apparently arising from a uterine fibroid; but she was in a very feeble state of health with mitral regurgitation, and was not fit for an abdominal section. The third was an enfeebled woman, aged 72, with a fibroid almost filling the pelvis and with a slight degree of prolapse. Many years previously she was told she had a fibroid, but was advised not to have it removed. With advancing years the tumour dropped into the pelvis and produced more and more discomfort from pressure symptoms, and now she is a confirmed invalid, spending most of her life in bed or on the couch, and

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