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The use of sex-chromosome anomalies for measuring radiation effects in different germ-cell stages of the mouse¹

Liane Brauch Russell

Biology Division, Oak Ridge National Laboratory
Oak Ridge, Tennessee

The special features that distinguish meiotic systems from other dynamic cell systems of higher organisms are, first, the fact that far more complex chromosomal changes are involved, and, second, the opportunity for studying effects on such systems by genetic means. Mutagenesis studies in meiotic systems of mammals began, strictly speaking, in the mid-1930's when the pioneer workers in mammalian radiation genetics, Hertwig, Snell, and Strandkov, were comparing progeny from matings made at various intervals after irradiation of the male (see [1] for review). However, detailed determinations and interpretations were not possible until much later, when the relations in gametogenesis could be accurately established and the direct effects of radiation on various cell stages studied [2-6].

The two sexes of mammals offer different opportunities for comparing the genetic effects of radiation in various germ-cell stages. In the male, all stages are continuously present throughout adult life. Genetic effects on the gonial stage can easily be studied separately by waiting appropriate intervals after irradiation. Similarly, effects on mature spermatozoa can be studied separately in offspring conceived immediately after irradiation. The intermediate stages include the meiotic system which is the subject of this symposium. For these intermediate stages, the timing for the mouse, worked out by Oakberg and others [4; 7-9], tells us that they cannot be represented in the ejaculate before certain intervals (e.g., spermatids not before 7 1/2 days, spermatocytes not before 22 days, etc.). However, at no time can one be sure of an ejaculate "pure" for specific irradiated meiotic stages.

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In the female, the situation is different. If there is a true oogonial stage (which is disputed by some investigators), it is no longer present in embryos older than 14 1/2 days post-conception. To get survival and fertility from earlier embryos is difficult with anything but very low doses of acute irradiation. The bulk of the meiotic prophase development of the female occurs during fetal life [10, 11]. By birth, leptotene and zygotene have been well completed; and even pachytene is found only with low frequencies in the day-old mouse. Diplotene is succeeded by dictyate, a pseudo-resting stage that makes its first appearance around the time of birth. By adulthood, oocytes present in the ovary of the female are all in dictyate [12], except every five days or so (i.e., at each estrus) for a few hours when a small number (around 10) continue into diakinesis and metaphase-I and are ovulated. In the female, there is no haploid germ-cell stage -- comparable to spermatid or spermatozoon -- since the second meiotic division is not completed until after sperm entry [13, review].

The contrast between the sexes is thus marked. Gonial and mature germ-cell stages are easy to segregate for study in the male. These are inaccessible in the female. On the other hand, certain of the intermediate events can be studied in rather pure cell populations in the female. These are the dictyate and -- with appropriate synchronization -- some of the stages between diakinesis and anaphase II.

The germ-cell system also includes events not commonly thought of in this connection, namely post-copulation stages up to syngamy preceding the first cleavage. Following sperm entry into the oocyte and the completion of the second meiotic division, the male and female pronuclei are separate genomes lying in a common cytoplasm. The pronuclear stage, which is of extremely short duration in many of the organisms used in mutagenesis studies (e.g., insects), is quite long in mammals and thus provides favorable material for sensitivity comparisons.

Several indices of genetic change have been employed in comparing germ-cell stages for radiation effects. These include dominant lethals in both sexes [14-16, reviews; 17, 18], translocations in the male [14-16], and specific-locus mutations [19-21].

The dominant-lethal category comprises a heterogeneous group of changes of unknown composition and can, moreover, be studied only with special precautions in females because of the complications of induced superovulation and indirect effects [47, 31]. The scoring of translocations requires large-scale fertility testing and is subject to some error. Because of these considerations, we embarked a few years ago on a program of making all possible germ-cell stage comparisons in the mouse by using sex-chromosome aberrations as an index of genetic damage.

METHODS

The discovery that the XO mouse is a viable, fertile female [22, 23] and that XXY is a viable male [24], both types being phenotypically recognizable in the presence of appropriate X-linked markers, led directly to experiments in which sex-chromosome aberrations could be used as an index to genetic damage [25-29, 14-16, 30].

The various cell stages investigated so far are summarized in Fig. 1. Meiotic events remaining to be surveyed are metaphase I, II, and immediately adjacent stages in both sexes. In the male, these are too short to be recovered in the necessarily mixed sperm samples. In the female, however, it is quite feasible to isolate some of them for study and this has been done in dominant-lethal experiments [31, 17]. We have recently attempted to do so for sex-chromosome anomalies, using hormone-synchronized ovulation, but various practical problems ascribable to this technique have temporarily delayed the collection of samples of sufficient size.

Some of the marker schemes that have been used in various of our experiments are shown in Figs. 2-4. In the irradiation of male germ-cell stages (Fig. 2), the use of a mate carrying two closely linked markers in repulsion is a useful device, since females homozygous for most sex-linked markers have reduced vigor. It can be seen that, with this scheme, all exceptional progeny resulting from loss or first-division nondisjunction is phenotypically detectable except in the relatively rare crossover classes.

Experiments on dictyate prophase stages in the female require two generations of marked matings (Fig. 3). The first produces the heterozygotes that must be irradiated in utero (in various stages of development) or as newborns. These are then allowed to grow up and are mated to males carrying a different marker. For irradiation of dictyate, the same scheme applies, except that the heterozygous females are irradiated as adults. Mates are not added until 24 hours after irradiation in order to avoid contamination from post-dictyate stages. It may be noted that the mating scheme in Fig. 3 allows detection not only of maternal loss and nondisjunction but also of paternal loss (not shown in figure). The latter would presumably be spontaneous, but could be mimicked by certain induced aberrations in the female (see below).

When "zygote" stages are irradiated (Fig. 4), the expected results would vary according to whether the damage induced was chromosome loss, chromatid loss, or nondisjunction. Only the first category is phenotypically detectable with certainty.

It should be noted that some of the expected exceptional phenotypes can also be produced by mechanisms other than those shown. The X/O phenotype can be the result of an $X/X^{Def(marker)}$ genotype, or of a deficiency in the Y involving male-determining regions. Various chromosome aberrations leading to non-random X inactivation, as in Searle's translocation [48], could mimic an X/O phenotype. Finally, there could be misclassification as a result of having a chance preponderance of cells in which the same X differentiated as the active one. Genetic and/or cytological tests of exceptional progeny are thus most desirable, whenever they can be made.

Spontaneous frequencies of various sex-chromosome anomalies in the mouse are quite unequal [26, 16]. By far the most common is loss of a paternal sex chromosome (X^P or Y), and frequencies in various stocks and experiments vary from about 0.1 to 1.1%. Non-disjunction of paternal X and Y leading to $X^M X^P Y$ [24] is very much rarer, the ratio of $X^M O$ to $X^M X^P Y$ being about 40:1 where the two could be compared in equivalent situations. Spontaneous maternal X aberrations are extremely rare, with $O X^P$ even less frequent than $X^M X^P Y$, and $X^M X^M Y$ never having been reported.

RESULTS

The results for various irradiated germ-cell stages in male gametogenesis, female gametogenesis and the zygote are summarized in Tables I, II, and III, respectively. In all cases, the last columns have been calculated on the same basis in order to facilitate comparison between Tables. The adjusted induced frequency per R involves subtraction of the control frequency which is always based on a strictly contemporary and otherwise comparable control group. This is considered necessary because of the rather wide variation in spontaneous X^{MO} frequency [26].

Not all phenotypically exceptional animals could be verified by genetic or cytological tests (see, e.g., some of the presumed X^{MO} animals in Tables I and II). Since, however, among these tested, the proportion misclassified was relatively small, the untested group also was included in calculations of frequencies. Among the 26 tested phenotypically XO animals in Table I are six animals that had 40 chromosomes but bred like XO. These are presumed to have X or Y deficiencies. Two of them, in fact, had a recognizably short chromosome [16].

Trisomies that would result from induced meiotic non-disjunction are detectable for only some of the possible genotypes. Thus, none of the XXX's (Figs. 2 and 3) or XYY's (Fig. 2) can probably be recognized phenotypically; and the same applies to a fraction of the XXY's, namely those where there has been a crossover in the female. Moreover, one cannot expect to find induced trisomies from post-divisionally irradiated stages, i.e., spermatids and spermatozoa. However, even allowing for all of these factors, it is evident that induced non-disjunction is quite negligible in comparison with induced loss: trisomy was never found after irradiation of females (Table II); and the single case observed after irradiation of males (Table I) could conceivably have been spontaneous. The subsequent discussion is thus based on induced sex-chromosome losses.

Of male germ-cell stages investigated (Table I), spermatids are most sensitive. The chromosome-loss frequency for all spermatocyte stages taken together is approximately equivalent to that for spermatozoa (on a per-R basis). Such a comparison should, however, be made with caution since the XO yield in the former case is measured in a selected cell population, in the latter case in an unselected one [15]. The first week of the spermatocyte sampling (possibly representing mostly post pachytene stages) gave a lower frequency of exceptions than the following two weeks.

Irradiation of female germ-cell stages was successful in producing OX^P , a type that almost never occurs spontaneously. The dictyate stage in mature follicles was found to give a considerably higher yield than did pre-dictyate prophase stages (Table II). As a matter of fact, dictyate oocytes in mature follicles appear to be at least as sensitive as are spermatids -- if not more so. For both of these stages, there is no direct germ-cell killing with the doses used, and one is therefore comparing chromosome-loss rates in presumably unselected populations. For earlier stages in both sexes, the possibility of selection does exist, and it may thus be fortuitous that leptotene-through-diplotene oocytes, as a group, yield approximately the same loss frequency as do late spermatocyte stages.

The earlier meiotic prophase stages in the female were studied in eight separate age-groups, namely days 13 1/2, 14 1/2, 15 1/2, 16 1/2, 17 1/2, 18 1/2, 19 1/2, 20 1/2 postconception, the last two of these being post-natal with the stocks used. The following fertility picture emerged in the course of this experiment. Females irradiated as 13 1/2-day embryos, and those treated as newborns or day-olds were very severely affected. Only a small percentage was productive at all; and even those animals that were, yielded very few young. On the other hand, among females irradiated with 250 R at intermediate stages, days 14 1/2 to 18 1/2 postconception, the percentage of productive ones was little, if at all, reduced from control; although the number of offspring per female was only about half normal, due to early cessation of breeding. At these intermediate stages, the bulk of the oocytes are probably in pachytene [11]. The very sensitive stages at each end may represent prepachytene on the one hand, and diplotene on the other. In newborn mice, Peters, on the basis of histological studies, considered pachytene and late diplotene quite sensitive and early diplotene resistant [49]. Strain differences very probably have a marked effect on the developmental schedule of the ovary.

As far as induced chromosome loss is concerned, the frequencies are not high enough to allow a meaningful comparison of different predictate stages. The OX^P's obtained to date came from females irradiated days 13 1/2, 17 1/2, and 18 1/2 postconception.

Irradiation of penetrated oocytes (Table III) can yield frequencies of sex-chromosome losses that are much higher than those obtained for any germ-cell stage irradiated in the gonads of either sex. Thus, peak incidences during that period are 3-4 times as great as those for irradiated spermatid or mature dictyate stages. Comparison of maternally and paternally contributed chromosomes indicates that sensitivity to X^M loss may be highest during final phases of the second meiotic division of the oocyte, while sensitivity to X^P or Y loss does not reach its peak until early pronuclear stage. (We are now making this comparison more accurately by using synchronized material.) A very clear result is the sharp drop in total XO's between early and late pronuclear stage. Loss of a maternal X, in fact, can apparently no longer be induced after 11 a.m. by 100 R (Table III) or even by 200 R given at 12:30, 2:00, or 3:00 p.m. [15]. Paralleling the drop in total sex-chromosome loss is a decrease in mortality, as measured either by litter size at birth or by fetal survival [15, 29].

It may be seen from Fig. 4 that irradiation of zygotes could, under certain circumstances, result in the formation of various mosaics. So far, no positive evidence for this has been obtained [15], and it has been tentatively concluded that irradiation of these stages is more likely to cause chromosome loss than chromatid loss or nondisjunction at cleavage.

DISCUSSION

Several types of anomaly that would be lethal if they involved autosomes [14] can be recovered in viable offspring if the chromosomes affected are the X and/or Y. It is this circumstance and the fact that many of the aberrant types can be recognized phenotypically by the use of appropriate markers that render induced sex-chromosome anomalies particularly

suitable for studying certain genetic effects of radiation and of other mutagens.

The question of whether sensitivity of sex-chromosomes is representative of chromosome sensitivity in general must, of course, be considered. For zygotes irradiated in early pronuclear stage, it can be calculated that the mortality results are in good agreement with induced XO frequency, if one assumes that the probability of eliminating autosomes and sex chromosomes is, on the average, the same and that loss of one or more autosomes is lethal. [15]. For a few other cell stages, the agreement was not quite as good, mortality being always slightly greater than expected on the basis of XO frequency [15, 29]. None of the disagreements within a given stage were, however, major. Moreover, comparison of relative sensitivities of different cell stages to the induction of sex-chromosome losses and of dominant lethals (presumed autosomal losses) in general gives good parallelism (see below). If truly substantiated cases of disagreement should eventually be found, these could be due to various causes. Thus, at certain stages, sex chromosomes might present an atypical proportion of the total chromosomal target, e.g., during male meiosis, when there is asynchrony of condensation of the sex bivalent. Alternatively, mortality after irradiation of certain stages might be due to causes additional to aneuploidy; or there might be a class of aberrations which would produce dominant lethality if they involved the autosomes but would be nondetectable (with the marker setup used) if they involved the sex chromosomes.

In the mouse, there is no good evidence that nondisjunction is induced by irradiation of meiotic stages. Despite the relative ease with which the XO type can be obtained, only one XXY animal was observed in all the various experiments. This is in contrast to the situation in *Drosophila*, where meiotic prophase irradiation has been shown to induce nondisjunction [32-36] and where, therefore, a portion of the XO's must be thought of as complementary to the sex-chromosome trisomics. Because of this situation, the fact that the highest XO frequency in *Drosophila* is found following irradiation of spermatocytes does not necessarily indicate that this stage is most sensitive to chromosome breakage. Not only nondisjunction in the purest sense, but perhaps other types of meiotic misdivision leading to loss only (e.g., "anaphase lagging") may contribute to the high XO yield from premeiotic irradiation.

Whether the latter occurs in the mouse, or whether all cases of sex-chromosome loss represent breakage phenomena is not known. Thus, various stages can be compared only in terms of the effect -- chromosome loss -- rather than the underlying cause for this effect.

The finding that the highest frequency of loss following irradiation of the male mouse occurs after treatment of spermatids parallels results obtained in dominant lethal studies [37-39, 18] and translocation experiments [40]. Studies of irradiated spermatocytes are complicated by the relatively high sensitivity to cell killing of these stages. Thus, even with a dose as low as 200 R, from 13 to 48% -- depending on exact prophase stage irradiated -- never reach the spermatid stage of development [9]; and some of those that do, go on to form abnormal

sperm. This is a particularly serious complication for dominant-lethal experiments, since the usual method of calculating preimplantation death cannot be applied. The easiest way out (and one used by most authors), that of merely ignoring this component of the total effect, may lead to results of doubtful validity. This particular difficulty is avoided when the index of genetic damage is sex-chromosome loss. However, there is still the possibility that the frequency determination is being carried out on a selected population.

The same is true of early oocyte stages, both dictyate and dictyate in early follicles. Oocytes in mature follicles, however -- both dictyate and post-dictyate -- are presumably not subject to selection by direct effects. In fact, it has been shown that after treatment of the stage most sensitive to the induction of dominant lethals, meiotic metaphase I, the average number of eggs normally ovulated and fertilized (as measured by viable two-cell embryos formed) was normal, even though only 0.1 offspring per female survived to fetal life [41].

It is unfortunate that technical difficulties to date have prevented the accumulation of good sex-chromosome-loss data for the oocyte stages that intervene between late dictyate and meiotic anaphase II. Dominant-lethal studies have shown metaphase I to be very much more sensitive not only than preceding stages [42, 31, 17] -- paralleling results on insects [43, 44] -- but also slightly more sensitive than the subsequent stages of anaphase I and metaphase II [17]. Metaphase II is probably included in the population of zygotes irradiated at 8:30 a.m. [29]. This population is highly sensitive to induced sex-chromosome loss (Table III); and if the parallelism with dominant-lethal results should hold, it might be expected that metaphase I would give an even higher yield of XO's. In the comparison between metaphase II and what was considered an early pronuclear stage, the dominant lethal data of Edwards and Searle showed a sharp drop in frequency [17]. The XO data show no drop between 8:30 and 11:00 a.m. [29]. At 8:30, most of the zygotes were already past metaphase II. It is also quite likely that what was considered early pronuclear stage in the dominant-lethal experiment [17] actually corresponds to our 3:30 p.m. irradiation, since over 12 hours had elapsed since metaphase II.

In speculating on the factors that determine differential sensitivity, the only clear fact that seems to emerge is that these are probably manifold and that the answer will be most complex. In one of the most favorable systems for study, that of the mammalian zygote, it appears that sensitivity changes may be related to nuclear growth and/or DNA synthesis [29, 45]. The relation between nuclear volume and sensitivity, however, can work in opposite directions. Thus, as the sperm head swells to become the early pronucleus, sensitivity increases greatly, but with further volume increase, it drops again sharply. This may point to DNA synthesis as a more important factor in the sensitivity change between early and late pronucleus, the presynthetic or synthetic phase being the sensitive one. High sensitivity in zygote stages, however, can be the result of other factors as well, since it is also found at the time of second-polar-body formation. This latter fits with Magni's suggestion that chromosomes just emerging from meiotic division are particularly vulnerable [46]. However, time relation to meiosis cannot be a major determinant, for the early paternal pronucleus, which is post-meiotic by several weeks, is as sensitive as the maternal

pronucleus, postmeiotic only by a matter of hours; and it is much more sensitive than the spermatid which is the direct product of male meiosis. The similarity of dictyate oocytes in mature follicles to spermatids, both with respect to frequency of induced sex-chromosome loss and the absence of direct radiation killing, is difficult to explain in view of the fact that one stage is premeiotic and the other postmeiotic. Finally, the exceedingly high sensitivity of metaphase I, which seems indicated by dominant-lethal data, cannot be accounted for by any of the interpretations suggested so far.

SUMMARY

Several types of anomaly that would be lethal if they involved autosomes can be recovered in viable offspring if the chromosomes affected are the X and/or Y. Marker systems have been developed for recognizing a high percentage of the possible anomalous types that would result from nondisjunction, monosomy, deficiency, and perhaps, certain rearrangements. Because sex-chromosome anomalies are thus especially suitable for studying effects of radiation and other mutagens, we have used them in extensive comparisons of various germ-cell stages in the mouse, including meiotic systems.

No good evidence has been obtained for X-ray induction of nondisjunction. On the other hand, the XO phenotype is easily produced. The great majority of these cases is the result of simple monosomy, but a few "XO" animals have also been observed that probably carry sex-chromosome deficiencies or, perhaps, other aberrations.

By far the highest yields of sex-chromosome losses are obtained by irradiating zygotes, from the time of sperm entry (second meiotic division) through early pronuclear stage. X^M may be relatively more sensitive than X^P or Y^P during the first part of this period; and there is reason to think that OX^P yield for irradiated metaphase-I of the female, when finally measured, may turn out to be even higher. There is a sharp drop in sensitivity between early and later pronuclear stages.

Among gonadal germ cells tested, the ones yielding the highest XO frequencies are the dictyate in mature follicles of the female, and the spermatid in the male. Leptotene-through-diplotene oocytes, and spermatocytes, taken as a group, give a lower, and roughly equal, yield. Among spermatocytes, post-pachytene stages give the lowest frequency of XO. These comparisons must, however, take account of the fact that XO yield from irradiation of spermatocytes and dictyate oocytes is presumably being measured in selected cell populations.

The factors that determine differential sensitivity are probably manifold and complex. It is very likely that they vary from one comparison to the next.

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TABLE I

Frequency of induced sex chromosome anomalies from irradiation of male germ-cell stages^φ

Post-irrad. days mated	Cell stage sampled	Dose (R)	No. of animals classified	No. with exceptional phenotype		Adjusted induced frequency per $r^+ \times 10^{-5}$	
				$\frac{M}{X^0}$	XXY	$\frac{M}{X^0}$	XXY
1-14 (mostly 1-7)	Spermatozoa (vas & epididymis)	600	1112	3+5*	0	2.0	- ^Δ
Contrcl		0	1285	1*	0		
15-21	Spermatids	200	2106	8+3*	0	4.3	- ^Δ
Contrcl		0	1683	1*	0		
22-28	Spermatocytes (postpachytene)	200	2118	2+1*	0	1.0	0
29-35	Spermatocytes	200	2370	8+1*	1	3.3	0.4
36-42	Spermatocytes	200	1726	5+1*	0	2.9	0
Contrcl		0	3423	1*	0		

^φIncludes some data from L. B. Russell and Saylor 1962, 1963 [28, 15].*Died before genetic or cytological tests could be completed.
Classified on basis of phenotype only.[†]Irradiated minus contemporary control. Based on 50% of offspring
of singly heterozygous; 100% of offspring of doubly heterozygous mates.^ΔInduction of XXY not expected since post-divisional stages were irradiated.

TABLE II

Frequency of induced sex-chromosome anomalies from irradiation of female germ-cell stages

Age irradiated	Stage sampled	Doses		No. animals classified	% loss		Adjusted induced frequency of OX^P per r x 10^{-5}
		Individual groups	Weighted average		X^M	X^P or Y	
Days 13 1/2-20 1/2, postconception*	Pre-leptotene through diplotene	150,200,250	221	2402	0.3	0.1 ^Δ -0.3	1.4
Adult	Dictyate (mature follicles) [Ⓢ]	100,400	342	331	2.4	0.0-0.3	7.1
Control		0	0	785	0	0 ^φ	

[†] Estimated frequency of occurrence, calculated by taking account of the fact that only half of all losses of X^M are detectable (O/Y being prenatally lethal).

* Groups separated by daily intervals.

^Δ The lower number represents only tested or cytologically verified animals.

^φ Range in other experiments is 0.1-1.1%.

[Ⓢ] Over 96% of classified young were conceived within 2 weeks after irradiation.

TABLE III

Frequency of sex-chromosome loss after 100 R irradiation at various stages between sperm entry and the first cleavage

Time irradiated ^Δ	Stage of development	Series ^φ	No. classified	% loss		Adjusted induced frequency per	
				X ^P or Y	X ^M *	$\frac{r \times 10^{-5}}{X^M}$	$\frac{r \times 10^{-5}}{OX^P}$
8:30 a.m.	Completion of second-polar-body formation. A few already in pronuclear stage	B	201	1.0	3.0	4.9	29.8
11:00 a.m.	Most zygotes in early pronuclear stage	A	422	3.3	1.9	23.0	19.0
		B	227	1.8	1.8	12.5	17.6
3:30 p.m.	All in pronuclear stage. Nuclear growth and DNA synthesis probably completed.	A	70	1.4	0	4.0	0
		B	193	0.5	0	0.1	0
Control		A	822	1.0	0		
		B	196	0.5	0		

^ΔAll on the day of the vaginal plug. Plug check about 8 a.m.

^φThe two series are separated by 3 years.

Series A: Mothers from 4 different strains, D, T, C3H, (101 X C3H)_F₁ [L. B. Russell and Saylor, 1963].

Series B: Mothers all (101 X C3H)_F₁ [L. B. Russell and Montgomery, 1966].

* Estimated frequency of occurrence, calculated by taking account of the fact that only half of all losses of X^M are detectable.

LEGENDS

- FIG. 1. Approximate time of occurrence of various germ-cell stages in the mouse, with wavy arrows indicating those irradiated in experiments with induced sex-chromosome anomalies. (Modified from [15]).
- FIG. 2. Mating scheme for experiments designed to detect sex-chromosome anomalies induced by irradiating various germ-cell stages of the male mouse. Exceptional phenotypes that are phenotypically recognizable are encircled with an unbroken line. A broken circle indicates those that are only questionably recognizable or detectable by further test of rare classes.
- FIG. 3. Mating scheme for experiments designed to detect sex-chromosome anomalies induced by irradiating various germ-cell stages of the female mouse. Circles and broken circles as in Fig. 2. (Modified from [15]).
- FIG. 4. Theoretical expectations for various abnormal events involving sex chromosomes of zygotes. (Modified from [15]).

	♀ GERMLINE	♂ GERMLINE
LATE FETUS, NEONATAL	MEIOTIC PROPHASE (through diplotene)	
POSTNATAL and ADULT	DICTYATE beginning follicles to most mature follicles DIAKINESIS (hours preovulation) MEIOTIC METAPHASE I	(all stages present at all times) SPERMATOGONIA A SPERMATOGONIA B MEIOTIC PROPHASE MEIOTIC METAPHASE I,II SPERMATIDS SPERMATOZOA
POST- COPULATION	MEIOTIC METAPHASE II SPERM PENETRATION OF VITELLUS PRONUCLEAR STAGES FIRST CLEAVAGE	

Fig. 1

Fig 2

Fig. 3

Fig. 4