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DEVELOPMENT IN AN ANIMAL MODEL.

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A CYTOGENETIC STUDY OF EARLY EMBRYONIC
DEVELOPMENT IN AN ANIMAL MODEL

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
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MAY 1977

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ABSTRACT

Several cytogenetic studies of human spontaneous abortions have reported 50 percent to contain chromosomal anomalies. Earlier losses due to chromosomal abnormalities or other causes which are not recognized as abortions remain unknown. Due to extreme difficulty in collecting material from man, there is a definite need for a good animal model. Chromosome defects have been observed in preimplantation blastocysts and embryos of other mammalian species including mouse, rat, hamster, rabbit and pig. The present study was designed to cytogenetically analyze early embryonic development by recovering preimplantation blastocysts and by collecting embryos during the implantation period in pigs.

Fifty-eight animals were cytogenetically analyzed at 10, 11, 12, 13, 16, 17, 18 and 19 days gestation. Using direct chromosome preparation, 338 blastocysts from thirty-one animals were analyzed. A variety of ploidies were observed in the same blastocyst ranging from $2N$ to $21N$. Forty blastocysts from seven animals were cultured for 24 hours. A variety of ploidies were observed in the same blastocyst ranging from $2N$ to large numbers of "high-order" polyploids greater than $32N$. Fifty percent of the preimplantation blastocysts in the direct preparation were polyploid mosaics, while 95 percent of the blastocysts after 24-hour culture were polyploid mosaics. This mosaicism was most likely due to chromosome preparation of trophoblast giant cells.

Five cases of chromosomally abnormal blastocysts included three triploids, one haploid, and one translocation mosaic. The frequency of

blastocysts demonstrating chromosome abnormalities (1.48 percent) most likely represented an underestimate of spontaneously occurring abnormalities. The polyploid mosaics were not included in the abnormal estimate. Using direct chromosome preparation, 192 embryos from twenty animals were analyzed. Polyploidy was insignificant and only four chromosomally abnormal embryos were observed. All four were monosomic mosaics and represented 2.08 percent of embryos studied.

The pooled estimate of mortality for blastocysts was 12.8 percent and that for embryos was 31.8 percent. These data suggest that a significant number of conceptuses were lost during the implantation process. The cytogenetic data failed to support the hypothesis that the increased mortality was due to chromosomally abnormal blastocysts failing to survive implantation. However, the frequency of abnormal blastocysts was most likely an underestimate due to the mixed population of trophoblast giant cells and embryonic disc cells in the blastocyst preparations.

A direct comparison to anomalies observed in human spontaneous abortions would not be appropriate. However, the value of swine as an animal model for early embryonic development remains to be fully explored.

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1. INTRODUCTION

Approximately 15% of all recognized pregnancies in man result in spontaneous abortion (Hamerton, 1971). Several cytogenetic studies of spontaneous abortions have reported 50% to contain chromosomal anomalies. Therefore, approximately 7 to 8% of all recognized conceptions may be considered as having chromosomal defects. Earlier losses due to chromosomal abnormalities or other causes which are not recognized as abortions remain unknown. However, chromosome defects have been observed in preimplantation blastocysts and embryos of other mammalian species including mouse, rat, hamster, rabbit and pig.

It has been shown that implantation of the blastocyst is a very critical stage of development. Due to extreme difficulty in collecting material from man, there is a definite need for a good animal model. Swine have been demonstrated as a useful biological model for the study of embryologic development of humans as well as for the study of many human diseases (Cornelius, 1969; Mitruka, et al., 1976). Other physiological similarities of swine and man which have been reported include: cardiovascular system, nutritional requirements, digestive system and formation of ulcers, immunological response, dental problems, and also effects of radiation (Bustad, et al., 1965; Bustad, 1966; Mitruka, et al., 1976). Pigs are particularly valuable for the study of early embryonic development because of detectable estrus, controlled timing of insemination, multiple ovulation and high fertilization rate.

The present study was designed to cytogenetically analyze early embryonic development by recovering preimplantation blastocysts and by collecting embryos during the implantation period in pigs.

2. REVIEW OF LITERATURE

Carr (1963) reported chromosome anomalies in a survey of spontaneous abortions and stillbirths in man. Similar findings of chromosome anomalies associated with spontaneous abortions were subsequently observed by others (Inhorn, et al., 1964; Kerr and Rashad, 1966; Boue, et al., 1967; Stenchever, et al., 1967; Makino, et al., 1967). In a summary of 20 unselected cytogenetic studies, Carr (1971a) reported 361 chromosome abnormalities in 1,485 spontaneous abortions, i.e., 42.1% trisomic, 23.8% XO monosomic, 15.5% triploid, 4.2% tetraploid, and 14.4% other anomalies. A high rate of chromosomal mosaicism (20% of abnormal) was noted in one study (Arakaki and Waxman, 1970). Based on two large published surveys, Jacobs (1972) derived an estimate that 42% of all spontaneous abortions are associated with detectable chromosome aberrations. The etiology of chromosome abnormalities in spontaneous abortions could be due to single gene disorders, immunological factors, hormones, radiation, maternal age, chromosomally defective sperm, or other factors (Carr, 1971a, 1971b; Jacobs, 1972). Chromosome banding techniques were also used on abortion material (Jonasson, et al., 1972; Lauritsen, et al., 1972; Kajii, et al., 1973; Therkelsen, et al., 1973; Creasy, et al., 1976). Banding allowed identification of individual chromosomes so that a more precise description of each chromosome abnormality became possible. However, the frequencies of abnormalities obtained from these studies were similar to those reported previously.

The relationship of the frequency of chromosome anomalies and the gestation period was investigated in human abortuses (Carr, 1971b). It was found that the incidence of chromosome anomalies remained at

40% up to 90 days of menstrual age, falling to 25% at 13-17 weeks and about 3.5% later. There was little information about very early embryonic development in man. Hertig (1967) examined 34 fertilized human ova. Morphologically, 10 were described as abnormal. However, chromosome analysis was not performed.

Preimplantation blastocysts and early conceptuses were studied in several species of primates, including baboon, macaque and rhesus monkeys. Gross anomalies were found in about 45% of the specimens studied (Hendricks and Kraemer, 1968; Heuser, et al., 1941). Some difficulties were experienced in primate reproduction and practicality of obtaining the appropriate material from primates.

Because of inherent high prolificacy, swine would be an ideal species for the study of early pregnancy. The length of gestation is approximately 114 days. The average number of ova shed during each estrus ranged from 10 to 20. The fertilization rate was normally close to 100% (Self, et al., 1955). Extensive data on embryonic mortality were available in this species. Mortality estimates were based on the difference between corpora lutea counts and fertilized ova (or embryos) recovered. Heavy loss occurred before 25 days of pregnancy, having been demonstrated by slaughter at different gestational ages (Hanly, 1961; Perry and Rowlands, 1962; Scofield, 1972). Several factors associated with loss were identified as: nutrition, exogenous progesterone, maternal age (Hanly, 1961), ovulation rate and uterine capacity, endocrine system, breeds of pigs, high temperature during early pregnancy (Scofield, 1972), uterine infection (Scofield, et al.,

1974), aged semen (Dziuk and Henshaw, 1958), delayed mating resulting in polyspermic fertilization (Pitkjanen, 1955; Hancock, 1959, Hunter, 1967) or possible polyspermy due to progesterone (Day and Polge, 1968; Hunter, 1972).

Bomsel-Helmreich (1961) analyzed the chromosomes of embryos resulting from delayed matings. From 13 sows mated 44 to 78 hours after the onset of estrus, 6% of the embryos were heteroploid on the 18th day of gestation. However, on the 26th day of gestation, no heteroploidy was found in 14 sows. She concluded that death of heteroploid embryos occurs at implantation. Smith and Marlow (1971) reported chromosomal analysis of 25-day-old pig embryos. From 9 gilts, a total of 76 embryos were recovered, 8 of which were degenerating or dead. A prenatal loss of 35.8% was estimated. One mosaic embryo was monosomic for the smallest metacentric chromosome and was the only abnormality observed. They concluded that most cytogenetically abnormal embryos rarely survive implantation. Few chromosome anomalies have been reported after implantation. However, Ruzicka (1968) reported a double trisomy in a kidney cell line derived from an embryonic pig which demonstrates one type of chromosome abnormality surviving implantation.

McFeely (1966) described a direct procedure for chromosome analysis of pig blastocysts. Subsequently, he reported 88 blastocysts collected 10 days after insemination from seven gilts sired by unrelated boars (McFeely, 1967). Of these, nine (10%) demonstrated chromosomal defects, i.e., four were triploid, three were tetraploid, one was mixoploid (2N/3N) and one had a structural anomaly in the form of a

deletion. In addition, 2.3% of the blastocysts were degenerating and lethal chromosome combinations could not be excluded in these cases. He concluded that the chromosome abnormalities accounted for approximately one-third of early embryonic mortality.

Several other species were examined for chromosome defects in development. Most of these studies involved observation of chromosome anomalies associated with a particular experimental condition. For example, superovulation was used in studies on mice (Vickers, 1969; Donahue, 1972) and rabbits (Fechheimer and Beatty, 1974; Fujimoto, et al., 1974). Hansen-Melander and Melander (1970b) suggested that blastocysts obtained following superovulation were not suitable as test subjects for many types of reproductive studies. Fujimoto, et al. (1974) supported this notion based on their findings that 10% of blastocysts from 9 superovulated rabbits contained chromosome defects while none were found in six control animals. However, Fechheimer and Beatty (1974) suggested that superovulation had no demonstrable effect in their study. Their sample size was much larger, having analyzed 463 blastocysts which contained 23 chromosome abnormalities.

Chromosome defects associated with delayed mating were analyzed in hamsters (Yamamoto and Ingalls, 1972). They found 15% of 98 blastocysts abnormal after delayed mating while only 0.7% in 134 controls. An increased chromosome aberration rate was also found in rats after delayed mating (Fugo and Butcher, 1966; Butcher and Fugo, 1967). In superovulated mice, Vickers (1969) found that delayed mating did not significantly increase the rate of heteroploids; however, the incidence of triploids was much higher. Increased triploidy

associated with delayed mating was also reported in rats (Piko and Bomsel-Helmreich, 1960) and rabbits (Shaver and Carr, 1967, 1969; Austin, 1967). Austin and Braden (1953) suggested that triploids resulted from diandry in the rat and rabbit after delayed fertilization. In the pig, digyny and diandry were discussed by Thibault (1959). Although digyny results from suppression of a polar body, diandry could result from either polyspermy or diploid spermatozoa. Fehheimer and Beatty (1974) reported that diploid spermatozoa were not a major cause of triploidy among rabbit embryos.

Triploidy was also observed at higher rates following in vitro fertilization in mice (Fraser, et al., 1976). They suggested polyspermy as the probably cause. However, Bomsel-Helmreich (1965, 1970) was able to induce triploidy during in vitro fertilization by experimentally suppressing the second polar body in rabbit ova.

Other studies reported chromosome anomalies in embryos as a result of increased maternal age in mice (Gosden, 1973), cytochalasin-B (Snow, 1973), breed of mice (Beatty, 1957; Wroblewska, 1971), aging of rabbit sperm (Martin and Shaver, 1972), and hormones (Widmeyer and Shaver, 1972).

3. MATERIALS AND METHODS

A feasibility study was performed using six crossbred sows from the University of Hawaii Experimental Farm. At 10-12 days gestation, the sows were sacrificed and the cervix, uterus, oviducts and ovaries were removed as a unit. The number of corpora lutea was counted and used as the criterion for the number of ova produced. The uterus was dissected after which the blastocysts were recovered by flushing the uterine horns with saline. The detailed procedure is outlined in Appendix I. A technique of direct chromosome preparation was used (McFeely, 1966). All animals were given a consecutive number for this study. Following the animal number, each blastocyst obtained from a particular animal was given a consecutive number. For example, 189-10 refers to blastocyst number 10 from animal number 189.

In December, 1974, a visit was made to the Pig Research Institute of Taiwan (PRIT), Chunan, Miaoli, Taiwan, Republic of China. At PRIT, access was obtained to a production herd of swine which consisted of 3,000 breeding females, producing 50,000 pigs annually for marketing. Three breeds were represented, i.e. Landrace, Yorkshire and Duroc. A system of rotational cross breeding was followed using purebred boars with crossbred females. All animals were kept in confinement with standard feeding and management. Selection was practiced for high productivity. Mating was performed by means of artificial insemination using 50 ml. fresh semen from randomly selected boars on the first day of estrus which was designated as day zero. A second insemination was performed 24 hours later with the same semen that had been preserved

in a skimmilk-glucose diluent at 15°C.

A total of 54 gilts and sows were available for sacrifice at 9-13 days gestation. Blastocysts were recovered as previously described, and arbitrarily allocated into two groups for chromosome preparation. Two methods were used; namely, direct chromosome preparation and chromosome preparation after 24 hour culture. The detailed procedure is outlined in Appendix IIA and IIB. An example of polyploidy from the first animal studied was published elsewhere (Moon, et al., 1975). Chromosome analysis was also performed on each adult pig utilized in this study using peripheral blood culture as outlined in Appendix III.

A subsequent visit was made in September, 1975, to the Pig Research Institute of Taiwan. Additional procedures were established for embryo dissection and chromosome analysis (Appendix I and IIA). Fifty-eight animals were available for sacrifice at 10-27 days gestation. Blastocysts and embryos of various gestational ages were photographed and processed for chromosome analysis.

A special arrangement was made with Lee Tah Farm Industries, Inc., to use 100 prepuberal crossbred gilts born within two months of each other. The farm was located in Lu Chu, Kaohsiung, Taiwan, approximately 200 kilometers south of PRIT. The mating plan was as follows: on any given day, all gilts showing signs of estrus were individually mated with randomly selected purebred boars. Only natural service was allowed. Daily mating records were examined from which animals were randomly assigned for sacrifice at 10, 11, 12, 16, 17, 18 and 19 days of gestation. The maximum number of animals sacrificed was limited to four per day

due to availability of facilities and manpower. The blastocysts and embryos were processed for chromosome analysis as described above. In order to detect any chromosome abnormalities present in the parental generation, blood was cultured from each gilt sacrificed and her mate.

Microscopic analysis was performed on slides obtained from PRIT and Lee Tah. One slide was prepared from each blastocyst and was scanned thoroughly. Every chromosome spread was recorded in one of three ways: 1) the chromosomes were well spread and an accurate count was made, 2) the chromosomes had few overlaps resulting in an estimated count, which was usually plus or minus one chromosome in the diploid to tetraploid range, and 3) the chromosomes were clumped or had too many overlaps to obtain an "estimated" count. In this case, the chromosome spread was recorded as an estimate of a ploidy. Two slides were made for each embryo and were scanned completely. However, only the chromosomes that were well spread or contained very few overlaps were recorded. There was a greater number of metaphases on the embryo slides than were on the blastocyst slides resulting in a more selective examination of chromosome spreads. Examples of tabulated chromosome counts for four randomly selected animals are displayed in Appendix IV. Lastly, the slides prepared from the blood cultures of the mated gilts and boars were examined. Ten well spread metaphases were counted from each individual and recorded. If any individual contained counts other than normal, the entire slide was analyzed completely to determine if any chromosomal abnormality was present.

Karyotypes were prepared following the procedure of Hansen-Melander and Melander (1974). Figures 3.1 and 3.2 show male and female karyotypes,

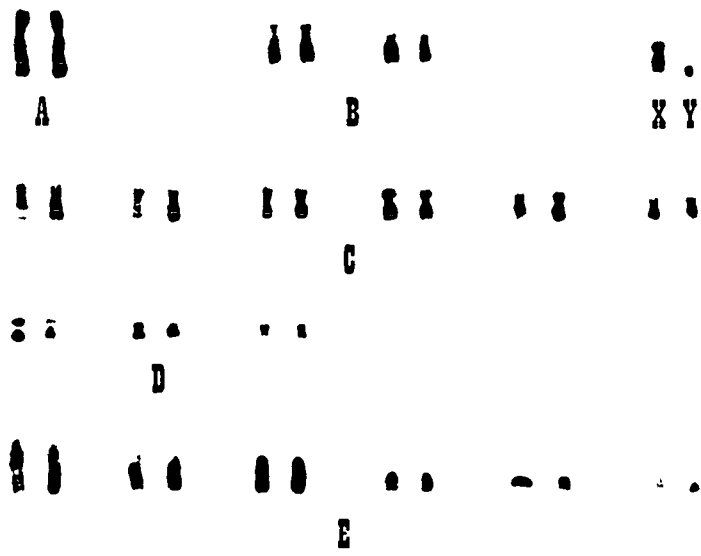


FIGURE 3.1
NORMAL MALE KARYOTYPE

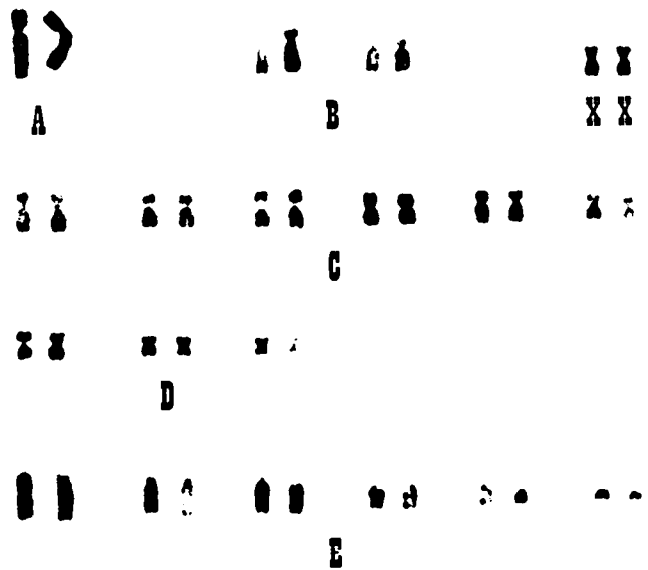


FIGURE 3.2
NORMAL FEMALE KARYOTYPE

respectively, demonstrating the arrangement of pig chromosomes into A, B, C, D, E, and sex chromosome groupings. Figure 3.3 shows a giemsa banded karyotype demonstrating each characteristic pair of chromosomes contained in the male complement.



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	X	Y
---	---	---	---	---	---	---	---	---	----	----	----	----	----	----	----	----	----	---	---

FIGURE 3.3

GIEMSA BANDED NORMAL MALE KARYOTYPE

4. RESULTS AND DISCUSSION

4.1 Description of Sample

A total of 218 animals were sacrificed. Ninety-six animals were deemed unsuitable for study because of failure of conception (22), uterine infection (13), failure of blastocyst recovery due to prolonged delay of evisceration (20), rapid elongation of blastocyst just prior to implantation (25), unknown causes (10), and six used for the feasibility study. Table 4.1.1 gives 122 animals studied at various gestational ages and counts of corpora lutea and conceptuses recovered. The rate of recovery in percentage provided an estimate of mortality during early pregnancy. Assuming that the probability of non-recovery remained the same at each age, the pooled estimate of mortality prior to implantation was 12.8 percent and that for post-implantation was 31.8 percent. These estimates were much lower than those reported at comparable gestational ages (Lerner, et al., 1959; Scofield, et al., 1972; Smith and Marlowe, 1971), indicating extremely good recovery. Also, animals used in this study were taken from large scale, specialized production herds in which constant culling was practiced.

Twenty-one animals not processed for chromosome analysis included eleven used for establishing embryo dissection technique and ten that were photographed. Forty-three animals processed for chromosomes, but unsuitable for analysis included fourteen used to establish culture technique, twenty-three with extremely low mitotic index and six sampled at 20-27 days gestation. Table 4.1.2 shows the number of blastocysts and embryos chromosomally analyzed at the various gestational ages. In ten percent of the blastocysts processed, no mitotic figures were found,

TABLE 4.1.1
NUMBER OF ANIMALS STUDIED

Age of Gestation	Number of Animals	Blastocysts or Embryos Recovered/Corpora Lutea Count	Mortality Estimate*
9 days	1	8/8	0
10 days	4	40/53	24.5%
11 days	42	504/567	11.1%
12 days	23	294/341	13.8%
13 days	1	11/14	21.4%
Total	71	857/983	12.8% **
16 days	3	33/49	32.7%
17 days	20	217/332	34.6%
18 days	16	178/232	23.3%
19 days	6	66/89	25.8%
20-27 days	6	70/125	44.0%
Total	51	564/827	31.8% **

*100% minus rate of recovery

**Mortality estimates for blastocysts and embryos were significantly different at 1% level.

TABLE 4.1.2
NUMBER OF BLASTOCYSTS AND EMBRYOS CHROMOSOMALLY ANALYZED

Age of Gestation	Number of Animals	No. Chromosomally Analyzed/No. of Blastos. Processed	No. Chromosomally Analyzed/No. of Embryos Processed
10 days	1	5/5	
11 days	27	267/294	
12 days	9	98/110	
13 days	1	8/11	
16 days	1		9/9
17 days	10		91/91
18 days	7		75/75
19 days	2		17/17
Total	58	378/420	192/192

therefore, a total of 570 blastocysts and embryos were chromosomally analyzed. All were obtained from Lee Tah Farms with the exception of 96 blastocysts (8 animals) which were cultured at the Pig Research Institute of Taiwan. A total of 9,182 metaphases analyzed included 6,499 from blastocysts after direct preparation, 923 from blastocysts cultured for 24 hours and 1,760 from directly processed embryos.

At 11 days gestation, blastocysts varied in size between individuals as well as within individuals. Figure 4.1.1 shows blastocysts recovered from four animals and placed in 60 mm. petri dishes for photography. The top two dishes contain blastocysts recovered from the left and right uterine horns of animal 188. The two center dishes were prepared similarly from animal 189. However, the bottom left dish is from animal 191 and the bottom right is from animal 192. The blastocysts shown were all recovered at 11 days gestation and demonstrate a size variation of approximately 1 mm. at the bottom left to an elongated, tangled mass of unknown length at the bottom right. From animal 181, recovered blastocysts ranged from 3 mm. to a tangled mass of unknown length (Figure 4.1.2). The largest intact blastocyst was 92 mm. in the top petri dish. Perry and Rowlands (1962) reported that ovulation occurred 24 to 36 hours after the onset of estrus in pigs. As the ova are shed consecutively, fertilization of each ovum must have occurred at different times. Therefore, fertilized ova, as well as cleaving zygotes may be present in the same animal, resulting in blastocysts of different sizes. The slaughter time for each day remained the same in the present study. Therefore, the size variation of blastocysts between animals was most likely due to ovulation which began at different times

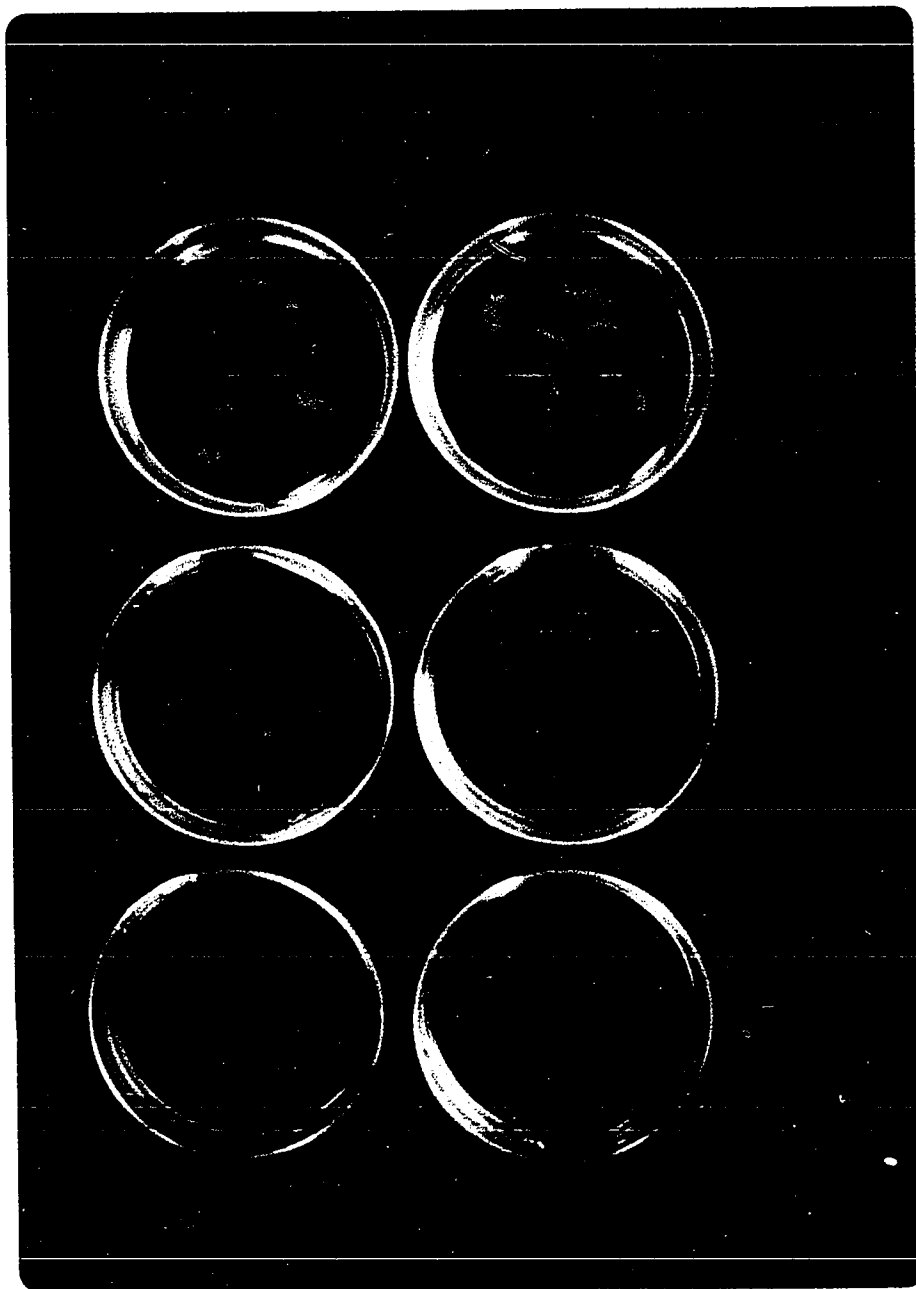


FIGURE 4.1.1

BLASTOCYSTS RECOVERED FROM FOUR ANIMALS
(188, 189, 191, 192) AT 11 DAYS GESTATION

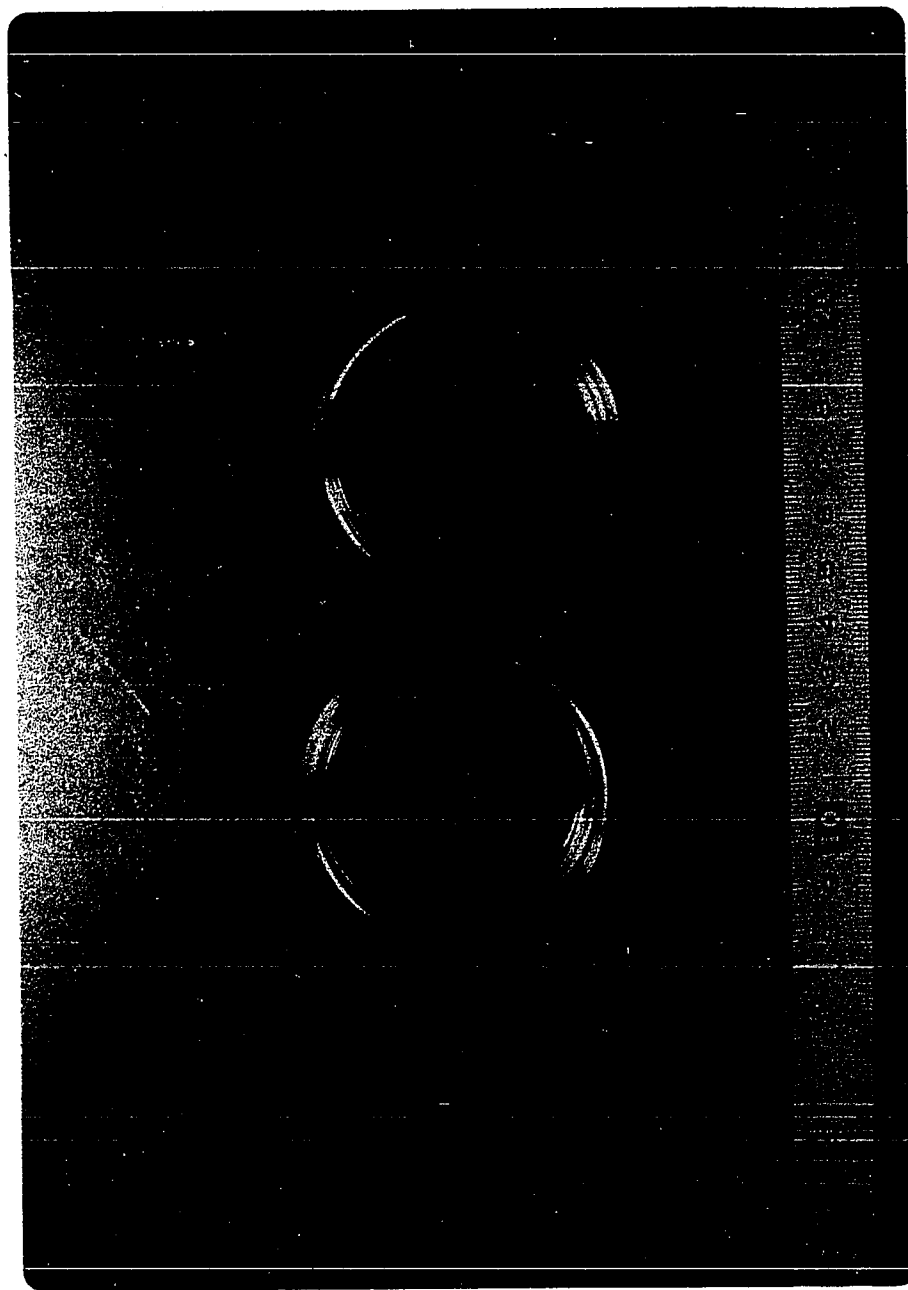


FIGURE 4.1.2

BLASTOCYSTS RECOVERED FROM ANIMAL 181 AT 11 DAYS GESTATION

of the day for each animal.

Embryo size was approximately 2 mm., 3 mm., 4 mm., and 5 mm. at 16, 17, 18 and 19 days gestation, respectively (Figures 4.1.3, 4.1.4, 4.1.5, and 4.1.6). Variation in embryo size was also observed between animals and within animals.

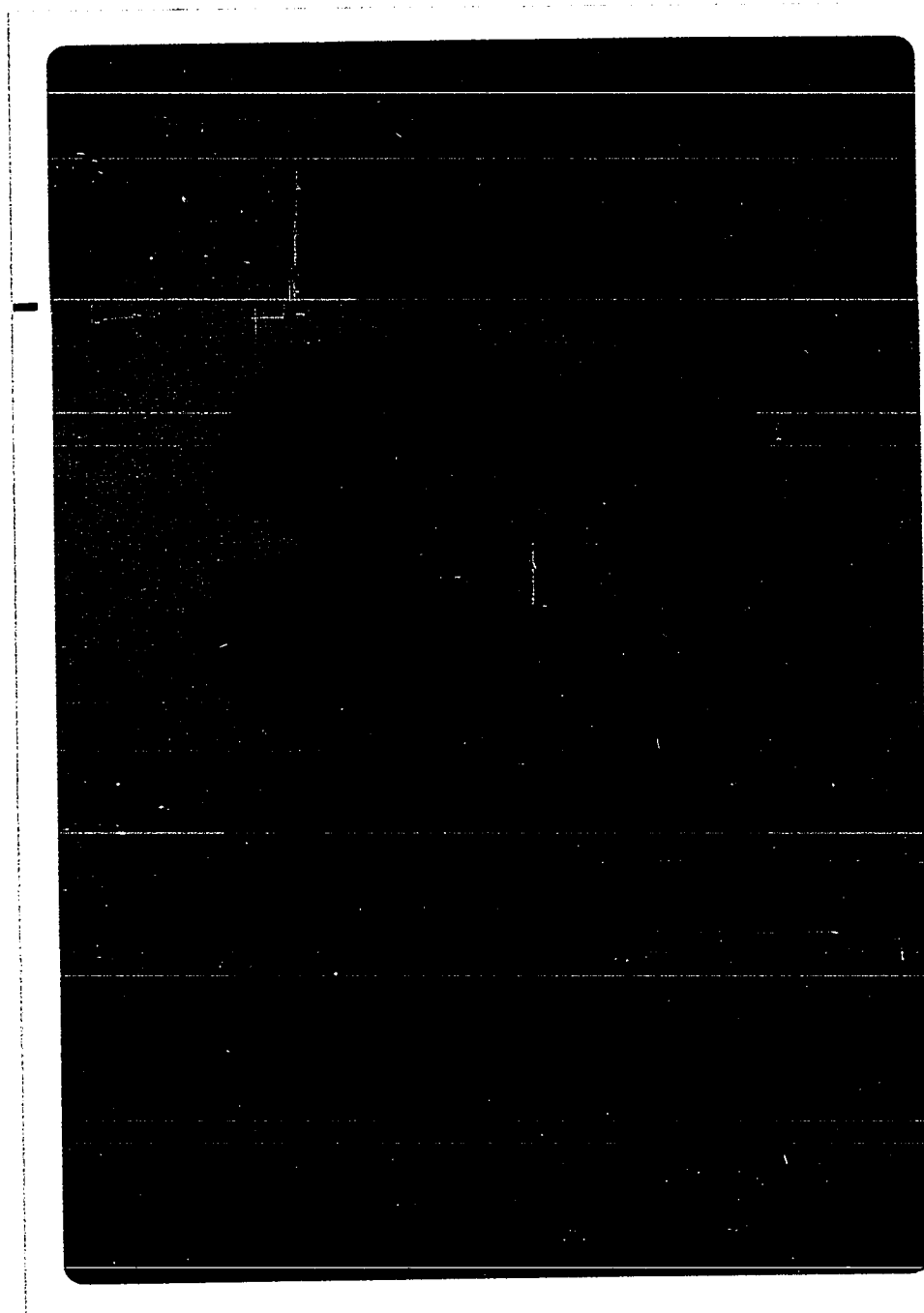


FIGURE 4.1.3
EMBRYOS--16 DAYS GESTATION

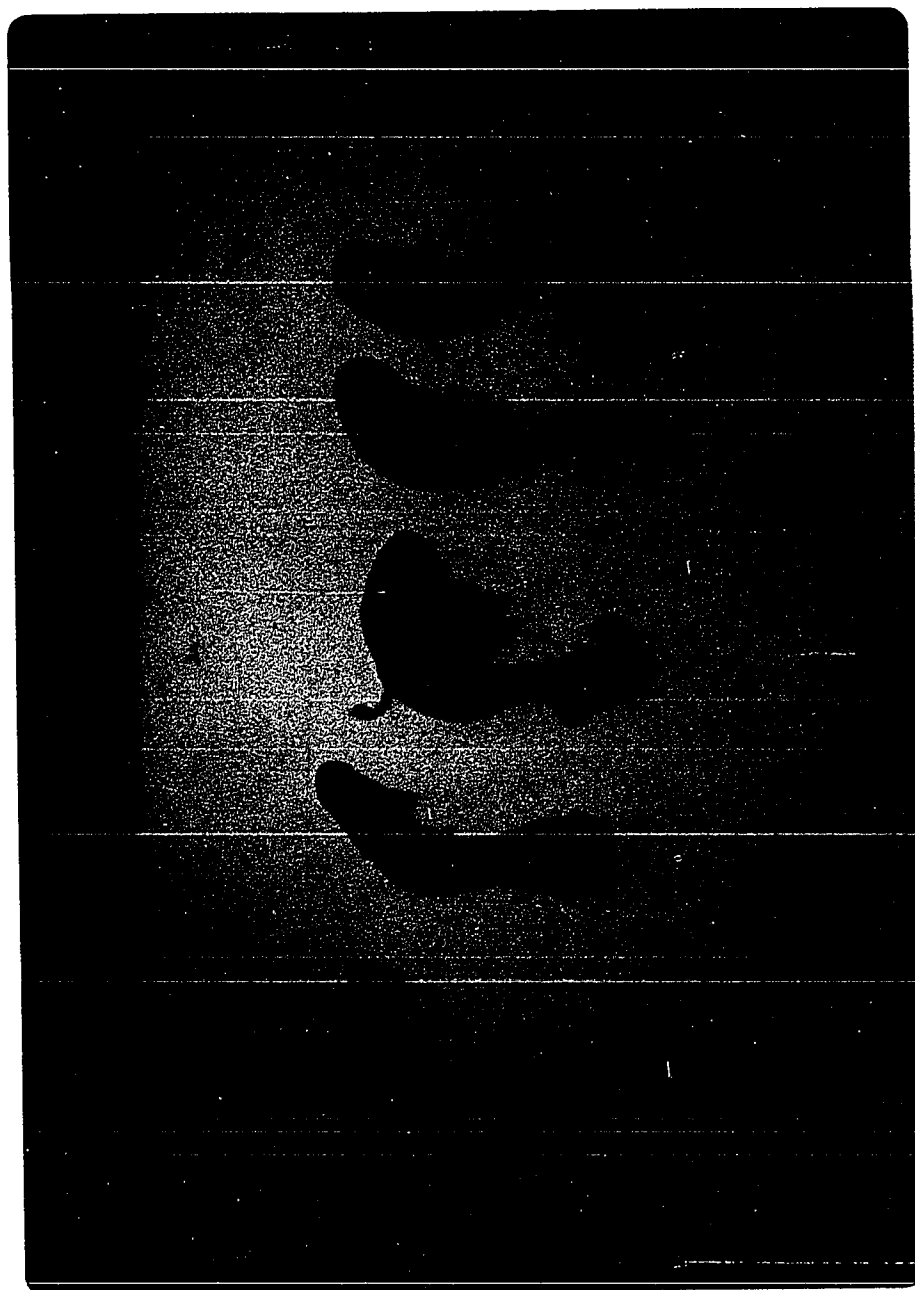


FIGURE 4.1.4

EMBRYOS--17 DAYS GESTATION

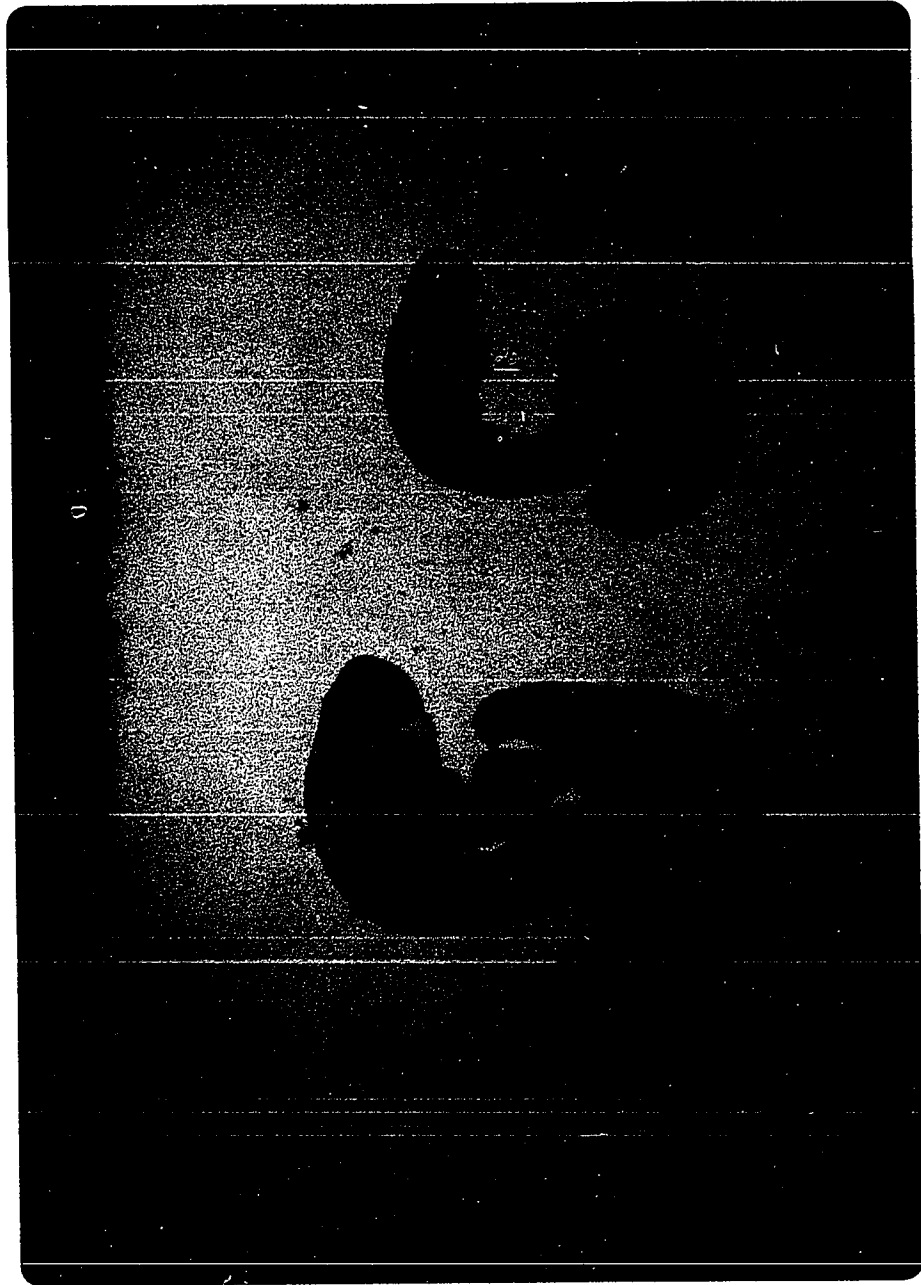


FIGURE 4.1.5

EMBRYOS--18 DAYS GESTATION



FIGURE 4.1.6
EMBRYOS--19 DAYS GESTATION

4.2 Blastocysts

4.2.1 Polyploidy in Direct and Cultured Preparations

Using direct chromosome preparation, 338 blastocysts from thirty-one animals were analyzed in the present study. A variety of different ploidies were observed, ranging from 2N to 21N. The majority of polyploids were 8N or less. Figure 4.2.1.1 shows four representative metaphases (2N, 6N, 7N and 8N). One hundred sixty-nine blastocysts (50 percent) contained polyploid cells as well as diploid cells. Of these, seventy-eight blastocysts (23.4 percent of the total) contained 2 or more different polyploid cell types, ranging up to eight different ploidies in a single blastocyst. However, accurate chromosome count was not possible in 31.19 percent of all polyploids. These cells provided rough estimates of chromosome number, all of which fell either in 3N or 4N. Cells with accurate count gave varying chromosome numbers which obviously clustered around each type of polyploidy. The distribution of chromosome counts are displayed by blastocyst for two randomly selected animals in Appendix VA. The data were then pooled and summarized by grouping cells in fixed class intervals with each polyploidy as the center. Of 6,499 metaphases examined, 10.75 percent were polyploids (Table 4.2.1.1). The frequencies were 3.48, 5.91, 0.57, 0.49, 0.09, 0.06, 0.06, 0.06, and 0.03 for 3N, 4N, 5N, 6N, 7N, 8N, 9-11N, 12-16N and 17-21N, respectively. On a blastocyst as well as an animal basis, the frequencies of these polyploids remained similar. Differences among animals for each type of ploidy were not found to be statistically significant at the 1% level except the less than 2N category. The frequency of less than 2N metaphases in any given individual ranged from zero to 28.2 percent. There were a total of 188 metaphases or 2.89 percent with chromosome counts less than that for diploid

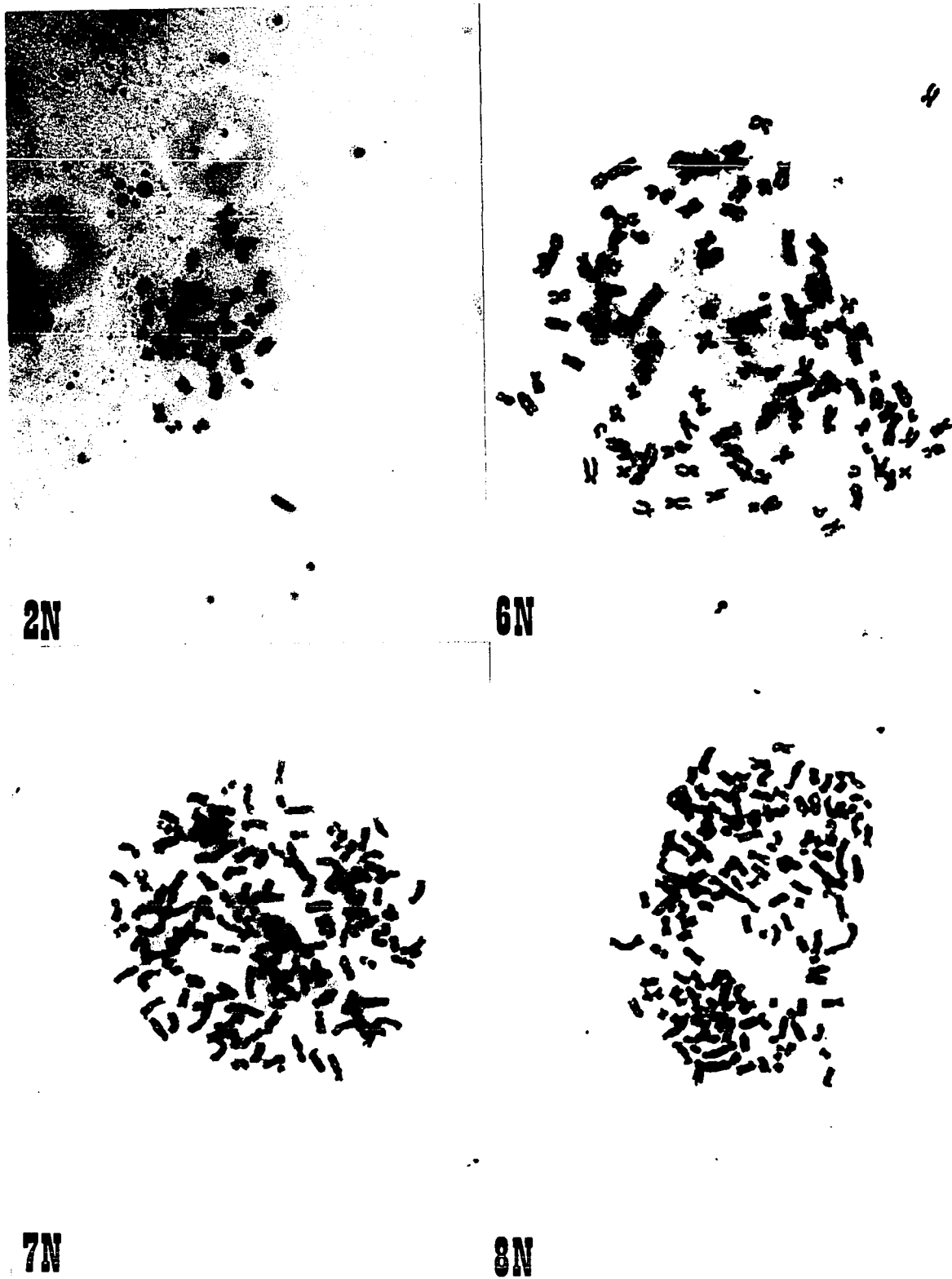


FIGURE 4.2.1.1

EXAMPLES OF PLOIDIES UNDER DIRECT PREPARATION (2N, 6N, 7N, 8N)

TABLE 4.2.1.1
 FREQUENCIES OF CHROMOSOME COUNTS
 AFTER DIRECT CHROMOSOME PREPARATION

Ploidies	By Metaphases	By Blastocyst	By Animal*
2N	.0289	.0377	.0426
2N	.8635	.8763	.8596
3N	.0348	.0274	.0285
4N	.0591	.0463	.0545
5N	.0057	.0042	.0062
6N	.0049	.0039	.0050
7N	.0009	.0010	.0017
8N	.0006	.0021	.0006
9-11N	.0006	.0003	.0004
12-16N	.0006	.0004	.0007
17-21N	.0003	.0001	.0003

*A frequency for each ploidy type was computed for each animal. The table contains the average frequency for all animals.

or near diploid.

Polyploid mosaics have also been observed in other species. In pigs, McFeely (1967) found nine chromosomally abnormal blastocysts among 88 studied, one being diploid/triploid mosaic. However, the number of metaphases analyzed for each type of abnormality was not given. In rats, Piko and Bomsel-Helmreich (1960) reported 6 mosaics out of 14 heteroploid blastocysts. Most of the mosaics contained diploid and triploid or near triploid cells. Polyploid mosaics $2N/3N$, $2N/4N$, $3N/6N$ and $2N/4N/8N$ were observed in rabbits (Fechheimer and Beatty, 1974; Hansen-Melander and Melander, 1971; Martin and Shaver, 1972). Mixoploidy was described in rabbit blastocysts and the findings were tabulated by chromosome count for diploid and near diploid and for polyploidy greater than $2N$ (Shaver and Carr, 1967; Martin and Shaver, 1972).

Several suggestions were given for such unusual mixoploids. McFeely (1967) suggested fusion of a diploid blastocyst with a triploid one to give rise to a $2N/3N$ mixoploid, based on the disparity of one between the number of blastocysts and the number of corpora lutea found in his material. He also mentioned that more complex mechanisms involving double fertilizations, fusion of polar bodies and loss of chromosomes during mitosis could be possible. Beatty (1972) described another theory for the origin of the $2N/3N$ mixoploid in which the ovum would first undergo immediate cleavage at the first meiotic division resulting in two cells, each with a set of haploid chromosomes. Only one of these two cells gave off a polar body and then both cells were fertilized, or possibly neither cell would give off a polar body and only one of the cells was fertilized. Other possibilities included various combinations of meiotic cleavage of an ovum with subsequent fertilization

by a diploid sperm. Fechheimer and Beatty (1974) considered the possibility of an accumulation of cells that had undergone chromosomal but not cytoplasmic division for the origin of 2N/4N and 3N/6N mosaics.

In the present study, 40 blastocysts from seven animals were cultured for 24 hours. A variety of ploidies were observed in the same blastocyst ranging from 2N to large numbers of "high-order" polyploids greater than 32N. Figure 4.2.1.2 shows four representative metaphases (2N, approximately 26N, approximately 32N and greater than 32N). Thirty-eight blastocysts (95 percent) contained polyploid cells as well as diploid cells. Of these, thirty-three blastocysts (82.5 percent of the total) contained 2 or more different polyploid cell types, ranging up to twelve different ploidies in a single blastocyst. Accurate chromosome count was made in 80.74 percent of the 923 cells examined. The remaining 178 cells were estimated for chromosome number and classified into five groups of ploidy, namely, 3N, 4N, 8N, 16N and 32N+. Cells with accurate count gave varying chromosome numbers from 8 to 600. Again the distribution of these numbers obviously clustered around each type of polyploidy. The distribution of chromosome counts are displayed by blastocyst for two randomly selected animals in Appendix VB. The data were then pooled and summarized by grouping cells in fixed class intervals with each ploidy as the center. Of 923 metaphases examined, 38.56 percent were polyploids (Table 4.2.1.2). The frequencies were 4.12, 7.80, 1.63, 1.41, 0.98, 5.85, 9.32, 1.95 and 5.53 for 3N, 4N, 5N, 6N, 7N, 8N, 9-16N, 17-31N and 32N or greater. On a blastocyst as well as an animal basis, the frequencies of these polyploids remained similar. Differences among animals for each type of ploidy were not found to be statistically

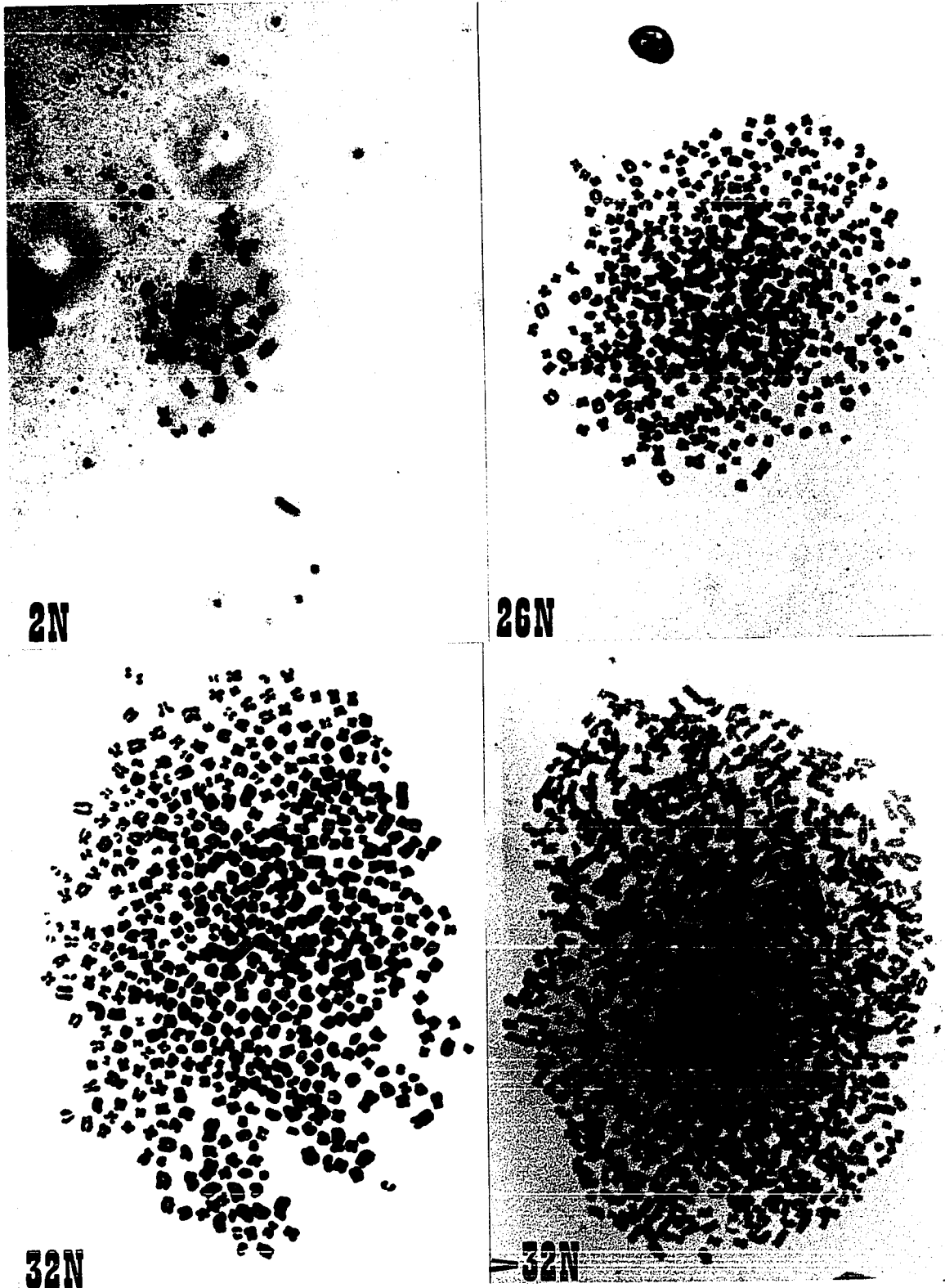


FIGURE 4.2.1.2

EXAMPLES OF PLOIDIES AFTER 24-HOUR CULTURE

(2N, APPROXIMATELY 26N, APPROXIMATELY 32N, GREATER THAN 32N)

TABLE 4.2.1.2
 FREQUENCIES OF CHROMOSOME COUNTS
 AFTER 24-HOUR CULTURE

Ploidies	By Metaphases	By Blastocyst	By Animal*
2N	.2839	.1818	.2464
2N	.3304	.4320	.4024
3N	.0412	.0403	.0373
4N	.0780	.0920	.0743
5N	.0163	.0143	.0113
6N	.0141	.0153	.0109
7N	.0098	.0060	.0064
8N	.0585	.0660	.0821
9-16N	.0932	.0913	.0759
17-31N	.0195	.0150	.0128
32N+	.0553	.0475	.0400

*A frequency for each ploidy type was computed for each animal. The table contains the average frequency for all animals.

significant at the 1% level. However, differences among animals for polyploidy as well as polyploidy greater than 8N were found to be statistically significant. The frequency of polyploid metaphases in any given individual ranged from 16.7 percent to 49.4 percent. The frequency of polyploid metaphases greater than 8N in an individual ranged from zero to 24.5 percent. Also, there were a total of 262 metaphases or 28.39 percent with chromosome count less than that for diploid or near diploid.

In comparison with direct preparation, some striking differences were noted in culture condition. There was approximately a four-fold increase in the frequency of polyploidy. A variety of "high-order" polyploids appeared in large numbers. Also, a ten-fold increase in the frequency of cells with chromosome number less than that for diploids was obtained.

It is known that the first cellular differentiation in mammalian development is the formation of the blastocyst. Prior to implantation, the blastocyst is comprised of two or three different cell types. Generally, the outside layer of cells, forming a sphere, is called the trophoblast. The cells which eventually form the embryo organize into an inner cell mass or embryonic disc in the blastocyst (Figure 4.2.1.3). The trophoblast are the cells that make contact during attachment and implantation in the uterine endometrium. Many of these cells eventually form a part of the placenta and chorion. In the pig, the placenta is of epitheliochorial type, which effectively shows no invasion (Billington, 1971). The chorion is merely in close opposition to the uterine endometrium and trophoblast cells form a



FIGURE 4.2.1.3

EMBRYONIC DISC OF PIG BLASTOCYST AT 11 DAYS GESTATION

single layer throughout. Trophoblast has been described as containing giant cells in pigs (Samuel and Perry, 1972) and in rodents (Barlow and Sherman, 1972; Barlow, et al., 1972; Hunt and Avery, 1971; Nagl, 1971; Schlesinger and Koren, 1967). These trophoblast giant cells may be uninucleate, but more frequently are multinucleate. The DNA content of trophoblast giant cells is enormous. In mice, Barlow and Sherman (1972) performed DNA measurements on the largest nuclei and found DNA amounts up to 850 times the haploid value, while Zybina (1964) described DNA values ranging from 64N to 1024N. The largest value reported was 4096N in giant cells of the rat (Nagl, 1971). These studies clearly show that the trophoblast contains various sizes of nuclei as well as some multinucleation. Many investigators have discussed the increased DNA in terms of increasing ploidy. This usually infers an increase in the number of chromosome complements; however, correspondingly large polyploid chromosome spreads have not been reported. Snow and Ansell (1974) reported "chromosomes" of trophoblast giant cells in mice. Actinomycin D was used to condense the chromatin into discrete bodies which represented "chromosomes". The number of these bodies did not exceed the diploid number, therefore, they suggested a considerable degree of polyteny in these giant nuclei.

It is apparent that cytogenetic analysis of the entire blastocyst would have to be performed on a mixed population of embryonic disc cells and trophoblast giant cells. The mixoploidy observed in the present study is possibly a result of chromosome preparation of both types of cells. The range of ploidies could then result from the

various sized nuclei and multinucleation of the giant cells in the blastocyst. Fechheimer and Beatty (1974) isolated the inner cell mass (embryonic disc) from rabbit blastocysts for chromosome analysis. The overall incidence of heteroploidy was 4.97 percent, which was not considerably different from the estimates derived from two earlier studies using the entire blastocyst in rabbits (Shaver and Carr, 1967, 1969).

Barlow and Sherman (1972), using dissected trophoblast cells, showed that not only the number but the size of giant cells increased during blastocyst development in mice. At seven days gestation, DNA content was determined which corresponded to either 2N or 4N cells with few 8N and 16N cells. However, at nine days, the majority of cells were 2N and 4N, but 8, 16 and 32N cells were well represented with few 64 and 128N cells. Giant cells constituted up to one-third of the trophoblast population in the first half of gestation. However, the result of tritiated thymidine labelling of trophoblast giant cell nuclei in mice and rats suggested that there was active DNA synthesis, but mitosis did not appear to follow (Jollie, 1964; Saccoman, et al., 1967).

Blastocysts are present in the pig about the fifth day of gestation. After the zona pellucida is shed about day six, the trophoblast begins a massive elongation characteristic of the pig blastocyst (Hunter, 1973). By day 13, when attachment begins, the elongated blastocyst may reach a length of 1.4 meters (Bonnet, 1901). It is amazing that while the pig blastocyst at day six is barely

visible to the naked eye, just seven days later it becomes approximately 1 meter in length. The divisions of trophoblast cells during this period of time must be numerous. Also, the number of trophoblast cells would far exceed the number of embryonic disc cells at this stage. Since chromosome preparation was made on blastocysts just prior to implantation, it may be concluded that the large polyploids, and probably the unusual mixoploids observed in the present study, are derived from trophoblast giant cells as suggested by McLaren (1975). It may represent a unique observation of pig blastocysts in which the trophoblast elongates tremendously prior to implantation.

Several mechanisms have been proposed for the formation of trophoblast giant cells. Cell fusion of diploid trophoblast cells was suggested (Avery and Hunt, 1969; Hunt and Avery, 1971; Jollie, 1964; Schlesinger and Koren, 1967; Saccoman, et al., 1967). Endoreduplication, replication of the genome without subsequent mitosis and cell division, was first proposed by Zybina (1964). Barlow and Sherman (1972) concurred that endoreduplication would be the most likely process of giant cell formation, although cell fusion could not be excluded. Chapman, Ansell and McLaren (1972) used genetic variants of the dimeric isozyme, glucose phosphate isomerase, to show that mouse trophoblast cells did not functionally incorporate maternal DNA nor form syncytial heterokaryons by cell fusion. Endoreduplication was also suggested as the probable mechanism of giant cell formation after the observation that mouse trophoblast cells were not formed by amplification of a portion of the total genome (Sherman, McLaren and Walker, 1972).

In the present study, chromosome preparations from blastocysts cultured for 24 hours provide another unique observation; namely, large numbers of "high-order" polyploids. The increased frequency and size of polyploids may be explained in two ways. First, these large cells could be present in the trophoblast, but are not amenable to direct chromosome preparation. Possibly, the 24-hour culture condition favors the larger nuclei for subsequent chromosome preparation. Second, the giant nuclei and cells of the trophoblast, regardless of the mechanism of their origin, could be more liable to fuse together when placed in culture. Subsequent chromosome preparation could then yield large polyploids from fused nuclei or multinucleated cells resulting from cellular fusion. Based on the following observations, the latter explanation seems most likely. Figure 4.2.1.4 shows examples of large and fused nuclei observed in cultured preparations. In addition, a polyploid metaphase (approximately 32N) located in frame C-1, was used for comparison of size with the nuclei. In frame A-4, a single giant nucleus is shown with adjacent smaller nuclei. The smallest nucleus in A-4 as well as the smallest independent nuclei in other frames are approximately 2N in size. Four medium-sized nuclei were apparently fused in both A-3 and B-3. The remaining frames show conglomerations of several different sized nuclei which appear to be fused resulting in unusually large "nuclei". Fused nuclei were not found in direct chromosome preparations.

In addition, certain cells displayed differential contraction of chromosomes suggesting more than one mitotic nucleus per cell. For

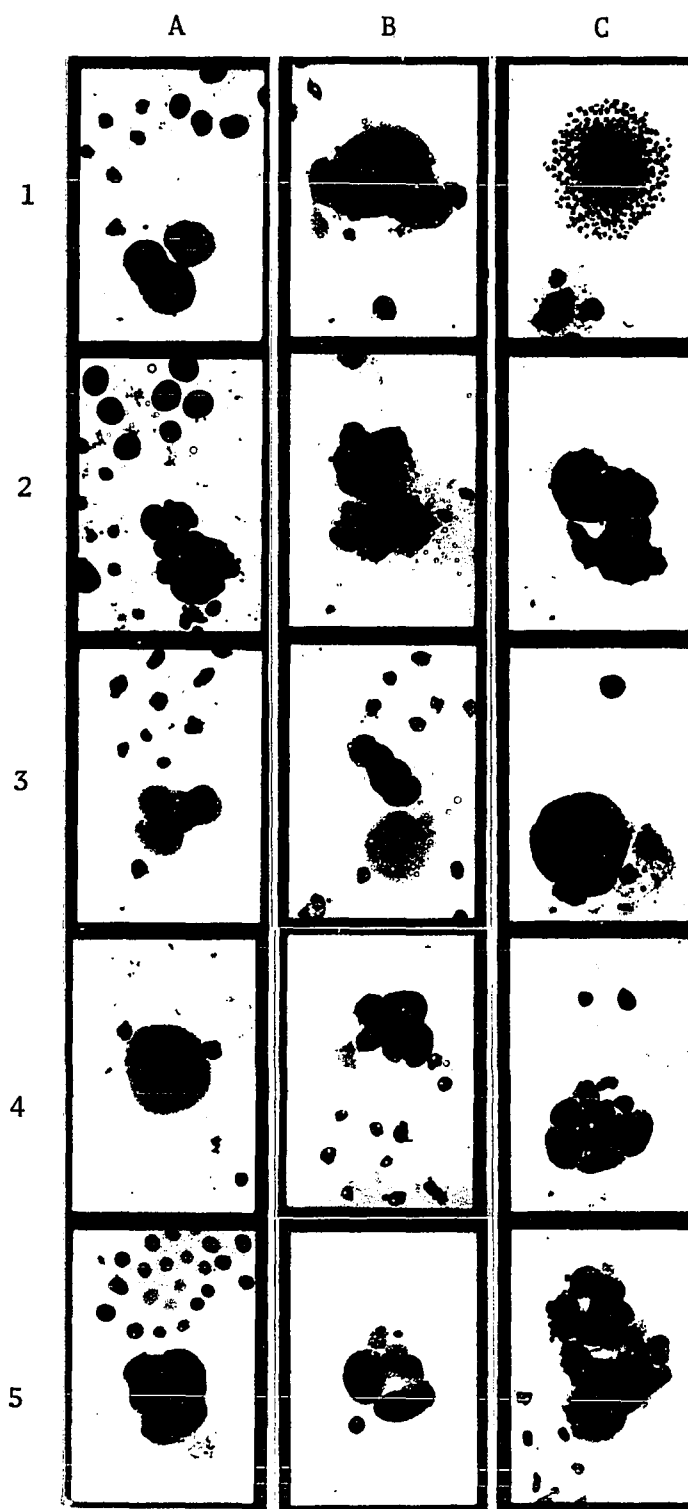


FIGURE 4.2.1.4 .

EXAMPLES OF LARGE AND FUSED NUCLEI AFTER 24-HOUR CULTURE
(PLUS ONE POLYPLOID METAPHASE)

example, Figure 4.2.1.5 shows chromosomes more contracted (arrow A) on one side of the metaphase than those on the other side (arrow B). The chromosomes in the center are a combination of the two degrees of contraction. The spread contains 153 chromosomes; therefore, it was recorded as 8N. However, it is likely that these chromosomes originated from two 4N nuclei which were located in the same cell. Upon mitosis, the two nuclei being slightly out of synchrony differentially contracted the chromosomes. Karyotypic analysis of this spread showed two different degrees of contraction for all chromosome groups (Figure 4.2.1.6). In each chromosome group, the chromosomes were arranged in two rows based upon gross morphology. In the bottom row, the chromosomes were characteristically shorter with a wider space between adjacent chromatids. There were 5 unpaired chromosomes which could have resulted from broken A-group chromosomes. Figure 4.2.1.7 shows another example of the same phenomenon. The spread contains approximately 230 chromosomes and was recorded as 12N. Karyotypic analysis was not possible, therefore, the number and size of the nuclei which formed this metaphase remained unknown.

In larger chromosome spreads, a similar phenomenon was observed. Figure 4.2.1.8 shows a localized area of chromosomes that appear broken and fragmented (indicated by the arrow). The same observation was made in metaphases in Figures 4.2.1.9 and 4.2.1.10. This phenomenon was reported in cells induced to multinucleate and is properly termed premature chromosome condensation or chromosome pulverization (O'Neill and Miles, 1970, 1971; O'Neill, 1972). They suggested that chromosome

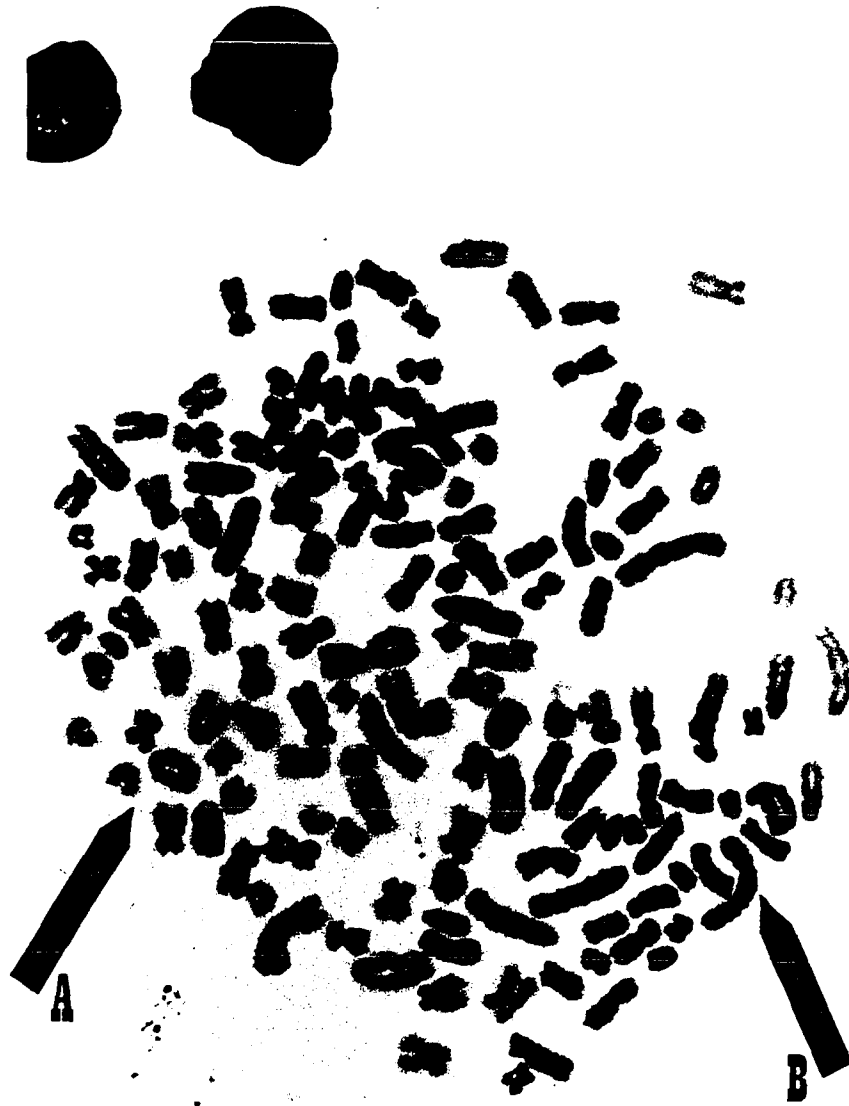


FIGURE 4.2.1.5

OCTOPLIOD METAPHASE DEMONSTRATING DIFFERENTIAL CONTRACTION OF CHROMOSOMES

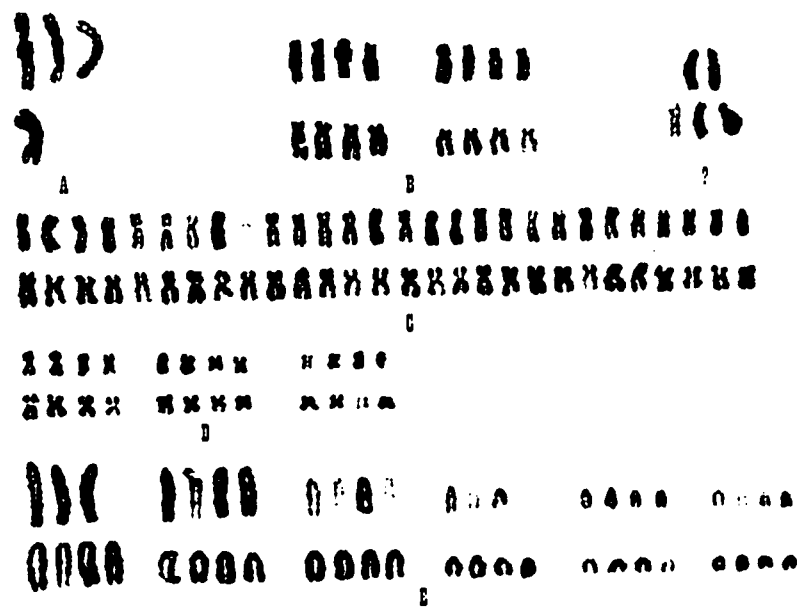
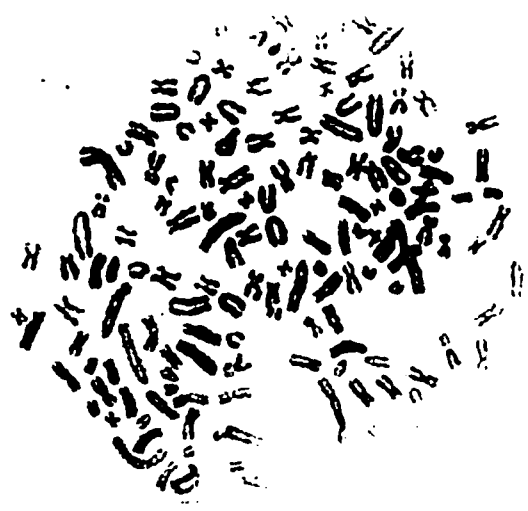


FIGURE 4.2.1.6

KARYOTYPE OF OCTOPOLOID METAPHASE DEMONSTRATING
DIFFERENTIAL CONTRACTION OF CHROMOSOMES



FIGURE 4.2.1.7

12N METAPHASE DEMONSTRATING DIFFERENTIAL CONTRACTION OF CHROMOSOMES



FIGURE 4.2.1.8

LARGE POLYPLOID METAPHASE DEMONSTRATING CHROMOSOME PULVERIZATION

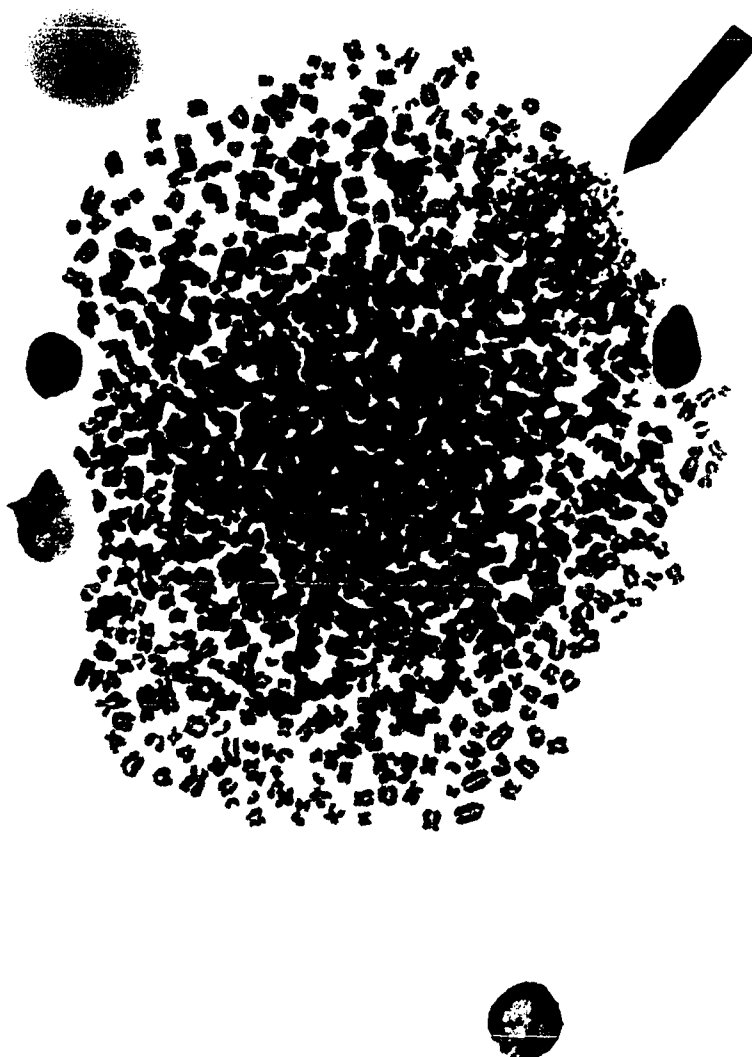


FIGURE 4.2.1.9

LARGE POLYPLOID METAPHASE DEMONSTRATING CHROMOSOME PULVERIZATION

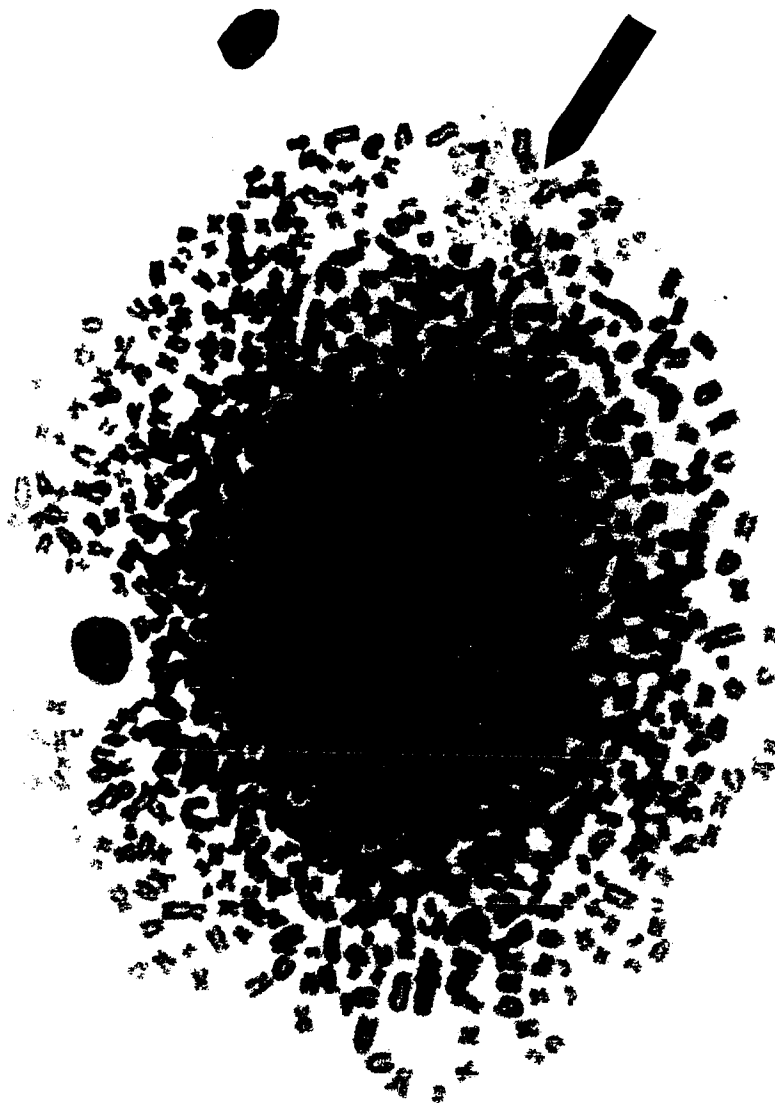


FIGURE 4.2.1.10

LARGE POLYPLOID METAPHASE DEMONSTRATING CHROMOSOME PULVERIZATION

pulverization resulted from mitosis of asynchronous nuclei, which were in various stages of the cell cycle, forcing the chromosomes to prematurely condense. The amount of chromosome pulverization observed in the present study was small suggesting a small polyploid nucleus or very few 2N nuclei were involved. Based on these observations, the large polyploids found in the present study may have resulted from fused nuclei or multinucleated cells.

Although polyploid cells were observed in 50 percent of all directly prepared blastocysts, obvious chromosome abnormalities were found in five blastocysts (Table 4.2.1.3). These abnormalities are described in the following sections.

TABLE 4.2.1.3
CHROMOSOMALLY ABNORMAL BLASTOCYSTS

Blastocyst Number	Gestational Age	Number of Metaphases	Chromosome Count	Karyotypes	Abnormality
172-2	11 days	12	57,Xyy	1	Triploid
212-6	11 days	33	57,Xyy	1	Triploid
		2	114 ?	0	
212-7	11 days	3	57 ?	0	Triploid
179-13	11 days	4	19,X	1	Haploid
189-7	11 days	17	37,XX	4	Translo- cation
		16	38,XX	3	Mosaic

4.2.2 Triploidy

In the present study, three blastocysts were observed as triploid which represented 0.79 percent of the 378 studied. Blastocyst 172-2 contained 12 metaphases, all near 3N. Thirty-three triploid metaphases were observed in blastocyst 212-6 which also contained two other cells that were hexaploid. In the third blastocyst (212-7), only three metaphases were found, but all were near 3N. It was not possible to confirm triploidy in 212-7 by karyotypic analysis, but it seemed most likely. Karyotypic analysis of the other two blastocysts demonstrated an additional member of each chromosome pair (Figures 4.2.2.1 and 4.2.2.2). The sex chromosome constitution of both blastocysts was XYY.

McFeely (1967) reported nine of 88 pig blastocysts demonstrating chromosome anomalies. Of these nine, four were triploids (XXY, XYY and 2 XXX), representing 4.5 percent of the sample. In the present study, the incidence of triploidy (0.79%) was much lower.

Triploidy was also observed in rabbits (Fechheimer and Beatty, 1974) and in mice (Donahue, 1972). The incidence of triploidy was 1.7 percent in rabbits and 1.2 percent in mice. From these studies of different mammalian species, triploidy was the most common abnormality observed. It was reported that the number of triploids as well as the frequency of chromosome abnormalities were increased by delayed mating in rats (Butcher and Fugo, 1967; Piko and Bomsel-Helmreich, 1960), rabbits (Shaver and Carr, 1967), and mice (Vickers, 1969). In vitro fertilization studies also showed an increased rate of triploidy

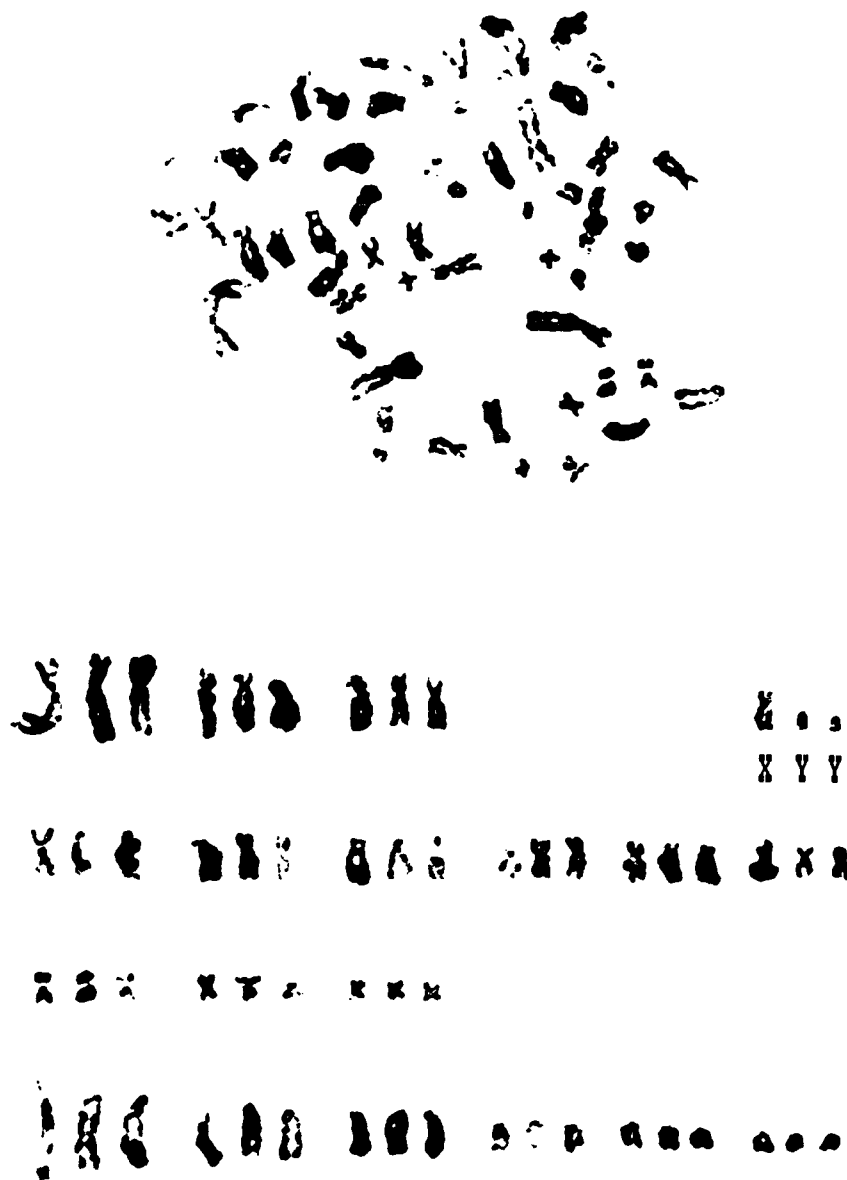


FIGURE 4.2.2.1

TRIPLOID KARYOTYPE--BLASTOCYST 212-6

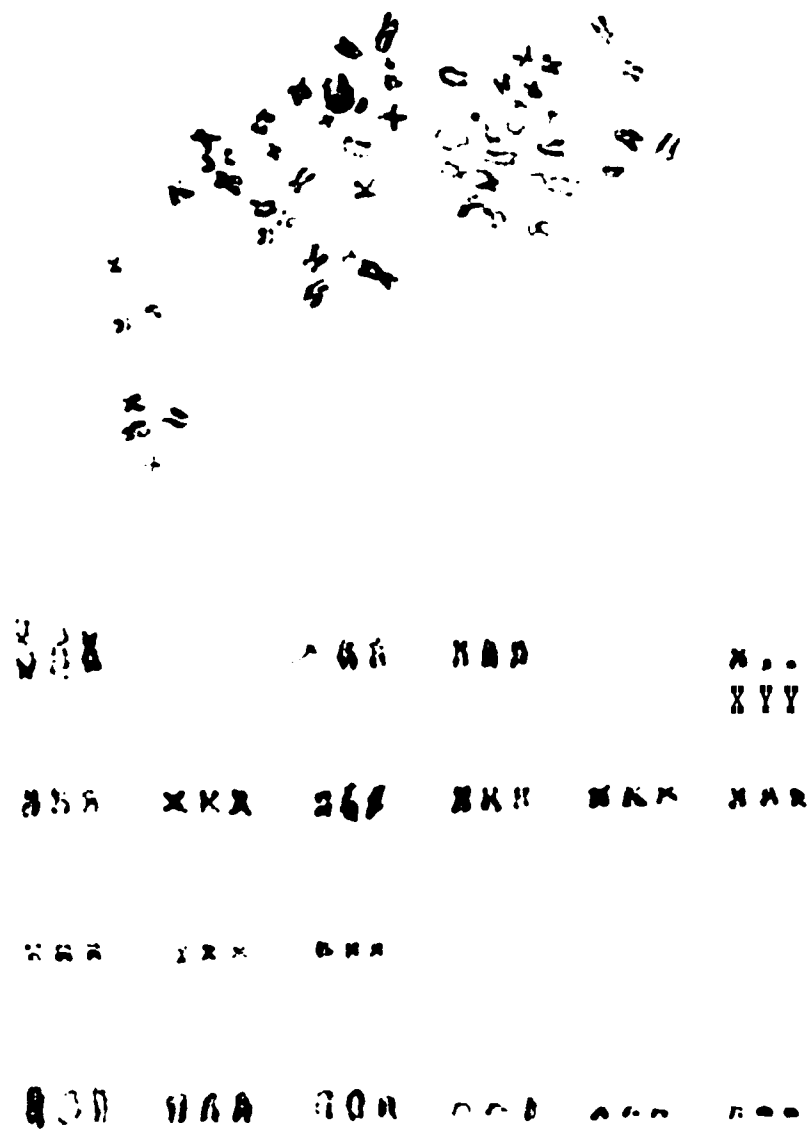


FIGURE 4.2.2.2

TRIPLOID KARYOTYPE--BLASTOCYST 172-2

suggesting polyspermy as a cause (Fraser, et al., 1976). It was reported that increased triploidy also occurred in certain strains of mice (Beatty, 1957; Wroblewska, 1971) and resulted from increased maternal age of mice (Gosden, 1973).

In man, Saadi, et al., (1976) reported triploidy in one still-born 69,XXX and two newborns, both 69,XXY, one of which lived 24 hours while the other only 3 hours. Other investigators reported a 69,XXY that lived 6 hours (Schindler and Mikano, 1970), a 69,XXX that lived 23 hours (Butler, et al., 1969), and a stillborn 69,XXY (Edwards, et al., 1967). Based on these studies, if a triploid was born, it did not survive more than one day. However, several older individuals have been reported to contain diploid and triploid cells (Schmid and Vischer, 1967; Schindler and Mikano, 1970; Dewald, et al., 1975). These individuals usually demonstrated malformations and survived to various ages, dependent upon the percentage of triploid cells in the body. Those with a higher percentage of diploid cells lived the longest. This was also true for individuals found to be 2N/4N mosaics (Kohn, et al., 1967; Atnip and Summitt, 1971; Kelly and Rany, 1974). In animals, two adult cases of diploid/triploid mosaicism were reported, one an intersex cat (Chu, et al., 1964) and the other an intersex mink (Nes, 1966). Few cytogenetic studies have been reported in adult mammals; therefore, the survival of triploids or 2N/3N mosaics remains unknown in animals.

Spontaneous triploidy may arise by suppression of either the first or second polar body which would result in digynic triploids when fertilized by a single sperm. Fertilization of a normal haploid ovum by two sperm or a diploid sperm would give rise to diandric

triploids. In the present study, both blastocysts subjected to karyotypic analysis contained XYY sex chromosomes. The most likely explanation for the origin of the triploids would be polyspermy for two Y-bearing sperm, although the mechanism for double fertilization remains unclear. Fertilization by a diploid (YY) sperm seems unlikely based on the findings of Fechheimer and Beatty (1974).

4.2.3 Haploidy

In blastocyst 179-13, only four mitotic cells were observed. The karyotype of one cell is shown in Figure 4.2.3.1. It contained only one member of each chromosome pair including a single X chromosome. The other three metaphases were estimated to be near haploid with overlapped chromosomes preventing karyotypic analysis (Figure 4.2.3.2).

Spontaneous haploidy in blastocysts was reported in other mammals. One haploid/diploid mosaic blastocyst (Fischberg and Beatty, 1951) and 6 haploid blastocysts (Beatty, 1957) were observed in crosses of the silver strain of mice. Matings of the silver strain of mice, especially silver females mated to non-silver males, resulted in the highest percentage of spontaneously heteroploid embryos. Vickers (1969) reported one haploid blastocyst from a PDE mouse. The blastocyst appeared degenerate containing cells with pycnotic nuclei and only three metaphases were observed. All three were haploid. Hansen-Melander and Melander (1971) reported a haploid/diploid mosaic blastocyst in a rabbit at 6 days gestation. During slide preparation, they were able to separate the trophoblast from the inner cell mass. The chromosome counts were: trophoblast - 40 haploid, 7 diploid and 1 tetraploid while inner cell mass contained 13 haploid, 6 diploid and 1 tetraploid. Seven haploid karyotypes were analyzed to reveal one member of each chromosome pair including the X chromosome.

The origin of spontaneous haploidy has been suggested as one of the three following possibilities: 1) parthenogenesis resulting in division of the maternal pronucleus, 2) fertilization by a sperm which contains inactive genetic material, and 3) expulsion of the male

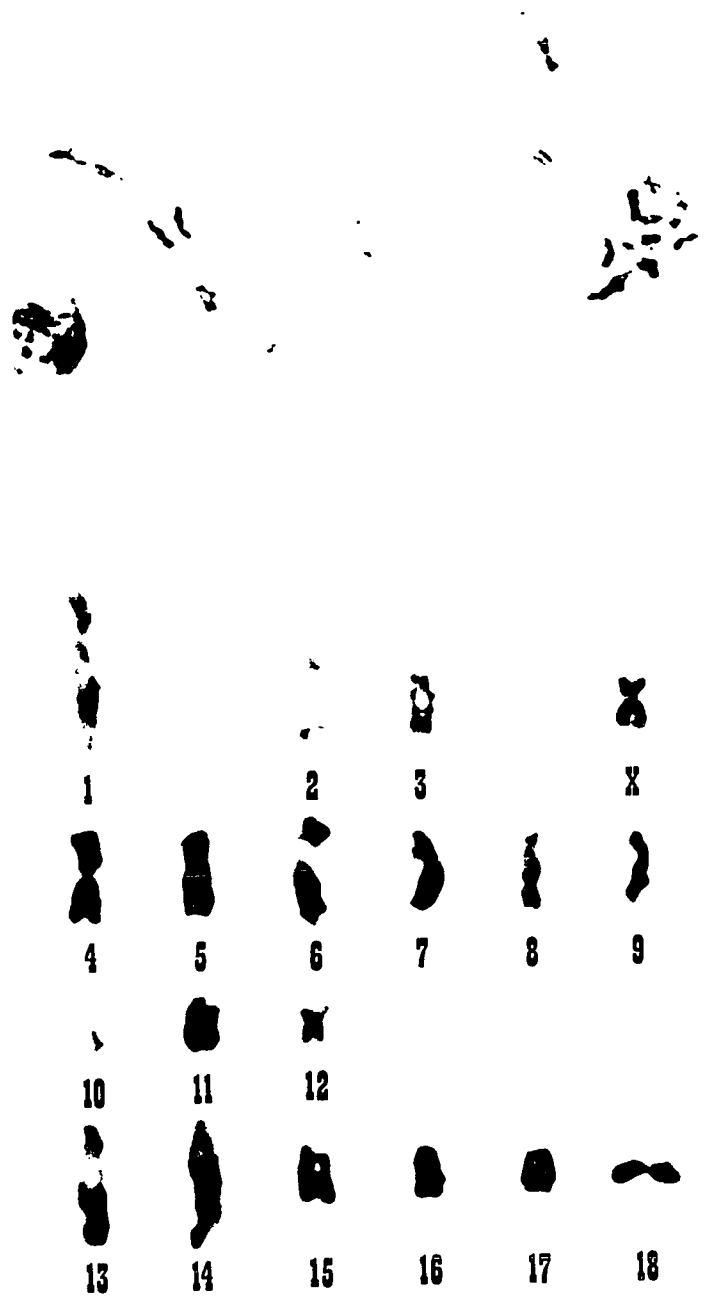


FIGURE 4.2.3.1

HAPLOID KARYOTYPE--BLASTOCYST 179-13



FIGURE 4.2.3.2

THREE METAPHASES ESTIMATED AS HAPLOID--BLASTOCYST 179-13

pronucleus from the ovum prior to combining with the female counterpart (Beatty, 1957).

Haploids were experimentally produced in mammalian ova, which survived only a few divisions (Beatty, 1957), and also to the morula stage (Kaufman, 1975; Tarkowski, 1975). More recently, Tarkowski (1976) described a technique of bisecting fertilized mouse ova and transferring them into pseudopregnant mice. Only six of these developed to the blastocyst stage by the fifth day of gestation. Three were haploid and three were haploid/diploid mosaics.

It may be noted that in mice the implantation of blastocysts takes place during the fifth to sixth day of gestation, which would be approximately the same stage at which the haploid blastocyst was observed in the present case. However, haploid/diploid mosaicism remained a possibility since only four metaphases were found, all being haploid.

4.2.4 Translocation

In blastocyst 189-7, sixteen metaphases with normal 2N chromosomes were observed while 17 metaphases contained only 37 chromosomes. Karyotypic analysis was performed on 4 cells with counts of 37 and revealed three chromosomes for which no homologue could be found in the complement (Figure 4.2.4.1). The largest of the three was a submetacentric chromosome similar in size to the first pair of chromosomes with a large secondary constriction in the long arms. The other two unpaired chromosomes were acrocentric and unequal in size. They corresponded in size and morphology to pairs 14-15 and 17-18. The remaining members of the complement corresponded to chromosomes of a normal pig karyotype. Gross measurements of the unpaired chromosomes were obtained and it appeared that the large submetacentric chromosome resulted from a Robertsonian translocation of the homologues to the unpaired acrocentrics. The result was a rearrangement with apparently minimal loss of chromatin material.

A similar karyotypic pattern was observed in adult pigs (McFee, et al., 1966; McFee and Banner, 1969). In a preliminary study of 36 European wild pigs, 26 had a 2N number of 36 while the remaining 10 had 2N of 37 (McFee, et al., 1966). The karyotype of those pigs with 37 chromosomes was similar to the 37-karyotype in the present study, i.e., a large unpaired submetacentric with two unpaired acrocentrics of unequal size. They concluded that the animals with 2N of 37 were hybrids resulting from matings of wild pigs (2N=36) and domestic pigs (2N=38). It was also shown that these hybrid animals were fertile. McFee and Banner (1969) cytogenetically analyzed blood and kidney cells

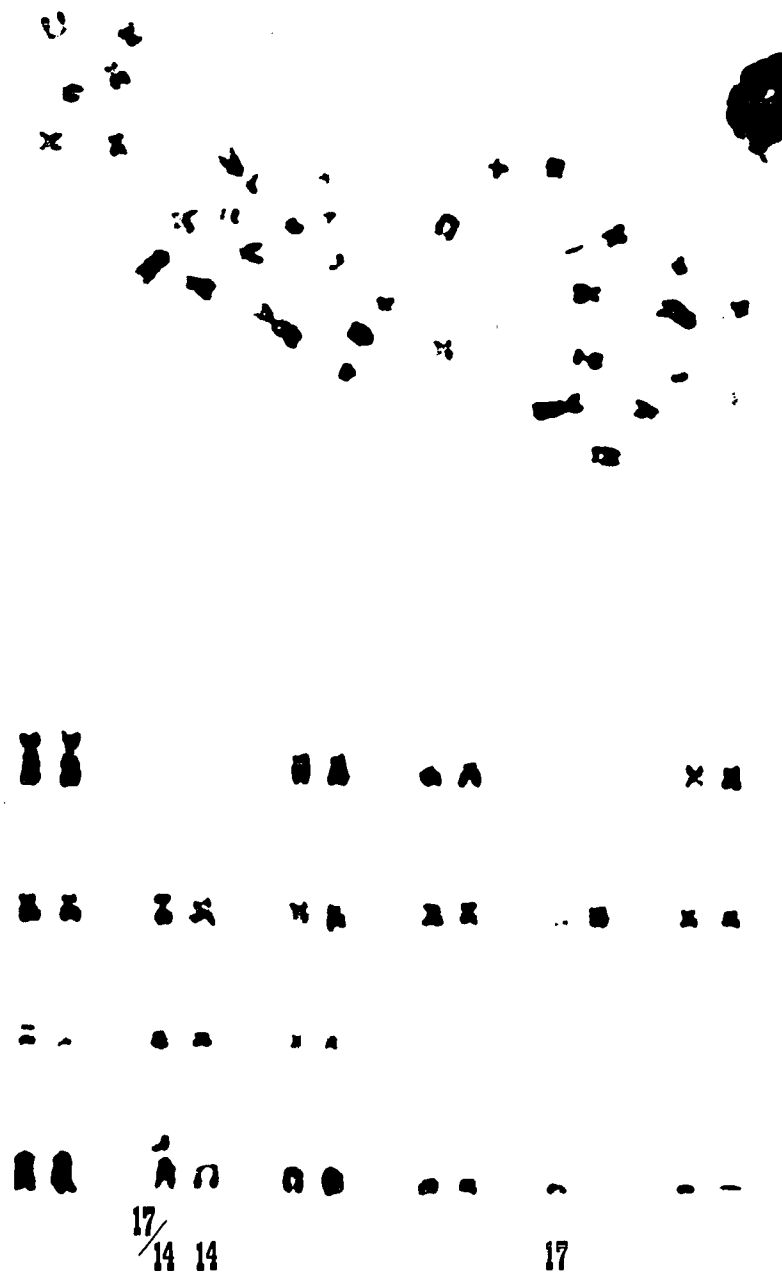


FIGURE 4.2.4.1

TRANSLOCATION KARYOTYPE--BLASTOCYST 189-7

of offspring derived from various matings of the wild, hybrid and domestic pigs. (For ease of reference, matings will be referred to by the 2N number of the animal.) All pigs farrowed from 36 x 38 matings were 37. The progeny derived from 36 x 37 and 37 x 38 yielded the parent numbers in approximately equal numbers. The interesting result of 37 x 37 matings were pigs with 36, 37 or 38 chromosomes in numbers approximating a 1:2:1 ratio. From these observations, McFee and Banner concluded that the three unpaired chromosomes in the 37-pigs act as a trivalent during meiosis. Thereby, the two unequal acrocentrics behaved as a unit during division, resulting in a 1:2:1 ratio of 36, 37 and 38 as observed. By mating wild pigs imported from Finland with domestic pigs in Sweden, a hybrid boar with 2N of 37 was produced (Gustavsson, et al., 1973). After conventional staining techniques, the karyotype was similar to that of the present study as well as McFee, et al. (1966, 1969). Using chromosome banding techniques, i.e., quinacine mustard staining and photoelectric recordings, Gustavsson established the origin of the three unpaired chromosomes from cultured lung cells. The large submetacentric chromosome was the result of a translocation of chromosomes 15 and 17, i.e., homologues of the other unpaired chromosomes.

Banding analysis was not performed on the translocation found in the present study nor those of McFee's study. Therefore, it was not possible to determine the origin of the unpaired metacentric. However, the 15/17 translocation described by Gustavsson was derived from a "hybrid" boar which suggests that the translocations described by McFee from hybrid pigs were most likely 15/17 translocations. The translocation

in the present study was observed in approximately equal numbers with the normal complement (16 normal, 17 were 37), hence, a mosaic condition. The chromosomes of the parents were normal. It seems likely that early in cleavage division a break and subsequent fusion occurred between the 14-15 and 17 acrocentric chromosomes in one cell, resulting in a Robertsonian translocation with minimal loss of chromatin. Visually, it appeared to be a 14/17 translocation; however, a 15/17 translocation was also a possibility.

4.2.5 Polymorphism

A suspected trisomic condition, $2N=39$, was investigated in 188-7. Karyotypic analysis revealed a medium-sized metacentric with a very large secondary constriction (Figure 4.2.5.1). As only one member of the pair demonstrated the constriction, it had originally been scored as two separate acrocentric chromosomes. Hansen-Melander and Melander (1974) described the sixth pair of chromosomes in pigs as often displaying a secondary constriction in the short arm at the centromere on one or both members. The chromosome in this study appeared to be a member of the sixth pair. Upon examination of the parents, the unique chromosome was also found in the mother, but not the father (Figure 4.2.5.2). Polymorphic chromosomes in banded preparations were utilized to explain the origin of selected abnormal karyotypes of spontaneous abortions in man (Jonasson, et al., 1972; Lauritsen, et al., 1972). However, the polymorphic chromosome observed in the present study represented only an interesting observation of a polymorphism shown to be inherited from the mother.

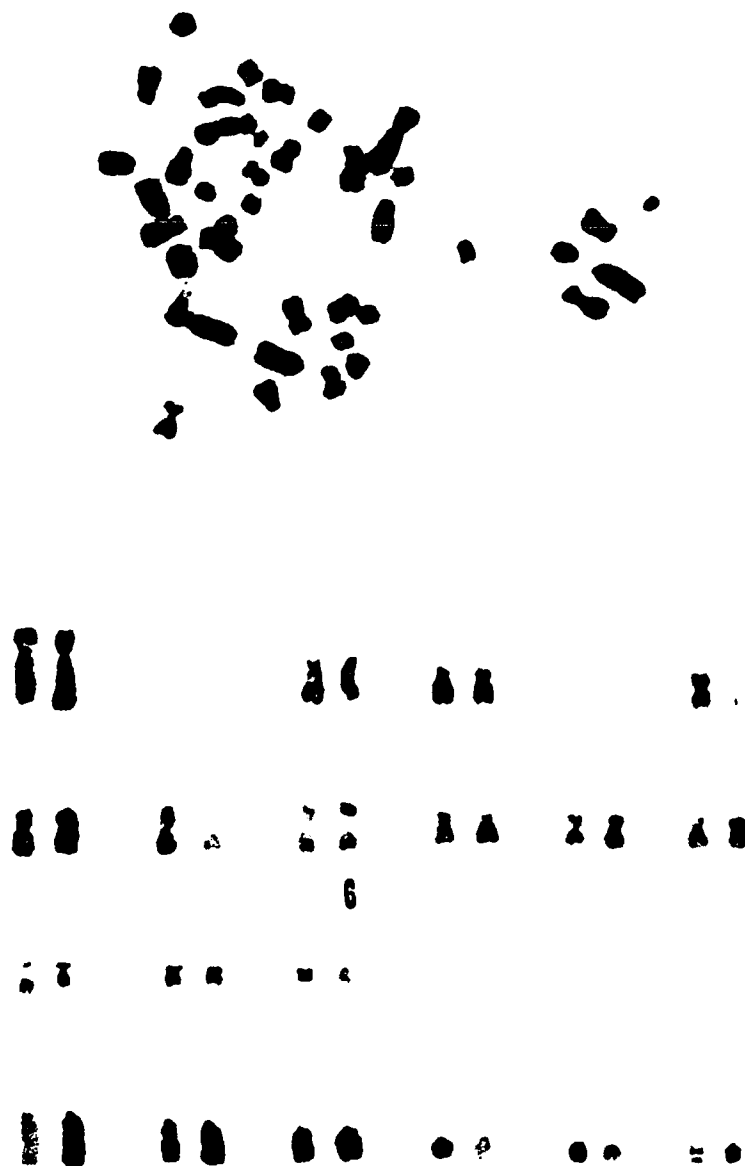


FIGURE 4.2.5.1

KARYOTYPE CONTAINING POLYMORPHIC CHROMOSOME NO. 6--BLASTOCYST 188-7

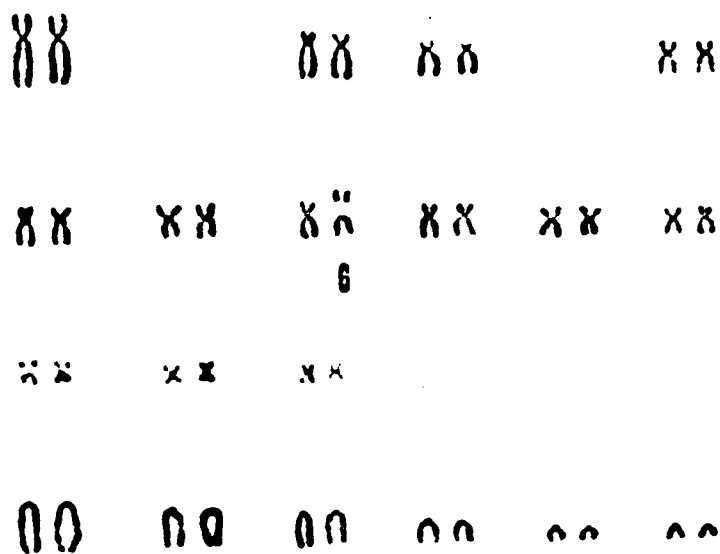
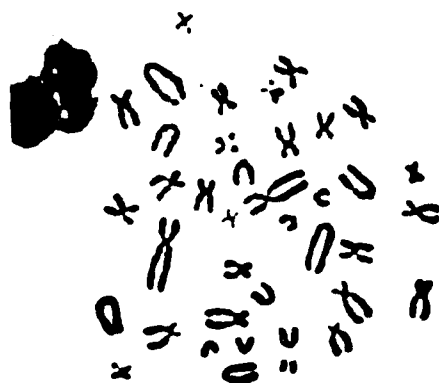


FIGURE 4.2.5.2

KARYOTYPE CONTAINING POLYMORPHIC CHROMOSOME NO. 6--GILT 188

4.3 Embryos

In the present study, 192 embryos were chromosomally analyzed from 20 gilts at 16-19 days of gestation. Unlike the blastocyst material, only 1.42 percent of 1,760 metaphases were polyploid (Table 4.3.1). The remaining 98.58 percent were diploid or near diploid. The frequencies were 6.42, 10.91, 16.82, 61.53, 2.61, 0.28, 0.57 and 0.85 for counts less than 36, 36, 37, 38, 39, 40, 3N and 4N, respectively. On an embryo as well as an animal basis, the frequencies of these counts remained similar. Differences among animals for each type of count were not found to be statistically significant at the 1% level. It was observed that the smallest metaphase contained 34 chromosomes while the largest contained 76.

Four chromosomally abnormal embryos were observed, namely, four monosomic 37/38 mosaics representing 2.08 percent of 192 embryos studied (Table 4.3.2). Embryo 131-8 was missing one chromosome from pair No. 13, the largest acrocentrics, in four karyotyped metaphases (Figure 4.3.1). No. 131-5 was missing one chromosome from pair No. 10, the small metacentrics characteristically displaying a large secondary constriction (Figure 4.3.2). This monosomy was observed in three karyotypes. Embryo 157-6 was missing one member from the submetacentric pair No. 2 in three karyotypes while the fourth embryo, 182-1, lacked one C-group chromosome in three karyotyped metaphases (Figures 4.3.3 and 4.3.4). It was not possible to identify each chromosome pair within the C-group, however, the morphology of the individual chromosome present suggested the missing homologue could be from pair No. 8.

Three monosomic mosaics were also observed in 202 hamster embryos produced by delayed matings which represented 1.49 percent of the sample

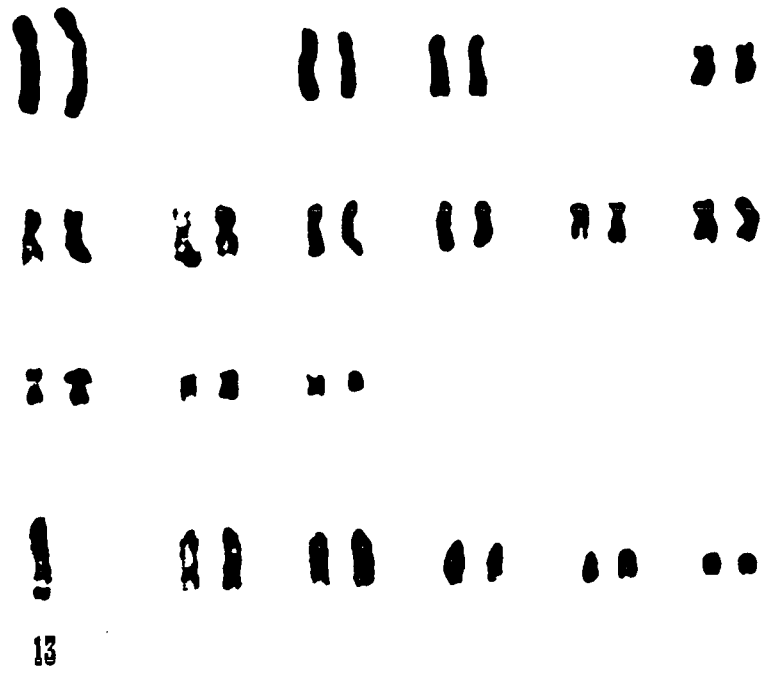


FIGURE 4.3.1

KARYOTYPE OF MONOSOMY 13--EMBRYO 131-8

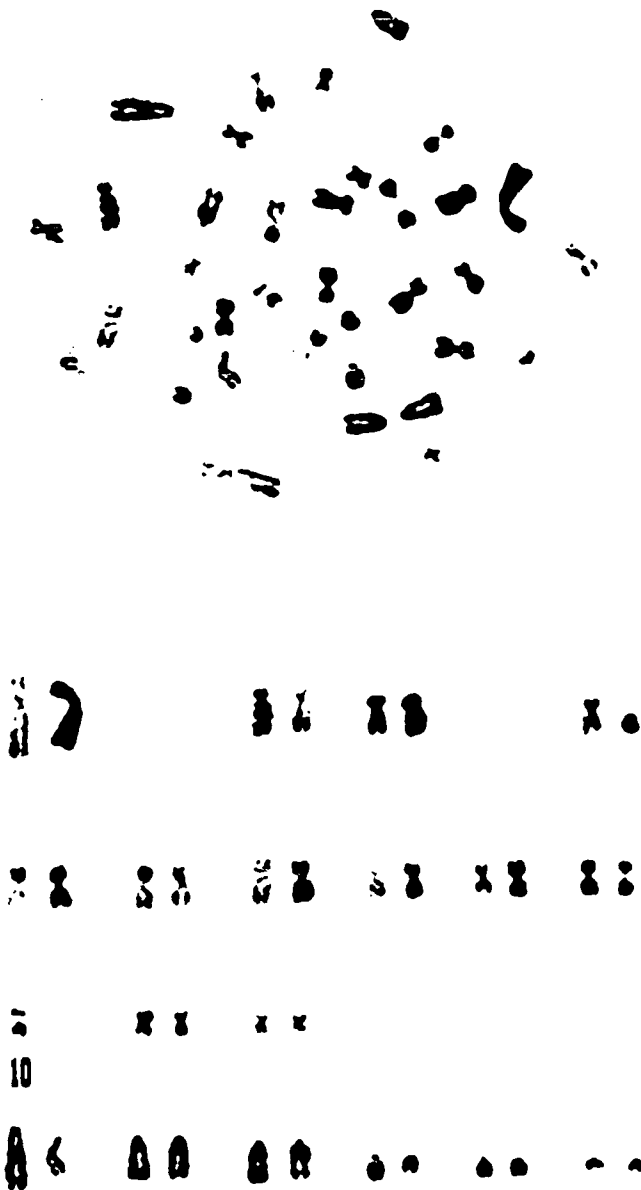


FIGURE 4.3.2

KARYOTYPE OF MONOSOMY 10--EMBRYO 131-5

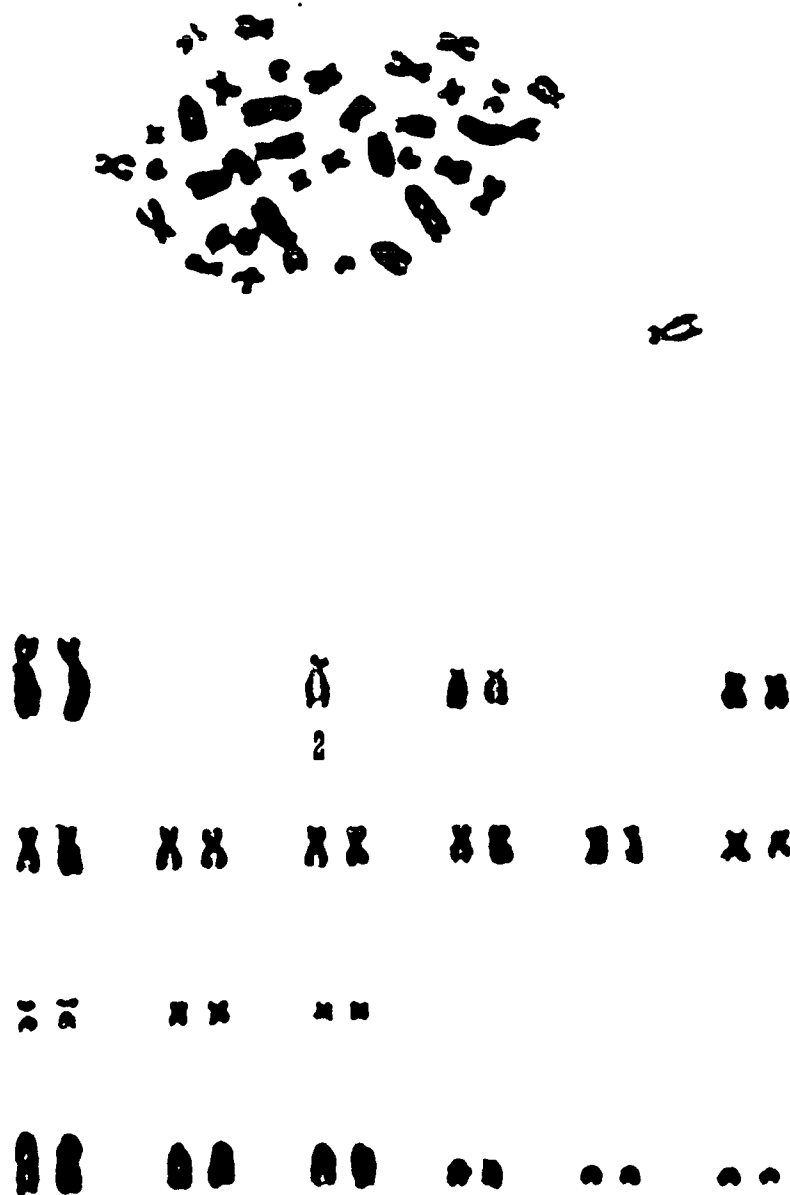


FIGURE 4.3.3

KARYOTYPE OF MONOSOMY 2--EMBRYO 157-6

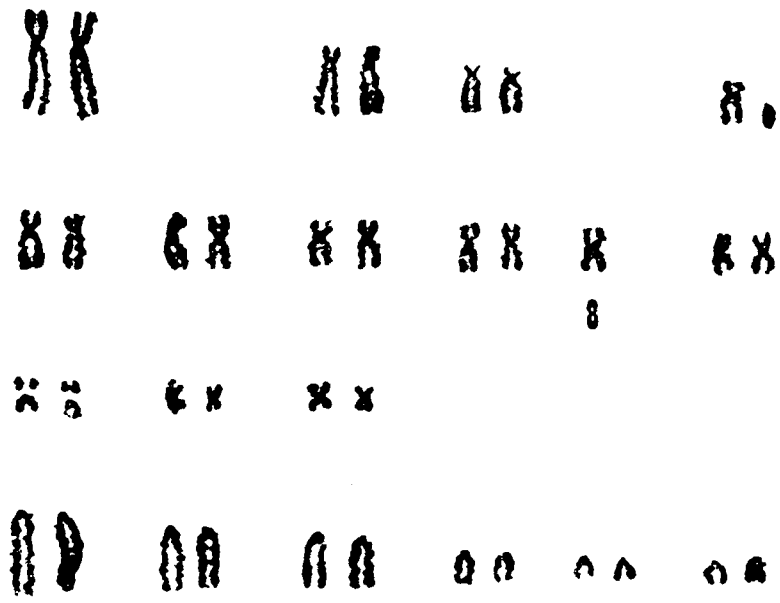
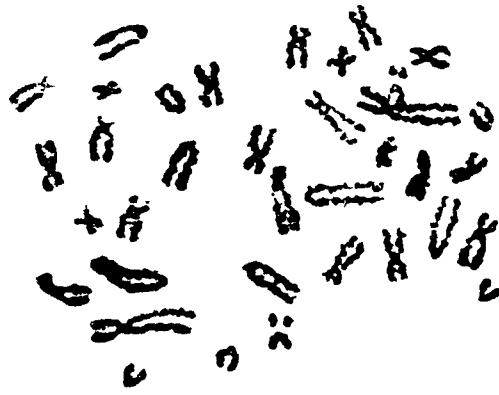


FIGURE 4.3.4

KARYOTYPE OF MONOSOMY 8--EMBRYO 182-1

TABLE 4.3.1
 FREQUENCIES OF CHROMOSOME COUNTS
 IN EMBRYOS

Chromosome Count	By Metaphase	By Embryo	By Animal*
36	.0642	.0922	.0698
36	.1091	.1014	.1058
37	.1682	.1622	.1649
38	.6153	.6106	.6178
39	.0261	.0198	.0259
40	.0028	.0012	.0026
3N	.0057	.0043	.0052
4N	.0085	.0069	.0085

*A frequency for each category listed was computed for each animal. The table contains the average frequency for all animals.

TABLE 4.3.2
 CHROMOSOMALLY ABNORMAL EMBRYOS

Embryo No.	Gestational Age	Chromosome Counts		Sex Chromosomes	Missing Chromosomes	Karyotypes
		37	38			
131-8	16 days	7	2	XX	#13	4
131-5	16 days	7	5	XY	#10	3
157-6	17 days	4	3	XX	#2	3
182-1	17 days	5	4	XY	C-group (#8?)	3

(Yamamoto and Ingalls, 1972). In rats, delayed ovulation produced 1.03 percent monosomic mosaics in 11 day embryos (390) while 0.24 percent was observed in 410 control embryos (Butcher and Fugo, 1967). In rabbit blastocysts, Fechheimer and Beatty (1974) reported one monosomic mosaic representing 0.22 percent while Shaver and Carr (1969) found one monosomic mosaic (0.47 percent) in 213 blastocysts produced by delayed matings.

In an embryonic pig, Ruzicska (1968) reported a double trisomy for the smallest telocentric chromosomes, no. 17 and 18. Although no gestational age was given, the abnormal complement was obtained from embryonic kidney cells. Smith and Marlowe (1971) reported one anomaly from 76 pig embryos at 25 days gestation, i.e. a 37/38 mosaic representing 1.32 percent of the sample. They observed 13 diploid metaphases and 13 metaphases missing one of the smallest metacentrics, no. 12. Based on these findings compared with the number of chromosome anomalies found in preimplantation stages, they concluded that most cytogenetically abnormal embryos rarely survive implantation. However, two Duroc boars were reported as chromosomally abnormal mosaics (Vogt, et al., 1974). One boar was a 37/38 mosaic missing the smallest telocentric chromosome, no. 18, in 7.7% of the cells examined. The other boar, a full-brother of the first, was a 37/38/39 mosaic. Both the missing and the extra chromosomes were no. 18's. Although both boars demonstrated reduced fertility, three daughters were available for cytogenetic analysis. Two daughters contained 38,XX/38,XX+ fragment chromosome cell lines and had small first litters. Henricson and Backstrom (1964) also reported

a Landrace boar with a chromosome abnormality associated with lowered fertility. The chromosome abnormality was a translocation which was later described as 38,Xy,t(11p+; 15q-) using banding analysis (Hageltorn, et al., 1973). Akesson and Henricson (1972) reported inherited translocation and reduced fertility in a boar which was the son of the boar just described. They analyzed 113 embryos and 111 piglets derived from matings with this son. Approximately 50 percent of the embryos and piglets demonstrated the translocation. However, 11 percent of the embryos contained an unbalanced karyotype while none was found in the piglets. Analysis of preimplantation blastocysts was unsuccessful. They concluded that defect karyotypes were lethal at the embryonic stage. Translocation heterozygosity was also reported in a stillborn piglet (Hansen-Melander and Melander, 1970a).

Based on the observation that monosomic mosaicism was observed in adult pigs (Vogt, et al., 1974), it was possible that the four monosomic mosaics in the present study could have survived. Translocation heterozygosity was also shown to occur in embryos and adult pigs (Akesson and Henricson, 1972). Chromosome analysis was performed on the adult pigs mated for the present study; however, all contained normal diploid chromosomes.

4.4 Adults

Chromosome analysis was performed on the adult pigs mated for the present study, i.e., fifty-eight females and twenty-seven males. Ten well-spread metaphases were counted from each individual with the exception of four females in which five metaphases each were analyzed. All adult pigs in the present study contained diploid chromosomes which appeared normal. However, an obvious chromosome polymorphism was observed in one animal (188). For description, see Section 4.2.5.

5. GENERAL DISCUSSION

Embryonic losses during the first few weeks of pregnancy in man remain unknown. Cytogenetic analysis of conceptuses from a suitable animal model could provide valuable information. Pigs are large mammals which can be used in significantly high numbers for an embryonic study. They also demonstrate many physiological similarities to man. Blastocysts and embryos can be obtained at any gestational age for comparison and as a result of multiple ovulations, a large number of samples are available for analysis. Direct chromosome preparations can be made from both blastocysts and embryos, unlike human spontaneous abortions which must be cultured for chromosome analysis. Multiple ovulations also provide the opportunity for comparisons between blastocysts within an individual. Such comparisons are limited in human material to twin studies which usually represent small sample sizes.

The animal model in the present study provided unique observations of polyploidy in blastocysts, including large numbers of "high-order" polyploids in cultured blastocysts. Although similar observations have not been made in human material, the value of swine as an animal model for early embryonic development remains to be fully explored.

Fifty percent of the preimplantation blastocysts in the present study were polyploid mosaics. This mosaicism was most likely due to chromosome preparation of trophoblast giant cells. In most cytogenetic studies of preimplantation blastocysts, chromosome preparations were made from a mixed population of embryonic disc cells and trophoblast giant cells. It is therefore possible that polyploids reported as

"abnormal" in other studies may be due, in part, to trophoblast giant cells. Any blastocyst which was abnormal due to a polyploid condition, such as pure triploidy, would be extremely difficult to recognize in this study due to the polyploid mosaicism. Therefore, the frequency of blastocysts demonstrating obvious chromosome abnormalities in the present study (1.48 percent) most likely represented an under estimate of spontaneously occurring abnormalities. A direct comparison to anomalies observed in human spontaneous abortions would not be appropriate. The frequency of pig embryos demonstrating obvious chromosome abnormalities was 2.08 percent which was similar to that reported by Smith and Marlowe (1971). They observed one anomaly in 76 pig embryos (1.32 percent). Several investigators have suggested that chromosomally abnormal conceptuses rarely survive implantation (Bomse-Helmreich, 1965, 1970; Smith and Marlowe, 1971). If one accepts this hypothesis and it is assumed that the abnormal embryo frequency in the present study and that of Smith and Marlowe (1971) represents a good estimate of post-implantation aneuploidy, then the frequency of chromosomally abnormal blastocysts should be greater than 2 percent. Again it seems most likely that the frequency of abnormal blastocysts found in the present study (1.48 percent) provided an under estimate of spontaneously occurring anomalies.

The pooled estimate of mortality for blastocysts was 12.8 percent and that for embryos was 31.8 percent. These data suggest that a significant number of conceptuses were lost during the implantation process. However, the cytogenetic data did not support the hypothesis

that the increased mortality was due to chromosomally abnormal blastocysts failing to survive implantation.

In the present study, the frequency of polyploid metaphases was reported in addition to the frequency of normals. This information was valuable in assessing the chromosome makeup of the sample. Only three other studies reported the number of abnormal metaphases found (Martin and Shaver, 1972; Shaver and Carr, 1967; Vickers, 1969). McFeely (1967) reported only the number of cells analyzed and those with the modal chromosome number. If it was assumed that the difference between these two numbers represented cells found abnormal, then he based his findings on very few metaphases. For example, he reported 154 cells analyzed in one animal with 147 demonstrating the modal chromosome number. The difference of seven cells would then have been used to describe a triploid XXX, a tetraploid XXXX, and a diploid XX/triploid XXX mosaic. Assuming that all seven cells were analyzable, an average of 2.33 cells were used to ascertain each abnormality. It seemed unlikely that such abnormalities could be confirmed with so few observations.

In the present study, karyotypic analysis was not possible on the large polyploids to determine if complete multiple sets of chromosomes were present. Karyotypes of two tetraploid metaphases revealed four members of each chromosome (Figures 5.1 and 5.2). Also, in several octoploid metaphases, eight characteristically large No. 1 chromosomes were counted demonstrating eight multiple copies of at least one chromosome of the complement.

The animal model in the present study provided unique observations

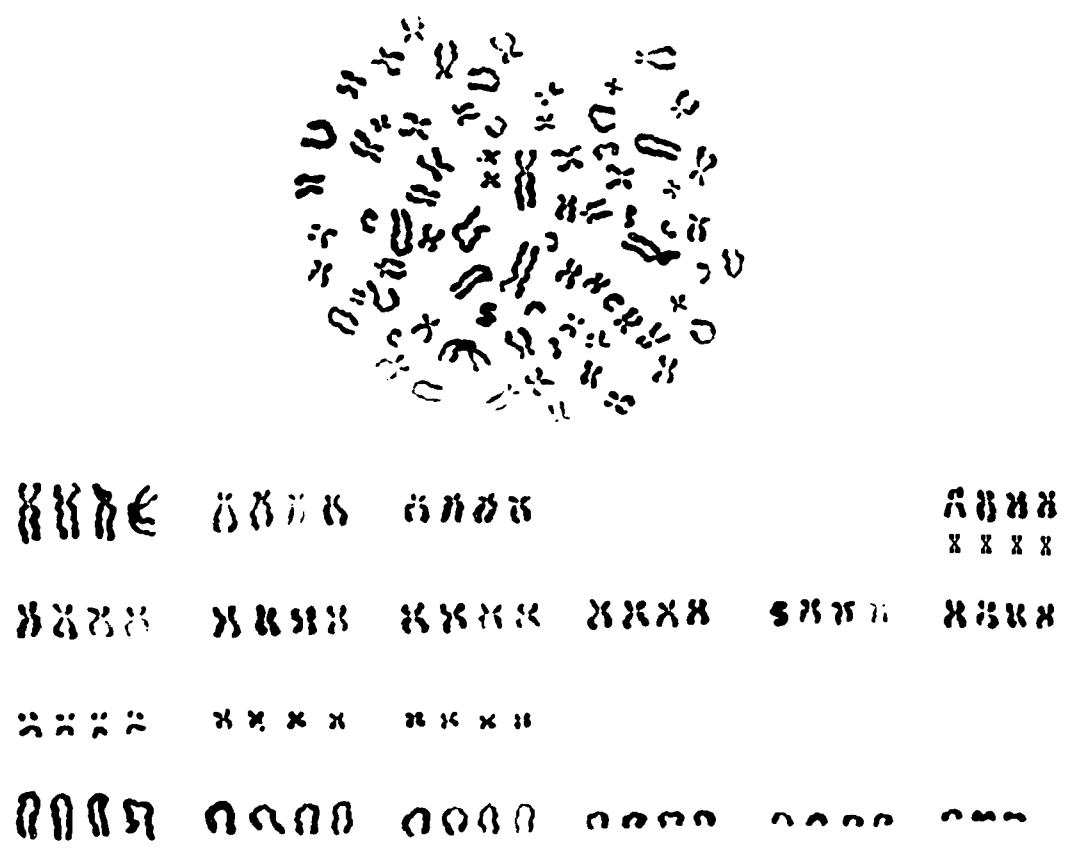


FIGURE 5.1
TETRAPLOID KARYOTYPE FROM ANIMAL NO. 167-7

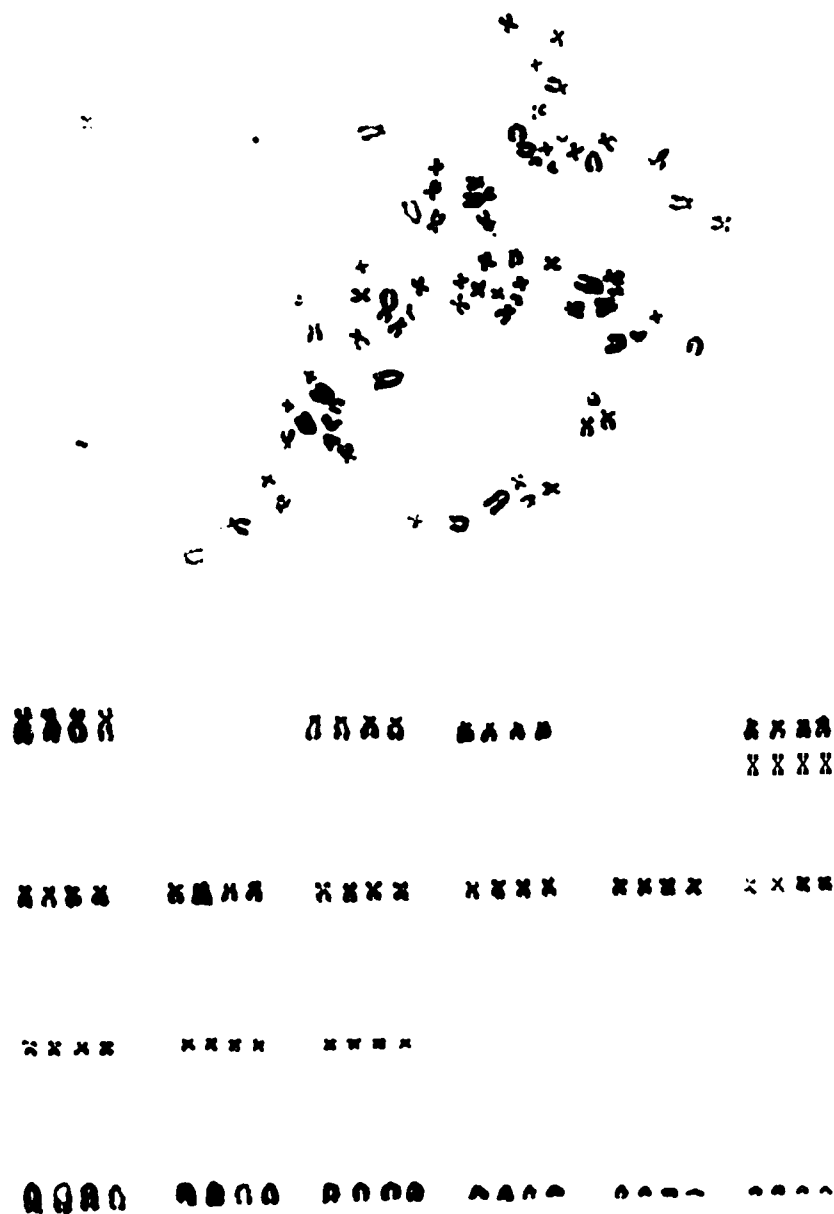


FIGURE 5.2

TETRAPLOID KARYOTYPE FROM ANIMAL NO. 202-3

of polyploidy. The polyploid mosaics observed most likely represented a "normal" condition resulting from trophoblast giant cells rather than an "abnormal" embryonic disc. The value of this animal model could be more fully explored by the following research:

- 1) Dissect embryonic disc cells from trophoblast giant cells and cytogenetically analyze each separately. Polyploid frequencies could then be obtained from each cell population.

- 2) Culture the cells from embryonic disc and trophoblast for various periods of time to test the hypothesis of culture-induced cell and nuclear fusion.

- 3) Culture embryo cells to determine if increased polyploidy or presence of large polyploids could be detected.

- 4) Investigate blastocysts of earlier gestational ages for chromosome abnormalities. Also, analyze the chromosomes of the trophoblast at the earliest stage of trophoblast formation.

APPENDIX I

RECOVERY PROCEDURE FOR BLASTOCYSTS AND EMBRYOS

1. Kill the animal by completely bleeding out via an anterior vena cava puncture.

2. Eviscerate the animal within 20 minutes of bleeding out.

3. Remove complete reproductive tract. The uterus of the swine is of the bicornate-type with a right and left uterine horn. At the distal end, each uterine horn reduces in diameter to become the fallopian tube which terminates in a thin umbrella-shaped membrane surrounding the major part of the ovary to facilitate the recovery of the ova once they are expelled from the follicle. At the proximal end, there is a uterine body which is a cavity common to both right and left uterine horns, thus allowing migration of developing blastocysts from one side to the other. Figure I.1 is a photograph of the reproductive tract from a non-pregnant gilt. Note the left ovary demonstrated recent ovulations. Figure I.2 is a photograph of the reproductive tract from a gilt at 11 days gestation. The uterine horns become extensively thickened and extended preparatory for the developing embryo.

4. Examine the ovaries to determine into which of three categories they should be classified:

- a) ovaries were of normal size with numerous pink-colored corpora lutea, suggesting that fertilization occurred on or near the scheduled time.

- b) ovaries were of normal size with numerous, small, pale corpora albicans. This observation suggests that ovulation occurred early and insemination was too late to fertilize the ova.

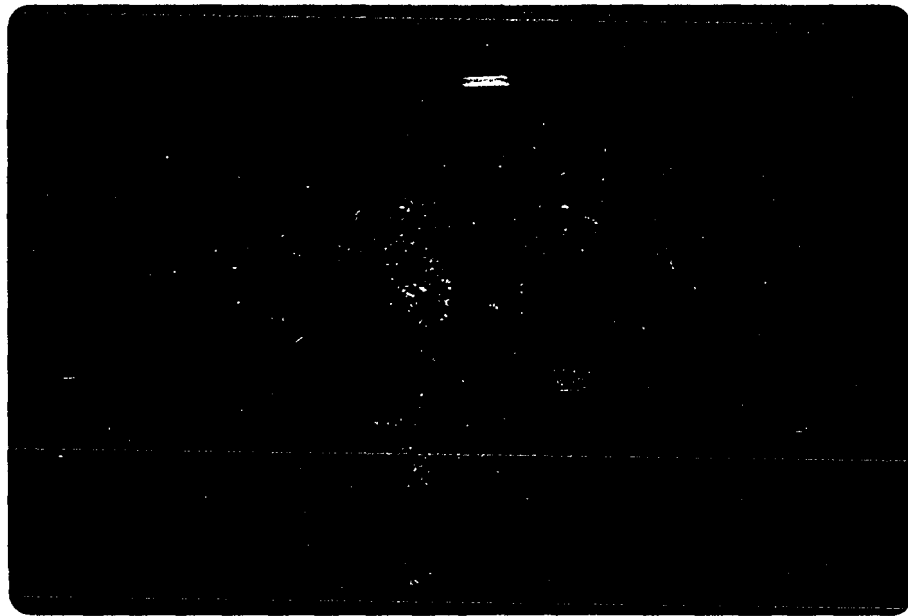


FIGURE I.1
REPRODUCTIVE TRACT FROM A NON-PREGNANT GILT



FIGURE I.2

REPRODUCTIVE TRACT FROM A GILT AT 11 DAYS GESTATION

c) ovaries were small to normal size with either no ovulation sites or bloody follicles resulting from recent ovulation. In this case, insemination was performed too early to fertilize the ova which had yet to be shed.

5. Count and record the number of corpora lutea of those tracts which appear to be fertilized.

6. Cut the mesometrium from each uterine horn and cut the cervix free. The result is a V-shaped organ with the uterine horns extended, an ovary at each end, and the body of the uterus as the common point.

7. Recovery of blastocysts:

a) clamp the distal end of the left uterine horn near the beginning of the fallopian tube with a hemastat.

b) cut the fallopian tube free and discard.

c) cut a small opening about 1 cm. in size on the mesometrial surface of the uterus about one-third the length from the distal end.

d) drape the section of uterus to be flushed over a ring stand in a vertical position and insert a glass funnel in the cut opening.

e) pour 100 ml. Hanks' solution (prewarmed to 37°C) slowly into the funnel and let stand for about two minutes.

f) remove hemastat from distal end and allow blastocysts to flow into a beaker.

g) pour an additional 100 ml. Hanks' solution into the funnel, directly through the vertical section of uterus into the beaker. Thus, each section of uterus is rinsed twice.

h) flush the next section of uterus (middle one-third) in the same manner.

i) rinse the remaining section of uterus (proximal one-third) in the same manner.

j) record the number of blastocysts recovered from the left horn of the uterus.

k) process the right uterine horn using the same procedure (one-third intervals) and record number of blastocysts recovered.

l) rinse the body of the uterus separately and record appropriately.

m) record the approximate size of the blastocysts by placing a metric ruler adjacent to the petri dish containing the specimens. The diameter of round blastocysts or the length of elongated blastocysts are recorded.

8. Recovery of embryos:

a) cut a small opening about 1 cm. in size on the mesometrial surface of the uterus about 10 cm. from the distal end.

b) drape the section of uterus to be flushed over a ring stand in a vertical position and insert a glass funnel in the cut opening.

c) pour 25 ml. Hanks' solution (prewarmed to 37°C) slowly into the funnel. The solution forms a bulge at the top of the uterine section.

d) force the bulge to the bottom of the uterine section by gently squeezing with the hand, thus forcing the contents out into a 60 mm. petri dish.

e) pour an additional 25 ml. Hanks' solution into the funnel

directly through the vertical section of uterus into the petri dish. Thus, each section of uterus is rinsed twice. The result is a tangled mass of embryonic membranes which may or may not contain embryos. Although the embryonic membranes are found throughout the length of the uterus, the embryos are evenly spaced in each uterine horn.

f) dissect each embryo from the attached membranes. It is important to cut the umbilical stalk as close to the embryo as possible, thus preventing these cells from being processed with the embryo.

g) continue rinsing 10 cm. sections of uterus in the same manner until the left uterine horn is completely flushed and all embryos are dissected out and the number recorded.

h) process the right uterine horn using the same procedure (10 cm. intervals) and record the number of embryos recovered.

i) record the approximate size of the embryos by placing a metric ruler adjacent to the petri dish containing the specimens.

APPENDIX II-A
METHOD OF DIRECT CHROMOSOME PREPARATION
OF BLASTOCYSTS AND EMBRYOS

1. Place blastocysts and embryos in Hanks' solution (prewarmed to 37°C) upon removal from the uterus until all are removed.

2. Incubate specimens at 37°C in 1 ml. of MEM culture media (supplemented with 10% fetal calf serum) containing 0.1 ug/ml colcemid for 1½ hours. A 2 ml. conical tube was used during the incubation.

3. Centrifuge at 1,000 rpm. for 5 minutes.

4. Discard supernatant.

5. Add 0.2 ml. fetal calf serum, then add 1 ml. distilled water.

Mix gently with cells. Note: individual pipettes must be used for each specimen throughout the entire procedure to avoid cross contamination of cells.

6. Incubate at 37°C for 20 minutes.

7. Centrifuge at 800 rpm. for 5 minutes.

8. Pipette off all but 0.1 ml. supernatant. Do not disrupt the cell button.

9. Add 1 ml. of fresh fixative (3:1 methanol to glacial acetic acid) dropwise down the inside of the tube. Do not disrupt the cell button. Let stand at room temperature for 10 minutes.

10. Gently resuspend the cells. Let stand at room temperature for 10 minutes.

11. Centrifuge at 600 rpm. for 5 minutes.

12. Discard supernatant, being careful not to disturb the cells.

13. Add 1 ml. of fixative. Gently resuspend the cells. Let stand at room temperature for 10 minutes.
14. Centrifuge at 600 rpm. for 5 minutes.
15. Discard supernatant.
16. Add 1 ml. of fixative. Gently resuspend the cells. Let stand at room temperature for 10 minutes.
17. Centrifuge at 400 rpm. for 5 minutes.
18. Discard supernatant.
19. Add enough fixative to yield a moderately dense cell suspension, usually 0.1 ml. or thereabouts.
20. Clean slides by dipping in 95% ETOH and wipe clean with a paper towel.
21. Drop cell suspension from a height of about 10 inches onto the clean slide.
22. Dry slides quickly by using a hair dryer to blow warm air across the surface at a distance of about 12 inches.
23. Stain slides in a 4% Giemsa stain (Gurrs Improved R66) for 5 minutes. Rinse slides in Gurr buffer for 5 seconds and finally rinse in distilled water for 10 seconds.
24. Air dry slides overnight.
25. Mount coverslips on slides with permount.

APPENDIX II-B
METHOD OF CHROMOSOME PREPARATION OF BLASTOCYSTS
AFTER 24 HOUR CULTURE

1. Place blastocysts in Hanks' solution (prewarmed to 37°C) upon removal from the uterus until all are removed.
2. Incubate specimens at 37°C in 2 ml. MEM culture media supplemented with 10% fetal calf serum for 23 hours.
3. Transfer blastocysts to 1 ml. MEM culture media (supplemented with 10% fetal calf serum) containing 0.1 ug/ml. colcemid and continue incubation for 1½ hours.
4. Harvest cells using the method of direct chromosome preparation described in Appendix II-A.

APPENDIX III
METHOD OF CHROMOSOME PREPARATION OF
PERIPHERAL BLOOD CULTURES

1. Obtain venous blood by ear puncture from vessels on the dorsal surface of the ear.
 - a) tie animal to the side of the enclosure using a nose halter.
 - b) swab the dorsal surface of the ear liberally with alcohol to cleanse the area.
 - c) clamp the ear near the head using the left hand, thus reducing the blood flow through the veins.
 - d) draw 5 ml. of blood from the most pronounced vein using a sterile 10 ml. syringe equipped with a 20 gauge needle.
 - e) remove the needle from the syringe.
 - f) transfer the blood to a sterile screw-cap test tube containing 1,000 units of sodium heparin to prevent clotting.
2. Allow blood sample to separate by gravity at room temperature for 2 hours.
3. Place 1 ml. of plasma (containing leukocytes) in 7 ml. MEM culture media supplemented with spinner salts and 10% fetal calf serum. Swirl gently.
4. Incubate at 37°C for 72 hours. The cultures were gently swirled each day during the culture period and the pH was maintained at approximately 7.2.
5. Add colcemid (0.1 ug/ml of media) 1½ hours prior to harvesting.
6. Transfer the culture to a graduated conical centrifuge tube.
7. Centrifuge at 1,000 rpm. for 5 minutes.

8. Discard supernatant.
9. Add 5 ml. of Hanks' solution prewarmed to 37°C and mix gently.
10. Centrifuge at 1,000 rpm for 5 minutes.
11. Discard supernatant.
12. Add 4 ml. of 0.17% NaCl hypotonic solution, resuspend cells gently and incubate at 37°C for 10 minutes.
13. Add 2 ml. of 0.7% KCl, resuspend gently and incubate at 37°C for 10 minutes.
14. Centrifuge at 800 rpm for 5 minutes.
15. Pipette off all but 0.2 ml. supernatant.
16. Gently resuspend the cells in the supernatant.
17. Add 4 ml. of fixative (3:1 methanol to glacial acetic acid) dropwise down the inside of the tube. Gently mix and disperse any cell clumps. Note: the fixative should be made up fresh for each harvest.
18. Let stand at room temperature for 10 minutes.
19. Centrifuge at 600 rpm. for 5 minutes.
20. Discard supernatant, being careful not to disturb the cells.
21. Add 4 ml. of fixative. Gently resuspend the cells. Let stand at room temperature for 10 minutes.
22. Centrifuge at 600 rpm. for 5 minutes.
23. Discard supernatant.
24. Add 2 ml. of fixative. Gently resuspend the cells. Let stand at room temperature for 5 minutes.
25. Centrifuge at 400 rpm. for 5 minutes.
26. Discard supernatant.

27. Add enough fixative to yield a moderately dense cell suspension, usually 0.2 ml. or thereabouts.
28. Clean slides by dipping in 95% ETOH and wipe clean with a paper towel.
29. Drop cell suspension from a height of about 6 inches directly onto the clean slide.
30. Dry slides quickly by using a hair dryer to blow warm air across the surface at a distance of about 12 inches.
31. Stain slides in a 2% Giemsa stain (Gurrs Improved R66) for 5 minutes. Rinse slides in Gurr buffer for 5 seconds and finally rinse in distilled water for 10 seconds.
32. Air dry slides overnight.
33. Mount coverslips on slides with permount.

APPENDIX IV

EXAMPLES OF TABULATED CHROMOSOME COUNTS BY ANIMAL

ANIMAL NO.	SAMPLE BLASTOCYST					TOTAL
SAMPLE NO.	CHROMOSOME COUNT	COUNTED	ESTIMATED	CLUMPED	SUBTOTAL	
1	35	0	2	0	2	
	36	0	2	0	2	
	37	1	0	0	1	
	38	3	0	7	10	
	39	0	1	0	1	16
2	38	2	0	5	7	7
3	29	0	1	0	1	
	33	0	1	0	1	
	34	1	0	0	1	
	36	0	1	0	1	
	37	1	2	0	3	
	38	3	0	7	10	17
5	30	0	3	0	3	
	32	0	1	0	1	
	36	0	1	0	1	
	38	0	1	13	14	
	50	0	1	0	1	
	76	0	0	1	1	21
6	38	0	0	2	2	2

ANIMAL NO. 34

SAMPLE BLASTOCYST

SAMPLE CHROMOSOME COUNTED ESTIMATED CLUMPED SUBTOTAL TOTAL
 NO. COUNT

7 *	12	1	0	0	1	
	13	2	0	0	2	
	17	1	0	0	1	
	27	1	0	0	1	
	31	0	1	0	1	
	33	3	0	0	3	
	34	1	1	0	2	
	35	2	2	0	4	
	36	2	0	0	2	
	37	1	0	0	1	
	38	11	1	1	13	
	47	1	0	0	1	
	66	1	0	0	1	
	72	0	2	0	2	
	73	0	3	0	3	
	74	0	1	0	1	
	75	0	1	0	1	
	76	0	0	2	2	
	78	1	0	0	1	
	79	0	1	0	1	
	91	0	1	0	1	
	115	0	1	0	1	
	131	0	1	0	1	
	133	0	0	1	1	
	136	0	1	0	1	
	152	1	0	0	1	
	190	0	1	0	1	
	228	0	1	0	1	
	304	0	0	2	2	
	608	0	0	2	2	56
8 *	20	1	0	0	1	
	21	1	0	0	1	
	23	1	0	0	1	
	24	0	1	0	1	
	38	2	0	2	4	
	40	1	0	0	1	
	57	0	0	1	1	
	107	0	1	0	1	
	135	0	1	0	1	
	197	0	1	0	1	
	212	0	1	0	1	
	220	0	1	0	1	
	305	0	1	0	1	
	368	0	1	0	1	
	608	0	0	1	1	18

ANIMAL NO. 34

SAMPLE BLASTOCYST

SAMPLE NO.	CHROMOSOME COUNT	COUNTED	ESTIMATED	CLUMPED	SUBTOTAL	TOTAL
9 *	7	1	0	0	1	
	9	1	0	0	1	
	16	1	0	0	1	
	24	2	0	0	2	
	25	1	0	0	1	
	34	1	0	0	1	
	38	0	0	1	1	
	40	1	0	0	1	
	46	0	1	0	1	
	56	0	1	0	1	
	61	1	0	0	1	
	67	1	0	0	1	
	72	1	0	0	1	
	95	0	1	0	1	
	153	0	1	0	1	
	160	0	1	0	1	
	256	0	1	0	1	
	400	0	1	0	1	
	608	0	0	1	1	20

ANIMAL NO. 34

SAMPLE BLASTOCYST

SAMPLE CHROMOSOME COUNTED ESTIMATED CLUMPED SUBTOTAL TOTAL
 NO. COUNT

10 *	5	2	0	0	2	
	8	1	0	0	1	
	11	2	0	0	2	
	14	1	0	0	1	
	16	2	0	0	2	
	17	2	0	0	2	
	18	1	0	0	1	
	19	1	1	0	2	
	20	2	1	0	3	
	21	1	0	0	1	
	28	1	0	0	1	
	31	0	1	0	1	
	32	1	0	0	1	
	34	1	0	0	1	
	36	0	1	0	1	
	37	2	1	0	3	
	38	1	0	5	6	
	39	1	0	0	1	
	44	2	0	0	2	
	52	1	0	0	1	
	57	1	0	0	1	
	58	0	1	0	1	
	62	0	1	0	1	
	68	1	0	0	1	
	70	0	1	0	1	
	73	1	0	0	1	
	74	1	0	0	1	
	75	1	0	0	1	
	101	0	1	0	1	
	110	0	1	0	1	
	115	1	0	0	1	
	130	0	1	0	1	
	152	1	0	2	3	
	170	0	1	0	1	
	245	0	1	0	1	
	250	0	1	0	1	
	264	0	1	0	1	
	306	0	1	0	1	
	330	0	1	0	1	
	400	0	1	0	1	
	600	0	1	0	1	
	608	0	0	2	2	60

ANIMAL NO. 34

SAMPLE PLASTOCOYST

SAMPLE CHROMOSOME COUNTED ESTIMATED CLUMPED SUITOTAL TOTAL
 NO. COUNT

11 #	12	1	0	0	1
	13	1	0	0	1
	15	1	0	0	1
	16	1	0	0	1
	17	1	0	0	1
	18	1	0	0	1
	19	1	0	0	1
	21	3	0	0	3
	23	1	0	0	1
	26	1	0	0	1
	29	1	0	0	1
	30	0	1	0	1
	36	1	0	0	1
	37	1	0	0	1
	38	2	0	1	3
	46	1	0	0	1
	49	1	0	0	1
	52	1	0	0	1
	62	0	1	0	1
	76	1	0	0	1
	86	1	0	0	1
	90	1	0	0	1
	178	0	1	0	1
	200	0	2	0	2
	224	0	1	0	1
	274	0	1	0	1
	300	0	1	0	1
	304	0	0	2	2
	350	0	1	0	1
	400	0	1	0	1
	430	0	1	0	1
	440	0	1	0	1
	538	1	0	0	1
	600	0	2	0	2
	608	0	0	5	5

ANIMAL NO. 34

SAMPLE BLASTOCYST

SAMPLE CHROMOSOME COUNTED ESTIMATED CLOMPED SUBTOTAL TOTAL
 NO. COUNT

12 *	7	0	1	0	1
	14	1	0	0	1
	16	1	0	0	1
	20	0	2	0	2
	22	1	0	0	1
	23	1	0	0	1
	26	1	0	0	1
	28	0	1	0	1
	31	1	0	0	1
	34	1	1	0	2
	35	0	1	0	1
	36	1	2	0	3
	37	0	3	0	3
	38	2	2	8	12
	40	1	0	0	1
	48	1	0	0	1
	95	1	0	0	1
	124	0	1	0	1
	148	0	1	0	1
	152	0	0	1	1
	227	0	1	0	1
	300	0	1	0	1
	310	0	1	0	1
	400	0	1	0	1
	450	0	2	0	2
	600	0	2	0	2
	608	0	0	2	2

47

ANIMAL NO. 34

SAMPLE BLASTOCYST

SAMPLE CHROMOSOME COUNTED ESTIMATED CLUMPED SUBTOTAL TOTAL
 NO. COUNT

13 #	5	1	0	0	1	
	7	1	0	0	1	
	9	1	0	0	1	
	11	1	0	0	1	
	13	1	0	0	1	
	16	1	0	0	1	
	22	1	0	0	1	
	23	0	1	0	1	
	28	1	0	0	1	
	38	0	1	0	1	
	48	0	1	0	1	
	53	1	0	0	1	
	57	1	0	0	1	
	96	0	1	0	1	
	152	0	0	1	1	
	176	0	1	0	1	
	190	0	0	1	1	
	300	0	2	0	2	
	304	0	0	3	3	
	310	0	1	0	1	
	600	0	1	0	1	
	608	0	0	3	3	
	690	0	1	0	1	28

ANIMAL NO. 39

SAMPLE BLASTOCYST

SAMPLE CHROMOSOME COUNTED ESTIMATED CLUMPED SUBTOTAL TOTAL

NO.	COUNT						
1	28	1	1	0	2		
	38	0	0	1	1		
	50	0	1	0	1		
	57	1	0	0	1	5	
2	38	0	0	1	1	1	
3	14	0	1	0	1		
	36	0	1	0	1		
	38	0	0	1	1		
	76	0	0	2	2		
	152	0	0	1	1	6	
4	38	0	1	6	7		
	76	0	0	1	1	8	
5	9	1	0	0	1		
	11	1	0	0	1		
	12	1	0	0	1		
	25	1	0	0	1		
	33	1	0	0	1		
	36	0	1	0	1		
	37	0	1	0	1		
	38	0	0	5	5	12	
6 *	16	1	0	0	1		
	28	0	1	0	1		
	34	0	1	0	1		
	38	1	0	5	6		
	114	0	0	2	2		
260	0	1	0	1	12		
7 *	29	0	1	0	1		
	34	0	1	0	1		
	35	1	0	0	1		
	38	0	1	0	1		
	67	0	1	0	1		
	70	0	1	0	1		
	300	0	1	0	1	7	

ANIMAL NO. 39

SAMPLE BLASTOCYST

SAMPLE NO.	CHROMOSOME COUNT	COUNTED	ESTIMATED	CLUMPED	SUBTOTAL	TOTAL
8 *	15	1	0	0	1	
	30	0	1	0	1	
	34	0	1	0	1	
	36	0	1	0	1	
	38	1	0	5	6	
	42	0	1	0	1	
	76	0	0	1	1	
	114	0	0	2	2	
	152	0	0	3	3	
	190	0	0	1	1	
304	0	0	2	2		
	608	0	0	1	1	21
10 *	34	0	1	0	1	
	38	0	0	4	4	
	76	0	0	3	3	
	114	0	0	1	1	
	150	0	1	0	1	
	152	0	0	2	2	
	200	0	1	0	1	
	225	0	1	0	1	
	226	0	1	0	1	
	300	0	1	0	1	
	304	0	0	1	1	17
11 *	9	1	0	0	1	
	11	1	0	0	1	
	12	1	0	0	1	
	30	1	0	0	1	
	35	1	0	0	1	
	38	3	0	1	4	
	52	0	1	0	1	
	152	0	0	1	1	
	300	0	1	0	1	
	304	0	0	1	1	
	608	0	0	3	3	16
12 *	11	1	0	0	1	
	13	1	0	0	1	
	19	1	0	0	1	
	22	1	0	0	1	
	30	0	2	0	2	
	36	1	0	0	1	
	38	0	1	2	3	
	152	0	0	1	1	
	266	0	0	1	1	
	304	0	0	1	1	
	400	0	2	0	2	15

ANIMAL NO. 30

SAMPLE BLASTOCYST

SAMPLE NO.	CHROMOSOME COUNT	COUNTED	ESTIMATED	CLUMPED	SUBTOTAL	TOTAL
13 *	30	0	1	0	1	
	36	1	0	0	1	
	37	1	0	0	1	
	38	1	0	1	2	
	50	0	1	0	1	
	152	0	0	1	1	
	190	0	0	1	1	
	304	0	0	1	1	9

ANIMAL NO. 161

SAMPLE BLASTOCOYST

SAMPLE CHROMOSOME COUNTED ESTIMATED CLUMPED SUBTOTAL TOTAL
 NO. COUNT

1	38	1	0	12	13	13
2	38	0	1	2	3	
	39	0	1	0	1	
	57	0	1	0	1	
	60	0	1	0	1	6
3	30	0	1	0	1	
	31	0	1	0	1	
	33	0	1	0	1	
	34	0	1	0	1	
	35	0	2	0	2	
	36	0	1	0	1	
	38	0	3	15	18	
	39	0	1	0	1	
	56	0	1	0	1	
	57	0	1	0	1	
	58	0	2	0	2	
	60	0	1	0	1	
	70	0	2	0	2	
	74	0	1	0	1	
	76	0	1	0	1	35
4	12	0	1	0	1	
	38	0	0	3	3	
	66	0	1	0	1	5
5	38	2	0	26	28	
	57	0	0	1	1	
	76	0	0	1	1	
	114	0	0	1	1	31
6	12	0	1	0	1	
	20	0	1	0	1	
	28	1	1	0	2	
	30	0	1	0	1	
	31	0	1	0	1	
	35	0	2	0	2	
	36	0	3	0	3	
	37	0	4	0	4	
	38	4	3	26	33	
	40	0	1	0	1	
	57	0	1	0	1	
	63	0	1	0	1	
	68	0	1	0	1	
	76	0	0	2	2	
	110	0	1	0	1	55

ANIMAL NO. 161

SAMPLE BLASTOCYST

SAMPLE NO.	CHROMOSOME COUNT	COUNTED	ESTIMATED	CLUMPED	SUBTOTAL	TOTAL
7	31	0	1	0	1	
	33	0	1	0	1	
	36	0	1	0	1	
	37	0	1	0	1	
	38	6	2	21	29	
	51	0	1	0	1	34
8	36	0	1	0	1	
	38	1	0	12	13	14
9	18	0	1	0	1	
	30	0	1	0	1	
	31	0	1	0	1	
	32	0	1	0	1	
	33	0	2	0	2	
	34	0	2	0	2	
	35	0	2	0	2	
	36	1	5	0	6	
	37	0	4	0	4	
	38	4	3	70	77	
	57	0	1	1	2	
	58	0	2	0	2	
	62	0	1	0	1	
	64	0	2	0	2	
	65	0	1	0	1	
	68	0	1	0	1	
	69	0	1	0	1	
	70	0	1	0	1	
	74	0	1	0	1	
	75	0	1	0	1	
	76	0	0	3	3	
	93	0	1	0	1	
	101	0	1	0	1	
135	0	1	0	1		
236	0	1	0	1	117	
10	38	0		7	7	7

ANIMAL NO. 201

SAMPLE BLASTOCYST

SAMPLE NO.	CHROMOSOME COUNT	COUNTED	ESTIMATED	CLUMPED	SUBTOTAL	TOTAL
1	35	0	1	0	1	
	36	0	4	0	4	
	38	1	4	41	46	
	40	0	1	0	1	
	72	0	1	0	1	
	74	0	1	0	1	
	93	0	1	0	1	55
2	23	1	0	0	1	
	38	0	1	44	45	46
3	8	1	0	0	1	
	14	1	0	0	1	
	21	1	0	0	1	
	31	1	0	0	1	
	36	0	1	0	1	
	37	1	1	0	2	
	38	5	2	52	59	
	39	0	1	0	1	
	41	1	0	0	1	
	50	0	1	0	1	
	52	1	0	0	1	
	63	0	1	0	1	
	72	0	1	0	1	
	76	0	1	3	4	76
4	18	1	0	0	1	
	21	1	0	0	1	
	28	1	0	0	1	
	36	0	2	0	2	
	38	3	1	54	58	
	76	0	0	1	1	64
5	29	0	1	0	1	
	32	0	1	0	1	
	33	0	1	0	1	
	36	0	1	0	1	
	37	0	1	0	1	
	38	10	4	49	63	
	39	1	0	0	1	
	45	0	1	0	1	
	57	0	0	1	1	
	58	0	1	0	1	
67	0	1	0	1	73	

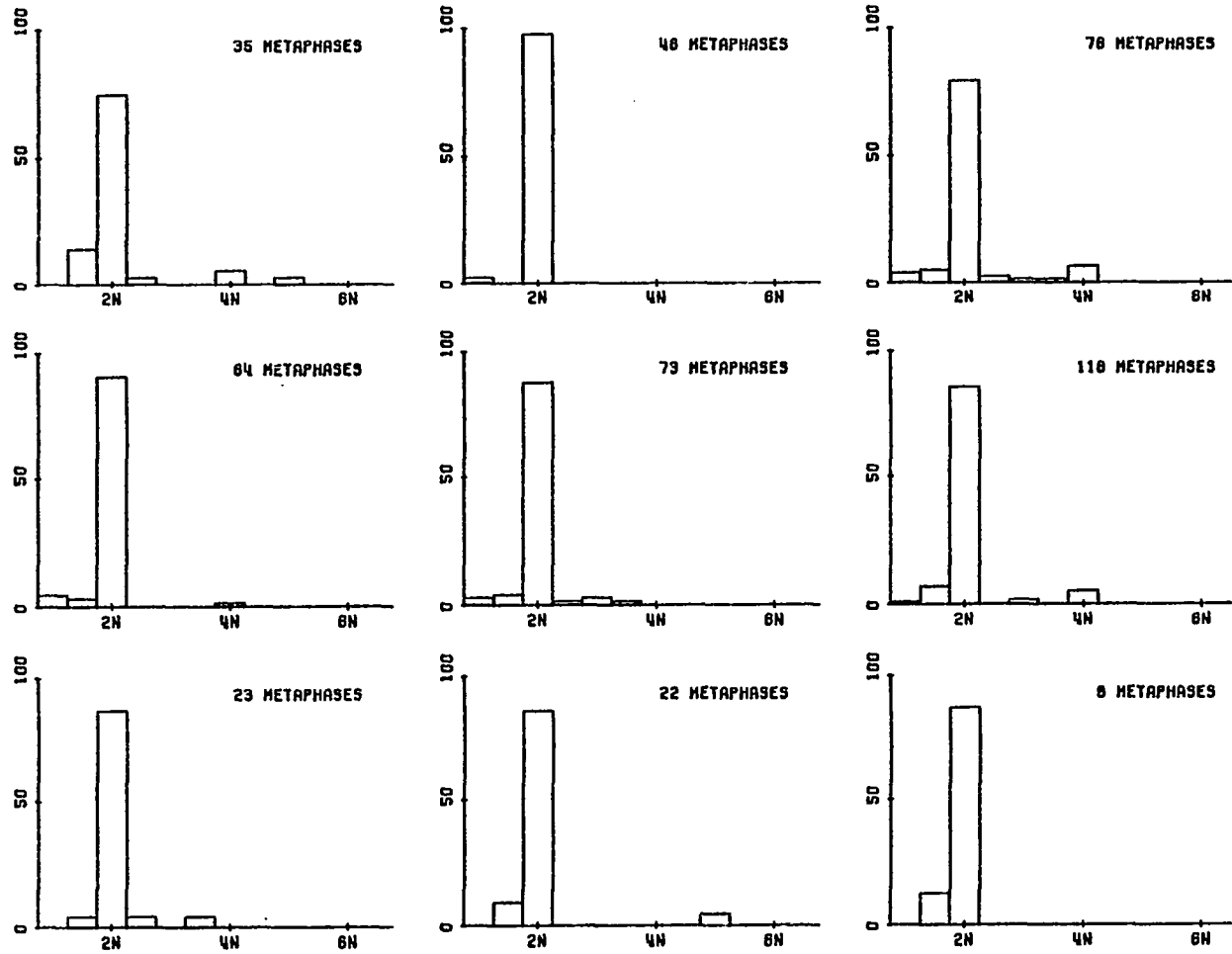
ANIMAL NO. 201

SAMPLE ELASTOCYST

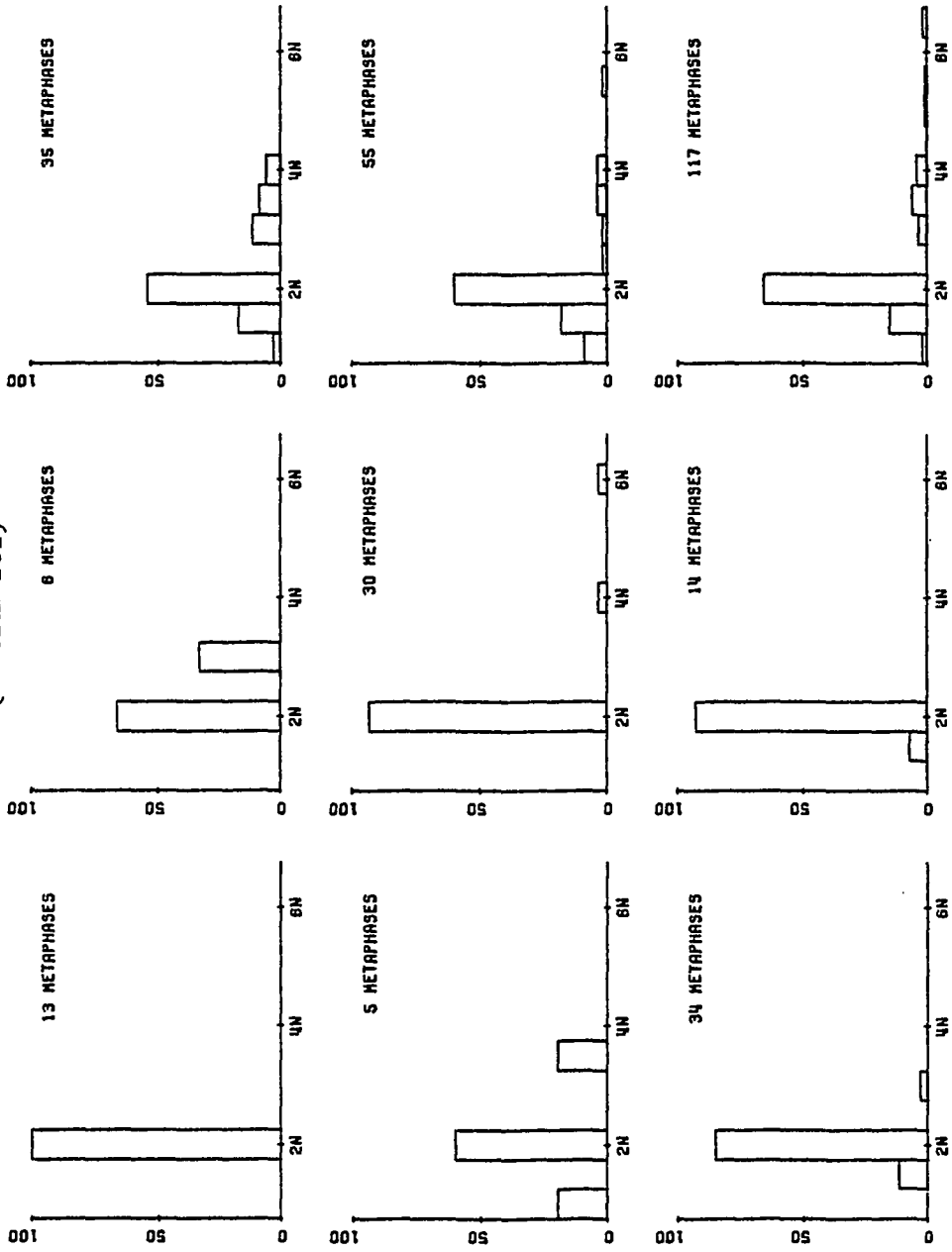
SAMPLE NO.	CHROMOSOME COUNT	COUNTED	ESTIMATED	CLUMPED	SUBTOTAL	TOTAL
6	28	1	0	0	1	
	33	1	0	0	1	
	35	1	0	0	1	
	36	0	2	0	2	
	37	2	3	0	5	
	38	10	3	86	99	
	39	0	2	0	2	
	57	0	0	2	2	
	74	0	2	0	2	
	75	0	1	0	1	
76	0	0	3	3	119	
7	36	0	1	0	1	
	38	1	1	17	19	
	39	1	0	0	1	
	42	0	1	0	1	
	62	0	1	0	1	23
8	35	0	1	0	1	
	36	0	1	0	1	
	38	0	1	17	18	
	39	0	1	0	1	
	100	0	1	0	1	22
9	38	0	0	7	7	7

APPENDIX VA

EXAMPLES OF DISTRIBUTION OF CHROMOSOME COUNTS FROM BLASTOCYSTS-DIRECT PREPARATION (ANIMAL 201)

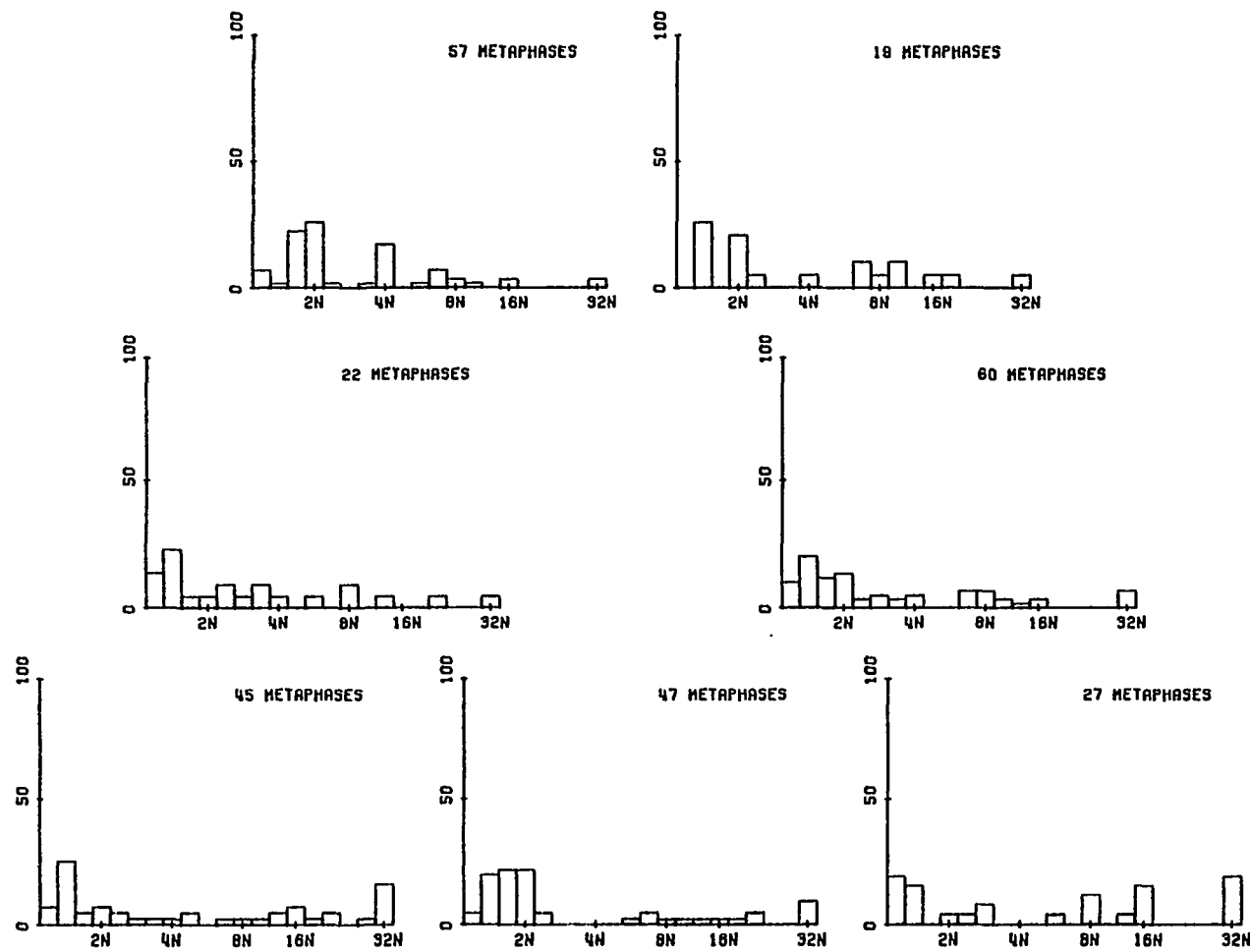


APPENDIX VA--CONTINUED
(ANIMAL 161)



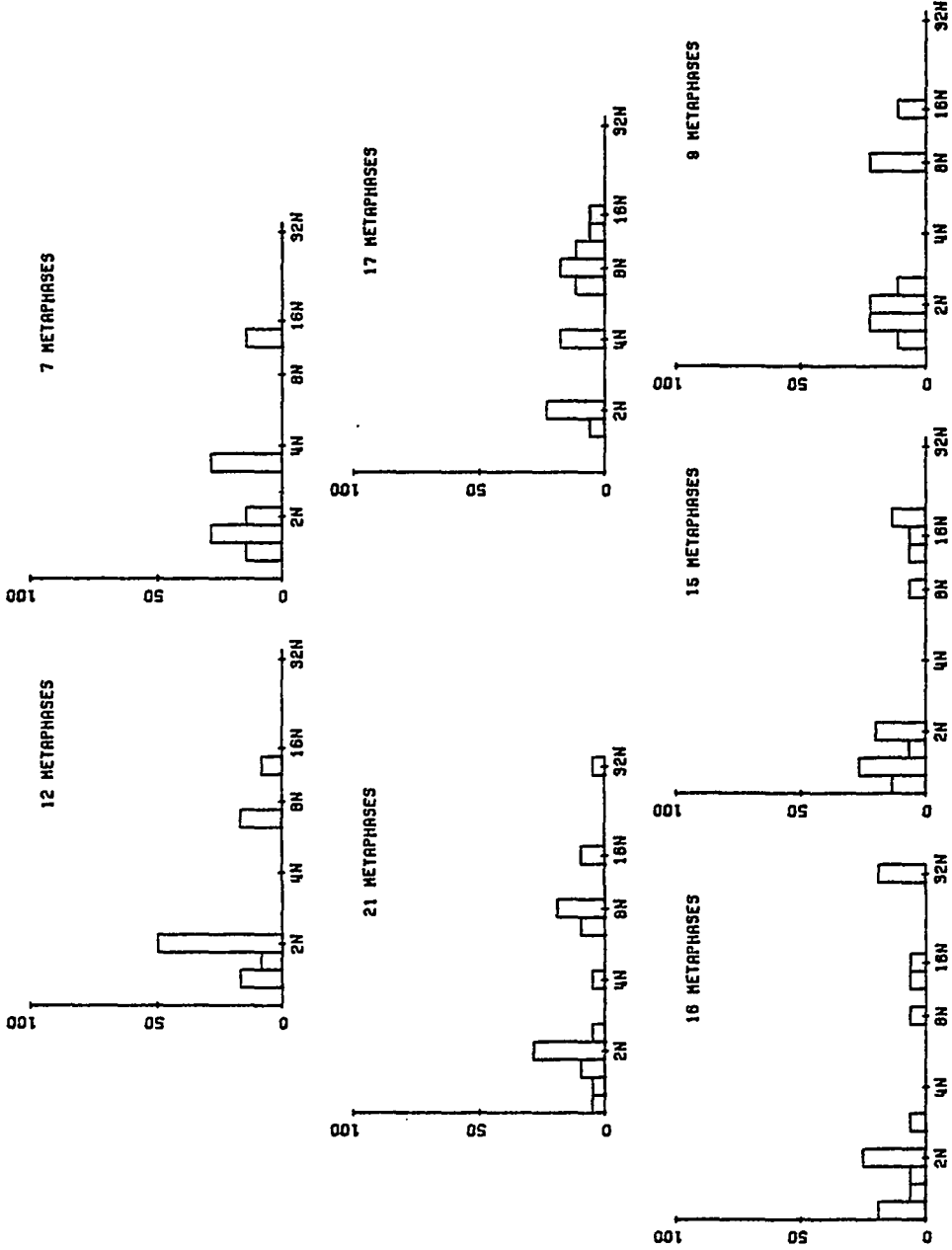
APPENDIX VB

EXAMPLES OF DISTRIBUTION OF CHROMOSOME COUNTS FROM BLASTOCYSTS-CULTURE PREPARATION (ANIMAL 34)



APPENDIX VB--CONTINUED

(ANIMAL 39)



LITERATURE CITED

- Akesson, A. and B. Henricson. (1972) Embryonic Death in Pigs Caused by Unbalanced Karyotype. *Acta Vet. Scand.* 13, 151-160.
- Arakaki, D. T. and S. H. Waxman. (1970) Chromosome Abnormalities in Early Spontaneous Abortions. *J. Med. Genet.* 7:118-124.
- Atnip, R. L. and R. L. Summitt. (1971) Tetraploidy and 18-Trisomy in a Six-Year-Old Triple Mosaic Boy. *Cytogenetics* 10, 305-317.
- Austin, C. R. (1967) Chromosome Deterioration in Aging Eggs of the Rabbit. *Nature* 213, 1018-1019.
- Avery, G. B. and C. V. Hunt. (1969) Differentiation of Trophoblast Giant Cells in the Mouse Studied in Kidney Capsule Grafts. *Transplantation Proc.* 1, 61-66.
- Barlow, P. W. and M. I. Sherman. (1972) Biochemistry of Differentiation of Mouse Trophoblast: Studies on Polyploidy. *J. Embryol. Exp. Morph.* Vol. 27, No. 2, 447-465.
- Barlow, P., D. A. J. Owen and C. Graham. (1972) DNA Synthesis in the Preimplantation Mouse Embryo. *J. Embryol. Exp. Morph.*, Vol. 27, No. 2, 431-445.
- Beatty, R. A. (1957) Parthenogenesis and Polyploidy in Mammalian Development. Cambridge University Press, London.
- Beatty, R. A. (1972) Parthenogenesis and Heteroploidy in the Mammalian Egg in Oogenesis. J. D. Biggers and A. W. Schultz, eds. University Park Press, London.
- Billington, W. D. (1971) Biology of the Trophoblast. *Adv. Reprod. Physiol.* Vol. 5, 27-66.
- Bomsel-Helmreich, O. (1961) IV Int. Congr. Anim. Reprod., Section 68, I, 1-4.
- Bomsel-Helmreich, O. (1965) Heteroploidy and Embryonic Death in Preimplantation Stages of Pregnancy, Ciba Foundation Symposium, London, 246-269.
- Bomsel-Helmreich, O. (1970) Fate of Heteroploid Embryos. *Advances in the Biosciences* No. 6, G. Raspe'ed., 381-403.
- Bonnet, R. (1901) Beitrage zur Embryologic des Hundes. *Anat. Hefte.* Vol. 16, 232-418.
- Boue, J. G., A. Boue and P. Lazar. (1967) Les Aberrations Chromosomiques Dans Les Avortements. *Ann. Genet.* 10, 179-187.

- Brinster, R. L. (1974) Embryo Development. *J. Anim. Sci.* 38, No. 5, 1003-1012.
- Bustad, L. K., R. O. McClellan and M. P. Burns, eds. (1965) Swine in Biomedical Research. Proceedings of a Symposium at the Pacific Northwest Laboratory, Richland, Washington, July 19-22. Frayn Printing Company, Seattle, Washington.
- Bustad, L. K. (1966) Pigs in the Laboratory. *Scientific American*, Vol. 214, No. 6, 94-100.
- Butcher, R. L. and N. W. Fugo. (1967) Overripeness and the Mammalian Ova II. Delayed Ovulation and Chromosome Anomalies. *Fert. & Steril.*, Vol. 18, No. 3, 297-302.
- Butler, L. J., C. Chantler, N. E. France and C. G. Keith. (1969) Liveborn Infant with Complete Triploidy (69,XXX). *J. Med. Genet.* 6, 413-421.
- Carr, D. H. (1963) Chromosome Studies in Abortuses and Stillborn Infants. *Lancet* 2:603-606.
- Carr, D. H. (1971a) Genetic Basis of Abortion. *Ann. Rev. Genet.* 5:65-80.
- Carr, D. H. (1971b) Chromosomes and Abortion, *Advances in Human Genetics*, No. 2, H. Harris and K. Hirschhorn, eds., Plenum Press, New York, 201-257.
- Chapman, V. M., J. D. Ansell and A. McLaren. (1972) Trophoblast Giant Cell Differentiation in the Mouse: Expression of Glucose Phosphate Isomerase (GPI-1) Electrophoretic Variants in Transferred and Chimeric Embryos. *Develop. Biol.* 29, 48-54.
- Chu, E. H. Y., H. C. Thuline and D. E. Norby. (1964) Triploid-Diploid Chimerism in a Male Tortoiseshell Cat. *Cytogenetics* 3, 1-18.
- Cornelius, C. E. (1969) Animal Models--a Neglected Medical Resource. *New England J. Med.* 281, 934-944.
- Creasy, M. R., J. A. Crolla and E. D. Alberman. (1976) Cytogenetic Study of Human Spontaneous Abortions Using Banding Techniques. *Hum. Genet.* 31, 177-196.
- Crombie, P. R. (1970) Ultrastructure of the Foetal-Maternal Attachment in the Pig. *Proc. Physiol. Soc.* 210, 101P-102P.
- Day, B. N. and C. Polge. (1968) Effects of Progesterone on Fertilization and Egg Transport in the Pig. *J. Reprod. Fert.* 17, 227-230.

- Dewald, G., M. N. Alvarez, M. D. Cloutier, P. P. Kelalis and H. Gordon. (1975) Diploid-Triploid Human Mosaic with Cytogenetic Evidence of Double Fertilization. *Clinical Genetics* 8, 149-160.
- Donahue, R. P. (1972) Cytogenetic Analysis of the First Cleavage Division in Mouse Embryos. *Proc. Nat. Acad. Sci.*, Vol. 69, No. 1, 74-77.
- Dziuk, P. J. and G. Henshaw. (1958) Fertility of Boar Semen Artificially Inseminated Following in vitro Storage. *J. Anim. Sci.* 17, 554-558.
- Edwards, J. H., C. Yuncken, D. I. Rushton, S. Richards and U. Mittwoch. (1967) Three Cases of Triploidy in Man. *Cytogenetics* 6, 81-104.
- Fechheimer, N. S. and R. A. Beatty. (1974) Chromosomal Abnormalities and Sex Ratio in Rabbit Blastocysts. *J. Reprod. Fert.* 37, 331-341.
- Fischberg, M. and R. A. Beatty. (1951) Spontaneous Heteroploidy in Mouse Embryos up to Mid-term. *J. Exp. Zool.* 118, 321-335.
- Fraser, L. R., H. M. Zanellotti, G. R. Paton and L. M. Drury. (1976) Increased Incidence of Triploidy in Embryos Derived from Mouse Eggs Fertilized in vitro. *Nature* 260, 39-40.
- Fugo, N. W. and R. L. Butcher. (1966) Overripeness and the Mammalian Ova I. Overripeness and Early Embryonic Development. *Fert. & Steril.* Vol. 17, No. 6, 804-814.
- Fujimoto, S., N. Pahlavan and W. R. Dukelow. (1974) Chromosome Abnormalities in Rabbit Preimplantation Blastocysts Induced by Superovulation. *J. Reprod. Fertil.* 40, 177-181.
- Gosden, R. G. (1973) Chromosomal Anomalies of Preimplantation Mouse Embryos in Relation to Maternal Age. *J. Reprod. Fert.* 35, 351-354.
- Gustavsson, I., M. Hageltorn, L. Zech and S. Reiland. (1973) Identification of the Chromosomes in a Centric Fusion/Fission Polymorphic System of the Pig (*Sus Scrofa* L.) *Hereditas*, 75, 153-155.
- Hageltorn, M., I. Gustavsson and L. Zech. (1973) Q and G Banding Patterns of a t(11p+; 15q-) in the Domestic Pig. *Hereditas* 75, 147-151.
- Hamerton, J. L. (1971) Human Cytogenetics. Vol. 2. Academic Press, New York.
- Hancock, J. L. (1959) Polyspermy of Pig Ova. *Animal Prod.* 1, 103-106.
- Hanly, S. (1961) Prenatal Mortality in Farm Animals. *J. Reprod. Fertil.* 2, 182-194.

- Hansen-Melander, E. and Y. Melander. (1970a) Mosaicism for Translocation Heterozygosity in a Malformed Pig. *Hereditas* 64, 199-202.
- Hansen-Melander, E. and Y. Melander. (1971) Case of Spontaneous Haploidy and Notes of Triploid-Diploid Mosaics in Rabbit Embryos. *Hereditas* 67, 83-88.
- Hansen-Melander, E. and Y. Melander. (1974) Karyotype of the Pig. *Hereditas* 77, 149-158.
- Hansen-Melander, E. and Y. Melander. (1970b) Rabbit Blastocyst as Test Object for Environmental Influences of Mammalian Chromosomes. *Hereditas* 65, 237-240.
- Hendricks, A. G. and D. C. Kraemer. (1968) Preimplantation Stages of Baboon Embryos (*Papio* sp.). *Anat. Rec.* 162, 111-120.
- Henricson, B. and L. Backstrom. (1964) Translocation Heterozygosity in a Boar. *Hereditas* 52, 166-170.
- Hertig, A. T., J. Rock, E. C. Adams and M. C. Menkin. (1959) Thirty-Four Fertilized Human Ova, Good, Bad and Indifferent, Recovered from 210 Women of Known Fertility. *Pediatrics*, 23, 202-211.
- Heuser, C. H. and G. L. Streeter. (1941) Development of the Macaque Embryo. *Contrib. Embryol.* 29, 15-55.
- Hofsaess, F. R. and T. N. Meacham. (1971) Chromosome Abnormalities of Early Rabbit Embryos. *J. Exp. Zool.* 177, 9-12.
- Hunt, C. V. and G. B. Avery. (1971) Increased Levels of Deoxyribonucleic Acid During Trophoblast Giant Cell Formation in Mice. *J. Reprod. Fertil.* 25, 85-91.
- Hunter, R. H. F. (1967) Effects of Delayed Insemination on Fertilization and Early Cleavage in the Pig. *J. Reprod. Fert.* 13, 133-147.
- Hunter, R. H. F. (1972) Local Action of Progesterone Leading to Polyspermic Fertilization in Pigs. *J. Reprod. Fert.* 31, 433-444.
- Hunter, R. H. F. (1973) Chronological and Cytological Details of Fertilization and Early Embryonic Development in the Domestic Pig, *Sus scrofa*. *Anat. Rec.* 178, 169-186.
- Inhorn, S. L., E. Therman and K. Patau. (1964) Cytogenetic Studies in Spontaneous Human Abortions (Abstr.). *Am. J. Clin. Pathol.* 42, 528.

- Jacobs, P. A. (1972) Chromosome Abnormalities and Fertility in Man, Genetics of the Spermatozoan, R. A. Beatty and S. Gluecksohn-Waelsch, eds. Bogtrykkeriet Forum, Copenhagen, 346-358.
- Jollie, W. P. (1964) Radioautographic Observations on Variations in Deoxyribonucleic Acid Synthesis in the Rat Placenta with Increasing Gestational Age. *Am. J. Anat.* 114, 161-171.
- Jonasson, J., A. J. Therkelsen, J. G. Lauritsen and J. Lindsten. (1972) Origin of Triploidy in Human Abortuses. *Hereditas* 71, 168-171.
- Kaufman, M. H. (1975) Early Development of Mammals, M. Balls and A. E. Wild eds. Cambridge University Press, Cambridge, 25-44.
- Kajii, T., K. Ohama, N. Niikawa, A. Ferrier and S. Avirachan. (1973) Banding Analysis of Abnormal Karyotypes in Spontaneous Abortion. *Am. J. Hum. Genet.* 25, 539-547.
- Kelly, T. E. and J. M. Rany. (1974) Mosaic Tetraploidy in a Two-Year-Old Female. *Clinical Genetics* 6, 221-224.
- Kerr, M. and M. N. Rashad. (1966) Chromosome Studies on Spontaneous Abortions. *Amer. J. Obstet. Gynec.* 94:322-339.
- Kohn, G., B. H. Mayall, M. E. Miller and W. J. Mellman. (1967) Tetraploid-Diploid Mosaicism in a Surviving Infant. *Pediat. Res.* 1, 461-469.
- Lauritsen, J. G., J. Jonasson, A. J. Therkelsen, F. Lass, J. Lindsten and G. B. Petersen. (1972) Studies on Spontaneous Abortions. Fluorescence Analysis of Abnormal Karyotypes. *Hereditas* 71, 160-163.
- Lerner, E. H., D. T. Mayer and J. F. Lasley. (1957) Early Embryonic Mortality in Strain Crossed Gilts. *Res. Bull. Mo. Agric. Exp. Sta. No.* 629.
- Makino, S., T. Ikeuchi, M. Sasaki, J. Mumamoto, H. Shimba, S. Fujimoto and S. Matsuda. (1967) Preliminary Study of the Chromosomes in Spontaneous Abortions. *Proc. Japan Acad.* 43, 552-555.
- Martin, P. A. and E. L. Shaver. (1972) Sperm Aging in Utero and Chromosomal Anomalies in Rabbit Blastocysts. *Dev. Biol.* 28, 480-486.
- McFee, A. F., M. W. Banner and J. M. Rany. (1966) Variation in Chromosome Number Among European Wild Pigs. *Cytogenetics* 5:75-81.

- McFee, A. F. and M. W. Banner. (1969) Inheritance of Chromosome Number in Pigs. *J. Reprod. Fert.* 18, 9-14.
- McFeely, R. A. (1966) Direct Method for the Display of Chromosomes from Early Pig Embryos. *J. Reprod. Fert.* 11, 161-163.
- McFeely, R. A. (1967) Chromosome Abnormalities in Early Embryos of the Pig. *J. Reprod. Fert.* 13, 579-581.
- McLaren, A. (1975) Personal communication.
- Melander, Y. (1951) Polyploidy after Colchicine Treatment of Pigs. *Hereditas* 37, 288-289.
- Mitruka, B. M., H. M. Rawnsley, D. V. Vadehra. (1976) Animals for Medical Research--Models for the Study of Human Disease. John Wiley & Sons, New York.
- Moon, R. G., M. N. Rashad and M. P. Mi. (1975) Example of Polyploidy in Pig Blastocysts. *J. Reprod. Fert.* 45, 147-149.
- Nagl, W. (1972) Giant Sex Chromatin in Endopolyploid Trophoblast Nuclei of the Rat. *Experientia* 28, 217-218.
- Nes, N. (1966) Diploid-Triploid Chimerism in a True Hermaphrodite Mink (*Mustela vison*). *Hereditas* 56, 159-170.
- O'Neill, F. J. and C. P. Miles. (1970) Virus-Induced Chromosome Pulverization in Human Diploid Cells. *Proc. Soc. Exp. Biol. Med.* 134, 825-830.
- O'Neill, F. J. and C. P. Miles. (1971) Origin of Nuclei in Spontaneous HeLa Cell Chromosome Pulverization. *J. Natl. Cancer Inst.* 46, 1085-1091.
- O'Neill, F. J. (1972) Chromosome Pulverization in Cultured Normal and Neoplastic Cells Treated with Cytochalasin-B. *J. Natl. Cancer Inst.* 49, 1733-1737.
- Perry, J. S. and I. W. Rowlands. (1962) Early Pregnancy in the Pig. *J. Reprod. Fert.* 4, 175-188.
- Piko, L. and O. Bomsel-Helmreich. (1960) Triploid Rat Embryos and Other Chromosomal Deviants after Colchicine Treatment and Polyspermy. *Nature* 186, 737-739.
- Pitkjanen, I. G. (1955) Ovulation, Fertilization and Early Embryonic Development in the Pig. *Izv. Akad. SSSR. Ser. Biol.* 3, 120-130.
- Polge, C. and P. J. Dziuk. (1970) Time of Cessation of Intrauterine Migration of Pig Embryos. *J. Anim. Sci.* 31, 565-566.

- Ruzicska, P. (1968) Double Trisomy in a Pig Embryo. *Mamm. Chrom. Newsl.* 9, 240-241.
- Saadi, A. A., J. F. Juliar, J. Harm, A. J. Brough, E. V. Perrin and H. Chen. (1976) Triploidy Syndrome--A Report on Two Live-Born (69,XXY) and One Still-Born (69,XXX) Infants. *Clinical Genetics* 9, 43-50.
- Saccoman, F. M., C. F. Morgan and L. J. Wells. (1967) Radioautographic Studies of DNA Synthesis in the Developing Extraembryonic Membranes of the Mouse. *Anat. Rec.* 158, 197-201.
- Samuel, C. A. and J. S. Perry. (1972) Ultrastructure of Pig Trophoblast Transplanted to an Ectopic Site in the Uterine Wall. *J. Anat.* 113, 1, 139-149.
- Schindler, A. and K. Mikano. (1970) Triploidy in Man--Report of a Case and a Discussion on Etiology. *Cytogenetics* 9, 116-130.
- Schlesinger, M. and Z. Koren. (1967) Mouse Trophoblastic Cells in Tissue Culture. *Fertil. Ster.* Vol. 18, No. 1, 95-101.
- Schmid, W. and D. Vischer. (1967) Malformed Boy with Double Aneuploidy and Diploid-Triploid Mosaicism 48,XXYY/71,XXXYY. *Cytogenetics* 6, 145-155.
- Scofield, A. M. (1972) Embryonic Mortality in Pig Production. ed. D. J. A. Cole, Butterworths, London. 367-383.
- Scofield, A. M., F. G. Clegg and G. E. Lamming. (1974) Embryonic Mortality and Uterine Infection in the Pig. *J. Reprod. Fert.* 36, 353-361.
- Selby, L. A., H. C. Hopps and L. D. Edmonds. (1971) Comparative Aspects of Congenital Malformations in Man and Swine. *J.A.V.M.A.* Vol. 159, No. 11, 1485-1490.
- Self, H. L., R. H. Grummer and L. E. Casida. (1955) Effects of Various Sequences of Full and Limited Feeding on the Reproductive Phenomena in Chester White and Poland China Gilts. *J. Anim. Sci.* 14, 573-592.
- Shaver, E. L. and D. H. Carr. (1967) Chromosome Abnormalities in Rabbit Blastocysts Following Delayed Fertilization. *J. Reprod. Fert.* 14, 415-420.
- Shaver, E. L. and D. H. Carr. (1969) Chromosome Complement of Rabbit Blastocysts in Relation to the Time of Mating and Ovulation. *Can. J. Genet. Cytol.* 11, 287-293.

- Sherman, M. I., A. McLaren, P. M. B. Walker. (1972) Mechanism of Accumulation of DNA in Giant Cells of Mouse Trophoblast. *Nature, New Biol.* 238, 175-176.
- Smith, J. H. and T. J. Marlowe. (1971) Chromosomal Analysis of 25-Day Old Pig Embryos. *Cytogenetics* 10:385-391.
- Snow, M. H. L. (1973) Tetraploid Mouse Embryos Produced by Cytochalasin B During Cleavage. *Nature* 244, 513-515.
- Snow, M. H. L. and J. D. Ansell. (1974) Chromosomes of Giant Trophoblast Cells of the Mouse. *Proc. R. Soc. Lond. B.* 187, 93-98.
- Stenchever, M. A., J. A. Hempel and M. N. MacIntyre. (1967) Cytogenetics of Spontaneously Aborted Human Fetuses. *Obstet. Gynecol.* 30, 683-691.
- Tarkowski, A. K. (1975) Induced Parthenogenesis in the Mouse, In the *Developmental Biology of Reproduction*, C. L. Markert and J. Papaconstantinou, eds. Academic Press, New York, 107-129.
- Tarkowski, A. K. and J. Rossant. (1976) Haploid Mouse Blastocysts Developed from Bisected Zygotes. *Nature* 259, 663-665.
- Therkelsen, A. J., N. Grunnet, T. Hjort, O. M. Jensen, J. Jonasson, J. G. Lauritsen, J. Lindsten and G. B. Petersen. (1973) Studies on Spontaneous Abortions, in *Chromosomal Errors in Relation to Reproductive Failure*. eds. A. Boue and C. Thibault. Paris INSERM, 81-94.
- Thibault, C. (1959) Analyse de la Fécondation de l'oeuf de la Truie Après Accouplement ou Insemination Artificielle. *Annls. Zootech. Series D, Suppl.*, 165-175.
- Vickers, A. D. (1969) Delayed Fertilization and Chromosomal Anomalies in Mouse Embryos. *J. Reprod. Fert.* 20, 69-76.
- Vogt, D. W., D. T. Arakaki and C. C. Brooks. (1974) Reduced Litter Size Associated with Aneuploid Cell Lines in a Pair of Full-Brother Duroc Boars. *Am. J. Vet. Res.*, Vol. 35, No. 8, 1127-1130.
- Widmeyer, M. A. and E. L. Shaver. (1972) Estrogen, Progesterone, and Chromosome Abnormalities in Rabbit Blastocysts. *Teratology* 6, 207-214.
- Witkowska, A. (1973) Parthenogenetic Development of Mouse Embryos In Vivo, I. Preimplantation Development. *J. Embryol. Exp. Morph.* Vol. 3, No. 3, 519-545.

- Wroblewska, J. (1971) Developmental Anomaly in the Mouse Associated with Triploidy. *Cytogenetics* 10, 199-207.
- Yamamoto, M. and T. H. Ingalls. (1972) Delayed Fertilization and Chromosome Anomalies in the Hamster Embryo. *Science*, Vol. 176, 518-521.
- Zybina, E. V. (1964) Sex Chromatin in Giant Cells of Trophoblast and in Cells of Early Rabbit Embryo. *Fedn. Proc. Fedn. Am. Socs. Exp. Biol. (Transl. Suppl.)* 24, 868.