

## Differences in the sex ratios of offspring originating in the right and left ovaries of Mongolian gerbils (*Meriones unguiculatus*)

M. M. Clark, M. Ham and B. G. Galef, Jr

Department of Psychology, McMaster University, Hamilton, Ontario L8S 4K1, Canada

Two experiments were undertaken to investigate the cause of the observed tendency of Mongolian gerbil dams to gestate more male than female fetuses in their right uterine horns and more female than male fetuses in their left uterine horns. It was found in Expt 1 that female gerbils that had both ovaries removed and portions of their right ovary placed in both ovarian capsules gestated significantly more male fetuses than did females that had both ovaries removed and portions of their left ovaries placed in both ovarian capsules. Expt 2 showed that female gerbils that had both their ovaries removed and then returned to their original locations gestated more males in their right uterine horns than in their left, while females that had the positions of their ovaries exchanged gestated more male fetuses in their left uterine horns than in their right. The data were consistent with the hypothesis that lateral asymmetries in gerbil ovaries rather than in gerbil uterine horns cause partial uterine segregation of gerbil fetuses by sex.

### Introduction

In many litter-bearing rodent species, the intrauterine position occupied by fetuses has profound effects on their reproductive behaviour when they reach adulthood (Tobet *et al.*, 1982; vom Saal and Moyer, 1985). For example, adult male Mongolian gerbils (*Meriones unguiculatus*) that are gestated in intrauterine positions between two males are more likely to impregnate females with which they are paired than are males that are gestated between two females (Clark *et al.*, 1992). During their lifetimes, female gerbils that developed in intrauterine positions between two female fetuses deliver and wean approximately twice as many young as do females that developed *in utero* between two males (Clark *et al.*, 1986; Clark and Galef, 1988).

Given the relatively greater reproductive success of gerbil fetuses that are gestated in relative isolation from fetuses of the other sex, it might be expected that gerbil dams have evolved an ability to protect their male and female fetuses from intrauterine contact with one another. In Mongolian gerbil dams the probability of contact between male and female fetuses is substantially reduced by the partial segregation of male and female fetuses in opposite uterine horns. Male gerbil fetuses are found in the right uterine horns of their mothers, while female gerbil fetuses develop in the left uterine horns significantly more frequently than would be expected by chance (Clark and Galef, 1990); preliminary data indicate that gerbils with their left ovaries extirpated deliver litters containing more males, while gerbils with their right ovaries extirpated deliver litters containing more females (M.M. Clark, unpublished). Consequently, gerbil fetuses are gestated adjacent

to fetuses that share their sex with greater frequency than they would be if males and females were gestated with equal probability in left and right uterine horns (Clark and Galef, 1990; Clark *et al.*, 1993).

The partial segregation of fetuses by sex seen in the gerbil uterus suggests some consistent lateral asymmetry either between the left and right uterine horns or the left and right ovaries of female gerbils. The literature provides much evidence of lateral asymmetry of both mammalian ovaries and mammalian uterine horns (Pearson, 1949; Wimsatt, 1975, 1979; Baird and Birney, 1985).

In hamsters, the right uterine horn contains more sperm after mating (O and Chow, 1987) than does the left. In Norway rats, the right uterine horn has more implantation sites (Buchanan, 1974) and, in mice, sustains more live embryos than does the left (Wiebold and Becker, 1987).

The right ovary of rats is larger (Mittwoch and Kirk, 1975) than the left and in rats, hamsters and mice the right ovary has more corpora lutea (Buchanan, 1974; O and Chow, 1987; Wiebold and Becker, 1987) than does the left. In humans, the right ovary is more prone to tumours (Willis, 1967) and, in shrews, has fewer follicles (Mohanty and Chainy, 1992) than does the left. Perhaps most striking is the finding in several mammals (humans, mice, rats, hamsters and guinea-pigs) of systematic differences in the probability of testes or ovotestes developing out of the potentially hermaphroditic ovarian rudiment on the right or left sides of true hermaphrodites. In humans (van Niekirk, 1974; van Niekirk and Retief, 1981), pigs (Hunter *et al.*, 1985), hamsters (Kirkman, 1958), and guinea-pigs (Jaffe and Papanicolaou, 1927) testes or ovotestes in hermaphrodites occur predominantly on the right side, while in some mouse strains they occur predominantly on the left (Ward *et al.*, 1987). Clearly, lateral asymmetries in either the

ovary or the uterus of gerbils might be responsible for the observed uterine sexual segregation of fetuses.

In the present experiments, we used a modification of procedures developed for autografting ovaries of house mice to determine the relative importance of ovarian and uterine lateral asymmetries in producing the partial segregation of fetuses by sex observed in the uterine horns of pregnant Mongolian gerbils.

## Materials and Methods

### Animals

Female Mongolian gerbils between 45 and 60 days old ( $n = 154$ ) were obtained from Tumblebrook Farms (Brookfield, MA). Each animal was placed in a polypropylene shoe-box cage (35 cm  $\times$  30 cm  $\times$  15 cm), housed in a temperature- and humidity-controlled colony room illuminated each day from 05:00 to 17:00 h, and left undisturbed for 2 weeks with free access to Purina Rodent Laboratory Chow and water.

### Surgery

At the start of the experiment, each animal was anaesthetized with 40 mg sodium pentobarbitol (MTC Pharmaceuticals, Cambridge, Ontario)  $\text{kg}^{-1}$  body mass and both ovaries were removed and reimplanted using a modification of surgical procedures developed by Jones and Krohn (1960) for autografting ovaries of house mice. Their procedures were modified to accommodate the ovaries of young Mongolian gerbils, which (unlike those of house mice) are neither surrounded by abundant fatty tissue nor free-floating.

Rather than introducing half an excised ovary into the ovarian fat pad, as is done in ovarian grafts in mice (Jones and Krohn, 1960), successful implantation of ovarian tissue in gerbils was achieved by placing half an ovary inside the capsular membrane of the recipient ovary and then using fine forceps to bring the edges of the membrane together to hold the grafted tissue in place. When removing an ovary from a gerbil, care was taken not to sever the fine filaments that connect the small ovarian fat pad found in gerbils to the ipsilateral kidney; this avoids adhesion of grafted tissue to the abdominal wall.

### Mating

One month after surgery, each female was placed in the cage of an adult male gerbil of proven fertility, but separated from him by a hardware-cloth partition. The barrier separating a pair was removed after 24 h and their cage was then monitored to determine the day on which mating occurred. Pair members were separated 3 weeks after mating.

### Caesarian delivery

Twenty-four days after a pair had been observed to mate (i.e. 1 day before anticipated vaginal delivery), a female that had gained weight at a rate consistent with impregnation

on the day of observed copulation was anaesthetized by halothane (Ayerst Laboratories, Montreal, Quebec) inhalation; the abdomen was opened, the uterus externalized and the fetuses delivered by Caesarian section. The sex of each fetus was determined by visual inspection of its anogenital distance (Clark and Galef, 1990). In Mongolian gerbils, unlike house mice, the sex of a pup on its last day of gestation is easily determined by inspection of its anogenital area. However, to ensure the reliability of sex assignment (1) sex determination was carried out by an observer blind to the previous treatment of dams, (2) a randomly selected 10% of animals were killed and their gonads exposed and examined by stereomicroscopy, and (3) a further 22% of animals (selected on the basis of their intrauterine position for use in future experiments) were reared by foster mothers. As reported previously (Clark and Galef, 1990), no errors were made in sex assignment on the basis of visual inspection of anogenital distance.

After all fetuses had been removed, the gerbil was killed by an overdose of anaesthetic (Somnotol, MTC Pharmaceuticals).

### Experiment 1

Both ovaries were removed from 32 animals. The left ovary was cut in half at right angles to its major axis; half of this ovary was placed within the capsular membrane that had contained the left ovary while the remainder of the left ovary was placed within the capsular membrane that had contained the right ovary. The remaining 32 animals in Expt 1 were treated identically to those described above except that each animal's right ovary was bisected and half of the right ovary was placed within the capsular membranes that had contained both right and left ovaries, respectively.

Across animals, (1) the order in which ovaries were removed, (2) the order in which ovarian tissue was inserted into left and right capsular membranes, and (3) the uterine horns in which anterior and posterior portions of extirpated ovaries were placed were counterbalanced.

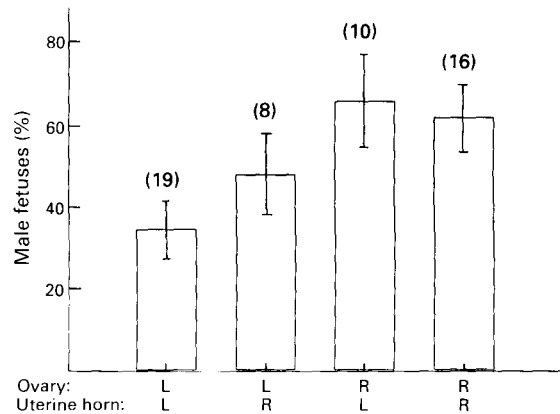
### Experiment 2

The methods of Expt 2 were identical to those of Expt 1 except that, after both ovaries were removed and sectioned across their major axis, (1) for each of the 45 animals assigned to the control group, half of both left and right ovaries were returned to the capsular membranes from which they had been removed, and (2) for each of the 45 animals assigned to the experimental group, half the right ovary was placed in the left ovarian capsular membrane and half the left ovary was placed in the right ovarian capsular membrane.

## Results

### Experiment 1

Females that had received autografts of tissue from their right ovaries into both ovarian capsules gestated litters containing a significantly greater proportion of males ( $61.4 \pm 7.1\%$  males per litter) than did females that had received autografts



**Fig. 1.** Percentage (mean  $\pm$  SEM) of male fetuses found in left (L) and right (R) uterine horns to which left and right ovarian tissue had been autografted. The number of uterine horns contributing fetuses to each group is indicated in brackets above the bars.

of tissue from their left ovaries into both ovarian capsules ( $36.5 \pm 6.6\%$  males per litter; Student's  $t$  test,  $t_{42} = 2.55$ ,  $P < 0.01$ ) (Fig. 1).

A  $2 \times 2$  ANOVA of the percentage of male fetuses that gestated in left and right uterine horns of females that had received autografts of tissue from either their left or right ovaries was performed. The analysis revealed a significant effect of the origin of ovarian tissue that females received on sex ratios of fetuses that they gestated ( $F_{2,49} = 5.01$ ,  $P < 0.03$ ). The same analysis revealed no effect either of the uterine horn receiving grafted tissue ( $F_{2,49} = 0.23$ , not significant) or of the interaction between the uterine horn receiving grafted tissue and the ovary contributing that tissue ( $F_{2,49} = 0.76$ , not significant) on the sex ratios of gestated fetuses.

Autografts of ovarian tissue into the same ovarian capsule from which they had been removed were more frequently successful than were autografts of tissue into contralateral ovarian capsules ( $F_{1,84} = 11.16$ ,  $P < 0.001$ ) (Table 1). However, despite the differential success of ipsilateral and contralateral autografts, the mean number of living fetuses gestated in a uterine horn, if any were gestated there, did not differ between uterine horns receiving ipsilateral ( $2.31 \pm 0.23$  fetuses) and contralateral ( $2.00 \pm 0.22$  fetuses) autografts of ovarian tissue (Student's  $t$  test,  $t_{51} = 0.85$ , not significant).

As expected, given that animals in this experiment had only half the ovarian tissue found in intact animals, the number of fetuses carried on day 24 of gestation by females receiving autografts was markedly less than the number carried at the same point in gestation by gerbil dams that had not been manipulated surgically before their Caesarian delivery. Such intact animals had an average ( $\pm$  SEM) of  $3.3 (\pm 0.1)$  fetuses in their left uterine horns and  $3.2 (\pm 0.1)$  fetuses in their right uterine horns (Clark *et al.*, 1991).

### Experiment 2

Females in the control group (those that had tissue from left and right ovaries returned to their original locations) gestated a greater proportion of male fetuses in their right uterine horns

**Table 1.** Sex composition of Caesarian-delivered litters of Mongolian gerbil dams receiving autografts of tissue from left or right ovaries

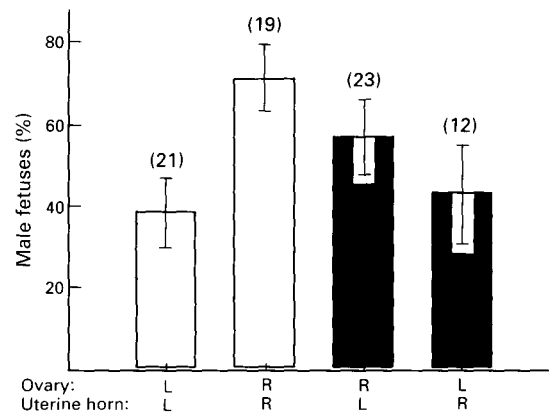
Dependent variable	Tissue		P
	Left ovary	Right ovary	
Number of animals	32	32	
Number with young	23	21	
Male young	$1.1 \pm 0.2$	$1.5 \pm 0.2$	NS
Female young	$1.6 \pm 0.2$	$1.0 \pm 0.2$	NS
Young in left horn	$2.0 \pm 0.4$	$0.9 \pm 0.4$	*
Young in right horn	$0.7 \pm 0.2$	$1.6 \pm 0.3$	*

Values are means  $\pm$  SEM.

P values are for two-tailed Student's  $t$  tests.

\* $P < 0.02$ .

NS: not significant.



**Fig. 2.** Percentage (mean  $\pm$  SEM) of male fetuses found in left (L) and right (R) uterine horns of females assigned to ( $\square$ ) control and ( $\blacksquare$ ) experimental groups. The number of uterine horns contributing fetuses to each group is indicated in brackets above the bars.

than in their left, while females in the experimental group (those that had tissue from both left and right ovaries placed in the contralateral ovarian capsule) gestated a greater proportion of male fetuses in their left uterine horns than in their right (Fig. 2).

A  $2 \times 2$  ANOVA revealed no main effect of either the experimental conditions or uterine horn on the sex ratios of fetuses gestated in left and right uterine horns (both  $F_{1,71}$  values  $< 0.99$ , neither were significant). However, as would be predicted on the hypothesis that the difference in sex ratio of fetuses gestated in the left and right uterine horns of intact Mongolian gerbil dams (Clark and Galef, 1990) is the result of asymmetry in their left and right ovaries, we did find a significant effect of the interaction between experimental conditions and uterine horn on the production of male and female fetuses in left and right uterine horns ( $F_{1,71} = 5.99$ ,  $P < 0.02$ ).

As in Expt 1, ipsilateral grafts of ovarian tissue were more likely to produce fetuses ( $3.6 \pm 0.4$  pups per female) than were contralateral grafts ( $2.4 \pm 0.2$  pups per female) and, in consequence, females assigned to the control group gestated

**Table 2.** Sex composition of Caesarian-delivered litters from Mongolian gerbil females assigned to control and experimental groups in Expt 2

Dependent variable	Group		P
	Control	Experimental	
Number of animals	45	45	
Number with young	25	27	
Male young	1.8 ± 0.3	1.4 ± 0.2	NS
Female young	1.8 ± 0.2	1.0 ± 0.2	*
Young in left horn	1.8 ± 0.3	0.8 ± 0.2	*
Young in right horn	1.8 ± 0.2	1.6 ± 0.2	NS

Values are means ± SEM.

P values are for two-tailed Student's *t* tests.

\**P* < 0.02.

NS: not significant.

significantly more fetuses than did females assigned to the experimental group (Student's *t* test,  $t_{50} = 2.98$ ,  $P < 0.02$ ) (Table 2).

### Discussion

The results of both experiments are consistent with the hypothesis that the difference in the sex ratios of fetuses found in the left and right uterine horns of Mongolian gerbil dams late in pregnancy (Clark and Galef, 1990) reflects asymmetries in the left and right ovaries of female gerbils rather than in their left and right uterine horns.

As noted, there is considerable evidence of lateral asymmetries in both the morphology and function of mammalian ovaries. Unfortunately, a catalogue of such differences does not help to explain the consistent difference in the sex ratio of fetuses developing in the right and left uterine horns of Mongolian gerbil dams. Possibly, ova shed in the right ovary of a female gerbil are more likely to be fertilized by Y-bearing spermatozoa than are ova shed by the left ovary. Alternatively, one or both ovaries may secrete some substance into their ipsilateral uterine horns that makes them differentially susceptible to implantation by male and female blastocysts. Regardless of the physiological or hormonal processes involved, the present results are consistent with the conclusion that there is a functional asymmetry in the ovaries of female Mongolian gerbils that plays an important role in determining the sex of the pups gestated and delivered by a female gerbil.

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