Litter Parameters and Spontaneous Abnormalities in Himalayan Rabbits

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A joint study was undertaken in three testing facilities to evaluate cumulative background data of Himalayan rabbits. All litters were derived from control does. The conception rate was high (84.0–95.1%) but the average numbers of corpora lutea (7.9–8.7), implantation sites (6.5–7.5) and viable fetuses (5.8–6.9) were somewhat lower than that of most other strains of rabbit. Altogether 90 malformed fetuses (1.12%) and 425 fetuses with variations (5.27%) occurred among 8,060 viable fetuses.

The Himalayan rabbit has been bred by us in closed colonies since 1966. In two previous papers [7, 10] the advantages of Himalayan rabbits, the general features of their fertility, reproduction and growth have been reported, and their practical suitability in reproduction toxicology testing, such as higher conception and weaning rate, and lower body weight have been discussed. In their compilation, Kameyama et al. [4] have published the teratologic background data of rabbits from various sources in Japan already including some data on Himalayan rabbits. The litter parameters and spontaneous abnormalities of Himalayan rabbits obtained from control does from teratologic studies are presented in this paper. The joint study combines data from 3 testing facilities of the Boehringer Ingelheim group, Labs 1–3.

Material and Methods

Rabbits of Labs 1 and 3 were purchased continuously for each study from the experimental animal breeding center of Dr. Karl Thomae GmbH, as was the first breeding group

of Lab 2 in 1971. Since then, the Himalayans were random-bred at Lab 2 in a closed colony, while at Dr. Karl Thomae GmbH, supplier for Labs 1 and 3, animals were not rigorously bred at random, but selected according to their litter size [7, 10].

Does were at least 16-weeks old, weighing 2328 ± 83 g (Lab 1), 2207 ± 263 g (Lab 2) and 2350 ± 97 g (Lab 3) at the beginning of trial. All does were inseminated twice in the morning and were considered fertilized when the buck both times wearily collapsed. A male usually succeeded in inseminating two females within 30 min. This day was counted as day 0 of pregnancy. Standardized pelleted dry food (fortified 9970 S, Altromin GmbH, Lage (Labs 1 and 3)), CR-1 (Clea Japan Co. Ltd., Osaka (Lab 2)), and tap-water from drinking bottles were available ad libitum.

All does were controls vehicle-treated during organogenesis and were killed on day 29 of gestation. Immediately after uterine incision, the number of fetuses, their placement in the uterine horns, correlation with the number of corpora lutea, the number of viable

and dead fetuses, and the number of early and late resorptions were determined per doe. Viable fetuses were weighed, given an identity number and examined for external abnormalities, sexed and examined for skeletal abnormalities using radiography. Thereafter, the fetuses were subjected to autopsy and examined for cephalic abnormalities by razor blade sections. Abnormalities were classified following Palmer [11, 12] modified according to nowadays standards.

Results

Deaths among pregnant does were rare: six at Lab 1, from unknown reasons, two at Lab 2, namely one from an accident and the other from catarrhal enteritis; 11 at Lab 3, namely four from misapplication, one from gastroenteritis and six from trauma. The litter parameters of Himalayan rabbits are shown in Table 1, the findings in fetuses listed in Tables 2 and 3.

Discussion

Between the three Labs compared, the conception rate of our Himalayan rabbits was between 84.0% and 95.1%. If compared with data published for other strains of rabbit (Table 4), this is distinctly higher than that of Japanese White (JW) and most other strains. Abortions (0.4–3.1%) compared well with most other colonies. The incidence of does with total resorption (0.4–1.7%) was either very low or lay between that of New Zealand White rabbits (NZW), which also had low values, and that of the other strains showing a higher percentage of fetal loss.

The average numbers of corpora lutea (7.9–8.7) and implantation sites (6.5–7.5) per litter were lower than for NZW, most JW and “hare-rabbits” but higher than for Silver Fawns. The percentage of dead implants to implantation sites was about 7.8–12.1%. This was rather high compared to the Silver Fawn rabbit, but compared well with NZW. The percentage lay within the wide range of JW rabbit data, and was markedly lower than that of the “hare-rabbits.” However, the average

small litter size of Himalayans (5.8–6.9) was rather small compared with all other strains except Silver Fawns (Table 4).

There were also marked differences between the 3 Labs keeping the Himalayan rabbits, although the animals originate from the same source [10]. Conception rates and litter parameters in particular, differed greatly. The different breeding strategy may account for differences in conception rate between Labs 1 and 3 versus Lab 2. The conception rate is remarkably higher in the latter compared with Labs 1 and 3 where the insemination became difficult—a problem unknown in Lab 2. Also, the slightly lower average body weight at Lab 2 may indicate that the does were younger and, therefore, their conception rate higher. Other interlaboratory differences like litter size cannot be attributed to the breeding procedures nor to feed ingredients. These factors are identical in Labs 1 and 3 where differences are more pronounced than between Labs 2 and 3. Whether geographic or minor housing differences may influence such parameters remains to be understood.

In rabbits the rate of abnormalities is relatively high, namely near or above 1% of viable fetuses [1, 11]. By external examination, 30 of our fetuses (Table 2) showed malformations (0.37%) and among them omphalocele was most common ($4/8060 = 0.05\%$). This is somewhat lower than the incidence of 0.08% omphalocele reported in NZW fetuses [12], and 0.11–0.25% in JW rabbits [4].

A total of 37 Himalayan fetuses showed skeletal abnormalities (0.46%). Among them, deformities of vertebral bodies or arches and ribs (0.16%) are rather common in rabbits, namely 0.12% in NZW [12] and 0.58% in JW [9]. Fused sternbrae (0.29%) were also reported by Palmer in much higher frequency in NZW (1.26%).

Among the 33 visceral abnormalities (0.41%), renal defects were relatively common in Himalayan rabbits in Lab 2, namely altogether 5/2247 (0.22%), but did not occur in either of the other colonies and were rare in JW, “hare-rabbits” and Silver Fawns [2, 4, 9, 15]. They were observed, however, in fetuses of NZW (0.11%) [12]. Cardiovascular malformations

Table 1. Litter parameters of Himalayan rabbits

	Lab 1	Lab 2	Lab 3
<i>Maternal animals</i>			
Females inseminated	592	427	475
Pregnant females	497 (84.0%)	406 (95.1%)	419 (88.2%)
Mortality	6 (1.2%)	2 (0.5%)	11 (2.6%)
Abortions + premature birth	2 (0.4%)	6 (1.5%)	13 (3.1%)
Does with dead implants only	2 (0.4%)	7 (1.7%)	—
Does with viable fetuses	487 (98.2%)	391 (96.3%)	395 (83.2%)
<i>Total number</i>			
Corpora lutea	4239	3101	3419
Implantation sites	3655 (86.2%)	2557 (82.5%)	2772 (81.1%)
Dead implants	285 (7.8%)	310 (12.1%)	329 (11.9%)
Early resorptions	234	208	246
Late resorptions	44	74	78
Dead fetuses	7	28	5
Viable fetuses	3370	2247	2443
Males: Females	1598:1772 (47.4%)	1126:1121 (50.1%)	1222:1221 (50.0%)
<i>Average per litter^{a)}</i>			
Corpora lutea	8.7 ± 0.6	7.9 ± 1.5	8.6 (6.9–10.3)
Implantation sites	7.5 ± 0.6	6.5 ± 2.0	6.5 (5.9–8.1)
Viable fetuses	6.9 ± 0.6	5.8 ± 2.0	5.8 (4.9–7.5)
Litter weight (g)	not calculated	201.6 ± 64.0	not calculated
Fetal weight (g)	38.0 ± 1.4	36.0 ± 4.7	39.2 (36.0–42.4)

a) : Means ± S. D. or tolerance limits 95%

Lab 1 : Dr. Karl Thomae GmbH, 1974–1984

Lab 2 : Nippon Boehringer Ingelheim Co., Ltd., 1972–1984

Lab 3 : Boehringer Ingelheim KG. 1977–1984

Table 2. Malformations found in fetuses of Himalayan rabbits

	Lab 1	Lab 2	Lab 3
Number of litters examined	487	391	395
Number of fetuses examined	3370	2247	2443
Number of litters with malformed fetus	33 (6.78%)	21 (5.37%)	27 (6.84%)
Number of fetuses with malformation	36 (1.07%)	24 (1.07%)	30 (1.23%)
(External examination)			
Anasarka	2 (0.06%)	—	—
Schistosoma reflexum + amelia & cleft palate	—	—	1 (0.04%)
Multiple arthrogryposis	—	1 (0.04%)	—
Cranioschisis	—	1 (0.04%)	—
Acephaly	—	—	1 (0.04%)
Acrania (Monster)	2 (0.06%)	—	—
Acrania with gastroschisis and absence of gallbladder	1 (0.03%)	—	—
Acrania and omphalocele	—	1 (0.04%)	—
Exencephaly + open eye & cleft plate	—	—	1 (0.04%)
Encephalocele	1 (0.03%)	—	—
Meningocele	1 (0.03%)	—	—
Anophthalmia + cleft lip & cleft palate & absent upper incisivi & hydrocephaly	—	—	1 (0.04%)
Brachygnathia	2 (0.06%)	—	—
Gnathopalatoschisis	1 (0.03%)	—	—
Cleft palate	1 (0.03%)	—	—
Scoliosis	—	1 (0.04%)	1 (0.04%)
Scoliosis + cleft palate	—	—	1 (0.04%)
Ectopia cordis	1 (0.03%)	1 (0.04%)	—
Gastroschisis	1 (0.03%)	—	—
Omphalocele	1 (0.03%)	—	—
Omphalocele and renal agenesis	—	1 (0.04%)	—
Omphalocele, fused sternbrae and renal caudaposition	—	1 (0.04%)	—
Absense of thumb (Oligodactyly)	1 (0.03%)	—	—
Micromelia	—	1 (0.04%)	—
Kinked tail	—	—	1 (0.04%)
(Skeletal examination)			
Composed anomaly of thoracic spine	—	3 (0.13%)	1 (0.04%)
Cleft and blocked vertebrae	1 (0.03%)	1 (0.04%)	—
Reduced number of vertebrae	—	—	3 (0.12%)
Hemivertebra	1 (0.03%)	—	—
Fused ribs	1 (0.03%)	1 (0.04%)	—
Branched ribs	—	1 (0.04%)	—
Fused sternbrae	9 (0.27%)	5 (0.22%)	9 (0.37%)
(Visceral and cephalic examination)			
Hydrocephaly	1 (0.03%)	—	—
Hydrocephaly + cleft palate	—	—	2 (0.08%)
Dextroposition of Aorta	—	2 (0.09%)	—
Truncus arteriosus persistent with septal defect of the heart	2 (0.06%)	—	—

Truncus arteriosus persistent with cor triloculare univentriculi	2 (0.06%)	—	—
Absence of gallbladder	10 (0.30%)	—	7 (0.29%)
Double gallbladder	—	—	1 (0.04%)
Renal agenesis	—	2 (0.09%)	—
Renal caudaposition	—	1 (0.04%)	—

Table 3. Variations found in fetuses of Himalayan rabbits

	Lab 1	Lab 2	Lab 3
Number of litters examined	487	391	395
Number of fetuses examined	3370	2247	2443
Number of litters with variated fetus	128 (26.28%)	75 (19.18%)	82 (20.76%)
Number of fetuses with variation	234 (6.94%)	96 (4.27%)	95 (3.89%)
Generally delayed ossification	2 (0.06%)	—	—
Enlarged fontanelle posterior	—	—	1 (0.04%)
Delayed ossification of the skull	1 (0.03%)	—	—
Slightly dilated cerebral ventricles	4 (0.12%)	—	—
Retina folds	154 (4.57%)	—	—
Dilated cardiac ventricles	13 (0.39%)	—	—
Abnormal lobulation of lungs	13 (0.39%)	2 (0.09%)	—
Agenesis of accessory lobe of lung	—	—	49 (2.01%)
Split of sternebrae	—	3 (0.13%)	1 (0.04%)
Asymmetry of sternebrae	—	3 (0.13%)	4 (0.16%)
13 th ribs	3 (0.09%)	65 (2.89%)	11 (0.45%)
Short 12 th ribs	2 (0.06%)	1 (0.04%)	—
Agenesis or short 12 th ribs	—	—	2 (0.08%)
Hypoplasia of gallbladder	12 (0.36%)	14 (0.62%)	—
Bilobed gallbladder	—	9 (0.40%)	2 (0.08%)
Dilated renal pelvis	—	—	3 (0.12%)
Flexure of fore paws	37 (1.10%)	2 (0.09%)	20 (0.82%)
Flexure of fore paws + 13 th ribs	—	—	1 (0.04%)
Flexure of fore paws + dilated renal pelvis	—	—	1 (0.04%)
Delayed ossification of the extremities	5 (0.15%)	—	—
<i>Number of fetuses with unossified sternebra V</i>	not calculated	176/963 (18.28%)	625/1339 (46.68%)

were found in eight fetuses (0.10%) of Himalayan rabbits, in two JW fetuses (0.58%)[9], in 21 NZW fetuses (0.04%) [12], or nine NZW fetuses (0.28%) [13] and in 37 Fauve de Bourgo- gne (0.32%) [12]. Such malformations are rarely described by other authors [3, 4].

Among variations, the frequency of the 13 th ribs (Table 3) differed largely between the three Himalayan colonies but was lower

than that of JW and NZW [4, 5, 9, 12, 13, 14], or Dutch belted rabbits (27.4%) [8]. The low incidence of the 13 th ribs was also reported in another Himalayan colony [4]. Hypoplastic and bilobed gallbladder were rather common in Lab 2 only. The extrahepatic biliary tract of the NZW also shows a wide range of variations [6]. No morphologic deviations of gallbladder were observed in JW [9,

Table 4. Comparison of maternal performance, litter parameters and malformation rates in various strains of rabbit

Testing facilities or Authors	Strains	Maternal animals				Average per litter				Malformation rates				Variation rate		
		Number of fetuses examin.	Pregnant females/ Insemin. %	Abort./ Pregnant Females %	Only dead implants/ Females %	Corpora lutea	Implant. sites	Dead implants %	Viable fetuses %	Ex- ternal %	Skeletal %	Visceral %	Total %	13th ribs %	Gall bladder %	
Lab 1:Thomae	Himalayan	3370	84.0	0.4	0.4	8.7	7.5	7.8	6.9	0.42	0.36	0.45	1.07	0.09	0.36	
Lab 2: NBI	Himalayan	2247	95.1	1.2	1.7	7.9	6.5	12.1	5.8	0.36	0.49	0.22	1.07	2.89	1.02	
Lab 3: BIKG	Himalayan	2443	88.2	3.1	—	8.6	7.0	11.9	6.2	0.29	0.53	0.41	1.23	0.45	0.08	
Sugisaki et al.[14]	JW	538	—	2.2	2.2	—	7.8	20.6	6.3	1.7	1.7	—	—	60.4	0	
Kameyama et al., [4]	JW	—	—	—	—	9-10	7-9	6-16	6-8	0.3-1.7	0.3-1.7	0-1.1	—	4-60	—	
Matsubara et al., [9]	JW	1217	78.6	5.1	—	8.5	7.1	8.5	6.5	0.57	0.69	0.16	—	4.03	0.08	
Palmer [12]	NZW	52626	92	3.1	1.4	10.5	9.3	15.6	7.8	—	—	—	0.9	—	1.19	
Stadler et al. [13]	NZW	5592	81.5	1.6	1.2	9.8	7.8	10.9	7.0	0.61	2.29	0.41	0.93	45.1	0.19	
Fritz et al. [2]	"hare- rabbits"	2207	79.1	—	2.4	—	8.6	20.5	6.9	—	—	—	0.91	—	1.68	
Fritz et al. [2]	Silver Fawn	3006	81.4	—	5.3	—	6.1	8.4	5.6	—	—	—	0.70	—	—	
Palmer [12]	Faune Bourgogne	11558	90	1.1	1.8	—	8.8	14.1	7.8	—	—	—	1.68	—	0.10	

14, 15], whereas Palmer [12] reported missing (0.32%) and bilobed (0.31%) gallbladder in NZW. “Hare”-rabbits had no gallbladder in 3.27% [2]. Retina folds (4.57%) were observed and histologically confirmed in Lab 1 only. The incidence of fetuses with unossified sternbra V (18.3–46.7%) compared well with one JW-NIBS colony (16.1%) [5], but was much higher than that of another JW-NIBS colony (3.0%) [9].

Such differences in type and number of abnormalities between laboratories working with the same strain of rabbit may have various reasons: differences in minor abnormalities like retina folds or hypoplastic gallbladder may be due to specific investigational methods of individual researchers. But this cannot hold for the majority of findings. For example, counting of ribs is done comparably in all laboratories with extremely differing results. Possibly, the interlaboratory variation may be an expression of the variability of this strain of rabbit.

The above data are additional to those previously published [7, 10] on the same strain, and show that the Himalayan rabbit is a useful test system in the field of reproduction toxicology.

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Himalayan ウサギの自然発生奇形および胎仔計測値

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Himalayan ウサギを使用している研究施設3カ所で、これまでに蓄積された同系ウサギの生殖能に関する背

景データを比較検討した。対象となる同腹仔はすべて試験対照群に用いた母動物から得られたものである。

Himalayan ウサギでは、他の系統と比較して妊娠率（84.0～95.1%）は高かったが、黄体数（7.9～8.7）、着床数（6.5～7.5）および生存胎仔数（5.8～6.9）の

平均値はやや低かった。生存胎仔8,060例の検索で、奇形胎仔は90例（1.12%）、また変異胎仔は425例（5.27%）に認められた。