Table 2. Mean testis, epididymus and seminal vesicle wights in sham-operated and vasectomized mice

| Treatment | | Mean testis weight ± SE (mg) | Mean seminal vesicle + coagulating gland weight ± SE (mg) | Mean epididymus weight ± SE (mg) |
|---|---|---|---|---|
| Experiment 1 | Sham | 103.44 ± 5.25 | 371.33 ± 13.82 | 44.02 ± 1.49 |
| | Vasectomized | 94.55 ± 3.78 | 340.80 ± 11.81 | *54.84 ± 3.48 |
| Experiment 2 | Sham | 102.39 ± 4.31 | 314.09 ± 28.22 | 38.67 ± 1.17 |
| | Vasectomized | 98.16 ± 4.79 | 313.75 ± 31.26 | *65.59 ± 3.97 |

*$p < 0.01$.

*Materials and methods.* This study was performed using adult male mice of the random bred Q strain. Bilateral vasectomy was carried out under Nembutal anaesthesia using a ventral approach. The vas deferens was cut between single proximal and distal ligatures placed approximately 5 mm apart. In the sham operation the vas deferens was mobilized with a pair of forceps but not interrupted. The animals were rested for 2 weeks following the operation before the recommencement of mating trials. In order to record coital frequency the males were placed in separate cages together with 4 normal adult females of the same strain. These females were subsequently checked daily for the presence of vaginal plugs. As soon as an animal had been mated she was removed from the cage and replaced by another female. At the end of the experiment the males were killed by cervical dislocation and their testes, epididymes and seminal vesicles removed, cleaned and weighed. 2 experiments were performed which differed only in the duration of the pre- and post-operative periods. The significance of the results was assessed by the analysis of variance.

*Results and discussion.* The coital frequencies (number of females mated in each 10-day-period) observed in experiments 1 and 2 are presented in table 1. The pre- and post-operatives scores observed for the vasectomized animals and the post-operative scores recorded for the sham-operated and vasectomized groups were not significantly different from each other. Testis and seminal vesicle weights (table 2) were similarly not influenced by vasectomy, and no significant correlation was observed between testis weight and coital frequency. In contrast, epididymus weight showed a highly significant increase ($p < 0.01$) following vasectomy (table 2), apparently as the result of fluid accumulation.

The results obtained in these experiments indicate that male sexual activity is not influenced by vasectomy, thereby confirming the clinical data. The measurement of coital frequency would seem a particularly useful test of sexual activity in a number of other contexts, such as assessing the influence of anti-androgens, oestrogens or progesterones on male sexual function, and may provide more meaningful information than the detailed analysis of coital behaviour.

## Ovarian HCG-binding in hemicastrated immature female rats*

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*Summary.* Hemigonadectomy in 9-day-old female rats results in a drastic increase in the weight and the number of HCG-binding sites of the remaining ovary during further development. However, on a per mg basis of ovarian weight, the number of HCG-receptors is identical in hemicastrated and control rats.

In the adult rat after unilateral ovariectomy, the other ovary enlarges ('compensatory hypertrophy')[3-5]. Even after removal of one ovary and a half the remaining ovarian fragment has been found to hypertrophy to the weight of one ovary in control rats[6]. The most favoured hypothesis to explain compensatory hypertrophy is that the transient decrease in blood steroid levels from removal of one ovary reduces the negative feedback effect of the steroids and thereby triggers an increase in gonadotrophin secretion of the pituitary[7-11]. This increase causes the remaining ovary to increase in size and secretory activity. Accordingly, oestradiol or oestriol treatment of the hemiovariectomized rat prevents the compensatory ovarian hypertrophy[9, 12].

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Binding of $^{125}$I-labelled HCG by ovarian homogenates from hemiovariectomized and control rats at various stages of development tested in the range of 2 to 70 ng of free hormone

| Age (days) | Number of binding sites (mol/mg wet weight [$\times 10^{15}$]) | | Dissociation constants ($K_D$ [$\times 10^{10}$]) | | Number of binding sites (mol/ovary [$\times 10^{15}$]) | | Ovarian weight (mg) | |
|---|---|---|---|---|---|---|---|---|
| | Hemiovariectomized rats | Controls | Hemiovariectomized rats | Controls | Hemiovariectomized rats | Controls | Hemiovariectomized rats | Controls |
| 20 | 4.20 | 4.69 | 5.62 | 5.80 | 36.33 | 25.51 | 8.65* $\pm$ 1.02 | 5.44 $\pm$ 1.02 |
| 26 | 6.51 | 6.43 | 5.32 | 5.62 | 106.31 | 57.36 | 16.33** $\pm$ 1.01 | 8.92 $\pm$ 1.03 |
| 32 | 8.40 | 9.30 | 5.25 | 5.69 | 287.28 | 209.25 | 34.20* $\pm$ 3.54 | 22.50 $\pm$ 3.29 |

For comparison of ovarian weight between experimental and control animals the t-test was done. *$0.001 > \alpha$ 0.0001, **$0.0001 > \alpha$. Mean values $\pm$ SE are included.

In the prepubertal female rat, the effect of hemiovariectomy has been studied by Ojeda and Ramirez[13]. Hemicastration of 10-day-old rats resulted in a significant ovarian hypertrophy only 10 days after the operation. In contrast to adult rats, in the immature rats hemiovariectomy is not followed by an increase in plasma gonadotrophin concentration. Since the effect of the gonadotrophins onto the ovary is mediated by specific membrane-bound gonadotrophin receptors, one might speculate that in the immature female rat the compensatory hypertrophy of the remaining ovary after hemiovariectomy is due to an increase of the number of receptors per ovarian cell, and/or due to an increase of the affinity of the ovarian gonadotrophin receptors. To test these possibilities, we studied the binding of human chorionic gonadotrophin (HCG) to the ovaries of control and hemiovariectomized immature rats during sexual maturation.

*Material and methods.* The animals used in this experiment were raised in the animal quarters of our institute and belong to the SIV-50 rat strain. 28 rats were hemiovariectomized (right ovary) under ether anesthesia at 9 days of age. 26 animals were selected from the same litters and served as controls. 1 h post-surgery, the young were returned to their mothers. All the animals were housed in air-conditioned and light-controlled animal rooms (light period from 6 a.m. to 9 p.m.). Animals to be killed after 20 days of age were weaned on the 20th day. Ovarian HCG-binding was determined in operated and control rats at day 20, 26 and 32 of age.

At autopsy ovarian weights were recorded and histological sections of the ovaries removed were performed. Our experimental procedure used for the demonstration of HCG-binding to ovarian homogenates has been described in detail recently[14,15]. Specific radioactivity of the $^{125}$I-labelled HCG (biological activity: 11,000 IU/mg) was 30–50 $\mu$Ci/$\mu$g. The ovaries removed were homogenized 1:10 (w/v) in cold Tris-HCl buffer (0.04 moles/l, pH 7.4 containing $MgSO_4$ 0.005 moles/l) and subsequently centrifuged at $100 \times g$ for 20 min. Aliquots of the supernatant corresponding to 5 mg tissue wet weight were incubated in duplicate in the homogenization buffer containing 0.1% bovine serum albumine (BSA) and varying amounts of labelled and unlabelled HCG, in a final volume of 1 ml, at 37 °C for 30 min. The reaction was stopped by the addition of 1 ml ice-cold buffer and the incubates were immediately filtered with suction through cellulose acetate filters (pore size 0.45 $\mu$m, Sartorius, Göttingen, BRD) previously washed with 10 ml 4% BSA. Then the filters were washed with 10 ml of the cold homogenization buffer and the radioactivity on the filters was counted in a liquid scintillation spectrometer. The amount of specifically bound $^{125}$I-HCG was determined as the difference between the radioactivity bound in samples containing a 1000fold excess of competing unlabelled HCG at each concentration of labelled hormone and in parallel samples with $^{125}$I-HCG alone.

*Results and discussion.* Hemiovariectomy in immature female rats results in a compensatory ovarian hypertrophy of the remaining ovary. While Ojeda and Ramirez[13], using rats operated on the 1oth day of age, observed the first distinct increase of ovarian size only on the 25th day of age, in our rats operated on the 9th day of age, a 1.6 fold-increase of ovarian weight was observable as early as the 20th day of age (table). This difference in ovarian weight between control and hemiovariectomized rats remained constant thereafter. According to our histological investigations, the compensatory hypertrophy in the immature female rats is due to an increase of medium and large follicles concomitant with the proliferation of theca and granulosa cells (not shown).

As can be seen from the table, ovarian compensatory hypertrophy after hemiovariectomy is accompanied by a similar increase in the number of HCG-binding sites per ovary. As compared with control rats, the number of HCG receptors in the remaining ovary is increased 1.4fold 11 days after the operation, 1.9fold 17 days and 1.4fold 23 days after the hemiovariectomy. However, on a per mg basis of ovarian weight, hemiovariectomized and control rats exhibit nearly identical HCG-binding sites. Scatchard[16] analysis of the results obtained in control and hemiovariectomized rats on the 20th, 26th and 32nd day of life reveal very similar dissociation constants ($K_D$) of the receptor-hormone complex in both groups, namely between 5.62 and $5.80 \times 10^{-10}$ $M^{-1}$ in control rats and between 5.25 and $5.62 \times 10^{-10}$ $M^{-1}$ in hemiovariectomized rats. From these results, it became evident that changes in the number of the LH-/HCG-receptors per cell, or an altered hormone affinity of these receptors, are not the underlying causes for ovarian compensatory hypertrophy in immature rats after hemiovariectomy.

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