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The influence of hormonal treatment with beta-human chorionic gonadotropin for cryptorchidism on future fertility in rats

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Summary

Introduction
There have been two treatment modalities for cryptorchidism such that surgical and hormonal; the latter being highly controversial. While some authors suggest that hormonal treatment increases the number and maturation of germ cells in cryptorchid testes, others believe just the opposite.

Objective
We aimed to find out the sperm counts and testicular index; briefly fertility potential of the normally descended contralateral testes in adulthood period in rats treated with Beta-HCG in early period of their lives.

Materials and methods
Three groups, each including 10 rats aged 22 days old, in which delactation and normal feeding can be started, were formed to be Group 1: Sham operated, Group 2: Experimental cryptorchidism (EC) and Group 3: Hormone-treated after experimental cryptorchidism was performed (HT-EC). Left testis was placed in the abdomen in group 2 and 3. In group 1, a sham operation was performed. The rats in EC-HT group received subcutaneous injections of 50 IU/kg Beta-HCG daily for 7 days. Right orchidectomy was performed when they reached reproductive period to evaluate fertility potential with sperm counts and testicular index. Testicular index was calculated according to the formula "testicular length × width/weight of rat". Epididymal sperm count was made with hemocytometer.

Results

Table Mean sperm count in cauda epididymis.

Groups	Sperm counts ($\times 10^6$/ml)
Group 1 (Sham)	425
Group 2 (EC)	265.89
Group 3 (EC-HT)	168.22

Mann–Whitney U test. $p < 0.05$ is threshold for significance.
$\mathbf{p} = \boldsymbol{0.008}$ for group 1–2, $\mathbf{p} = \boldsymbol{0.001}$ for group 1–3, $\mathbf{p} = \boldsymbol{0.017}$ for group 2–3.

Discussion
We evaluated the physical characteristics and fertility potential (sperm counts) of contralateral normal testes during adulthood in rats that underwent experimental unilateral cryptorchidism during infancy. A relationship between testis weight and sperm counts were also investigated. We could not find any direct correlation of sperm count with either testicular weight or testicular index in our study.

Although the rats had normal testes at birth, we found decreased sperm counts in contralateral normal testes in EC group. This suggests that unilateral cryptorchidism may cause some systemic effects that reach the other testis. Hormone treatment was not beneficial. This is comparable to Nambirajan et al. who reported histological changes and decreased spermatogenic cell count in contralateral scrotal testes in experimentally induced unilateral cryptorchidism in early period of life in rats. Heiskanen et al. reported that treatment with Beta-HCG leads to decreased total sperm counts in the future due to increased germ cell apoptosis caused by hormonal withdrawal after treatment. Cortes et al. also reported decreased number of germ cells in 1–3 year-old boys who underwent surgery after unsuccessful Beta-HCG treatment. The reasons could be delayed testicular descent or adverse effect of hormone treatment though. Our results concurred with them.

Apparently, our model has failed to mimic the pathophysiologic mechanisms of congenital cryptorchidism in humans. Furthermore, we applied hormone treatment in normal rats with normally descended testes. Therefore, the "by-product" information of our study is that, unnecessary use of Beta-HCG during infancy may impair future fertility.

Conclusion
Our study suggests that Beta-HCG treatment may decrease sperm counts and decrease the future fertility potential. We could not find any direct correlation of sperm count with either testicular weight or testicular index.

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Introduction

There are two treatment modalities for cryptorchidism: surgical and hormonal [1], with the latter being highly controversial. Beta-Human Chorionic Gonadotropin (Beta-HCG), Gonadotropin Releasing Hormone (GnRH) and Luteinising Hormone Releasing Hormone (LHRH) agonists have been used to provide testicular descent and to enhance fertility potential of individuals [2]. Whereas some authors suggest that hormonal treatment increases the number and maturation of germ cells in cryptorchid testes [3], others believe just the opposite [4] by referring to reports of increased apoptosis of spermatogonia and decreased numbers of germ cells after hormonal treatment [5,6].

We hypothesized that hormone treatment in rats that underwent experimental cryptorchidism would enhance fertility potential in the long term. We aimed to find out the sperm counts and testicular index, that is the fertility potential of normally descended contralateral testes in adulthood in rats treated with Beta-HCG in the early period of their lives. To our knowledge, ours is the only study investigating the issue in this regard.

Material and methods

After obtaining local ethics committee approval, 30 Sprague—Dawley rats aged 22 days old, in which delactation and normal feeding could be started, were included in our study. This age in rats mimics infancy in human beings. Temperature of the environment was kept at 21—23 °C, and each cage contained five rats. Periods of 12 h of daylight and 12 h of darkness were provided. Three groups, each including 10 rats were formed: Group 1: Sham operated (SO), Group 2: Experimental cryptorchidism (EC), and Group 3: Hormone-treated after experimental cryptorchidism was performed (HT-EC).

Anesthesia was achieved by administering a mixture of 40 mg/kg of ketamine and 10 mg/kg of xylocaine intraperitoneally. Left testes were freed completely, and placed in the abdomen through a 1-cm incision in Groups 2 and 3. In Group 1, a sham operation was performed, in which left testes were delivered out of the incision, and replaced in the scrotum only after manipulation. The rats in Group 3 received subcutaneous injections of 50 IU/kg Beta-HCG daily [7] for 7 days, starting on the first postoperative day after cryptorchidism was performed.

All rats were kept in appropriate conditions until they reached the reproductive period, which starts at 12 weeks old; this age mimics adulthood in human beings. Right orchidectomy was performed 12 weeks after the first operation to evaluate fertility potential with sperm counts and testicular index. Testicular index was calculated according to the formula "testicular length × width/weight of rat", which has been reported to be an indicator of fertility potential in rats [8,9], and the aim was to test whether or not this parameter reflects sperm counts.

The caput and caudal part of the epididymis was dissected and cut into 1 mm^3 pieces with a sharp razor blade, then kept in Kreb's Ringer phosphate (KRP) buffer. Spermatozoa from the epididymal pieces were removed by vortexing gently in buffer, and the suspension was allowed to settle for 1 h. The suspension was placed on hemocytometer to count the sperm [10].

SPSS version 16.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analyses and $p < 0.05$ was accepted as threshold for significance. Comparison between the groups was made with Mann—Whitney *U* test.

Results

Two rats died just after the first operation in Groups 2 and 3. Sperm counts from caput and cauda epididymides, weights of testes and testicular indices were determined. Mean cauda epididymal sperm counts (CaESC) were 425×10^6 in Group 1 (SO), 265.89×10^6 in Group 2 (EC) and 168.22×10^6 in Group 3 (HT-EC).

Groups 2 and 3 had significantly less CaESC than Group 1 ($p = 0.008$ and $p = 0.001$). Furthermore, Group 3 had less CaESC than EC group and the difference was statistically significant for CaESC ($p = 0.017$). In terms of caput epididymal sperm count (CpESC), sperm counts gradually decreased from Group 1 towards Group 3, the only significant difference being between Group 1 and Group 3.

Our data show that experimental cryptorchidism affected the sperm counts negatively and Beta-HCG treatment further decreased the numbers ($p < 0.05$) (Figs. 1 and 2).

There were no significant differences between the groups in terms of testicular index and testis weight ($p > 0.05$) (Fig. 3).

Discussion

We evaluated the physical characteristics and fertility potential (sperm counts) of contralateral normal testes during adulthood in rats that underwent experimental unilateral cryptorchidism during infancy. Germinal cells and tubules constitute up to 98% of testicular tissue, suggesting that testicular volume may be an indicator of spermatogenesis. Testicular index related to shape and volume has been

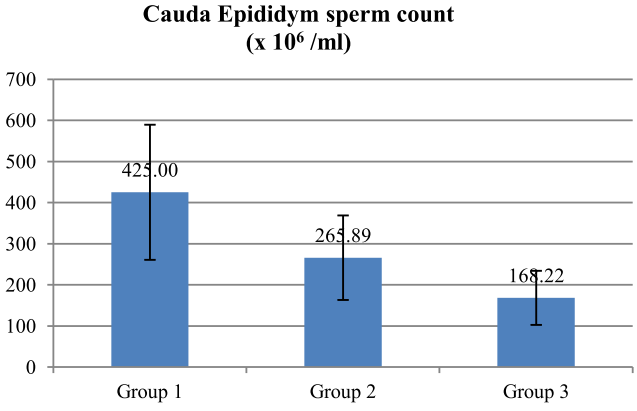

Figure 1 Mean sperm count in cauda epididymis of Group 1: Sham operated (SO), Group 2: Experimental cryptorchidism (EC), and Group 3: Hormone-treated after experimental cryptorchidism (EC-HT). $p = 0.008$ for Groups 1—2, $p = 0.001$ for Groups 1—3, $p = 0.017$ for Groups 2—3.