

# Effects of Experimental Cryptorchidism on Testicular Function in Adult Rats

BERNARD JEGOU, GAIL P. RISBRIDGER, AND DAVID M. DE KRETZER

The effects of surgically-induced cryptorchidism on testicular function in adult rats was studied, with particular emphasis on the temporal relationship between the onset of cryptorchidism and a number of parameters of Sertoli cell, Leydig cell, and pituitary function. Sertoli cell function assessed by measurement of testicular ABP content, ABP production rate, testicular fluid production, and FSH receptor levels was found to be disrupted very rapidly (one to two days) after inducing cryptorchidism. By four days, ABP production rate and fluid production were found to represent only 12% and 65% of control levels, respectively. However, these parameters were maintained at these levels even 70 days following cryptorchidism. The binding of  $^{125}\text{I}$ -FSH to testes homogenates was found to decrease steadily to less than 10% of control levels after 70 days of cryptorchidism. Serum FSH levels were elevated 14 days postcryptorchidism and there was a highly negative correlation between the rise and both ABP and fluid production. Serum levels of LH rose after 14 days of cryptorchidism, although testosterone levels were not significantly altered throughout the experiment. The binding of iodinated hCG to testes homogenates also decreased significantly within six days of inducing cryptorchidism and remained low (16%) after 70 days of cryptorchidism. The results demonstrate rapid changes in aspects of both Sertoli and Leydig cell function after surgically-induced cryptorchidism.

**Key words:** testis, cryptorchidism, Sertoli cell, Leydig cell, androgen binding protein, LH receptors, FSH receptors.

Experimental cryptorchidism is known to result in disruption of spermatogenesis, accompanied by elevated FSH levels (Moore, 1924; Clegg, 1963; Amatayakul et al, 1972; Altwein and Gittes, 1972; Walsh and Swerdloff, 1973). More recently, changes in Leydig cell function that accompany hypertrophy have been shown to result in low or

*From the Department of Anatomy, Monash University, Clayton, Victoria, Australia*

normal levels of serum testosterone and increased levels of serum LH (Kerr et al, 1979a). Despite the observation of morphologic changes in Sertoli cells (Kerr et al, 1979b) after cryptorchidism and impaired function as shown by decreased androgen binding protein (ABP) production (Hagenas and Ritzen, 1976; Kerr et al, 1979b), the persistence of these cells in the tubules is still taken to indicate their relative resistance to damage. Furthermore, since it has been postulated that the Sertoli cell may influence Leydig cell function, the present study reexamines the temporal relationships between a number of parameters of Leydig cell and Sertoli cell function after the induction of cryptorchidism in the rat.

## Materials and Methods

### *Experimental Animals*

Ninety-day-old Sprague-Dawley rats were used throughout this investigation.

*Induction of cryptorchidism.* Animals were randomly divided into two groups and classified either as controls or as rats made cryptorchid for one to four, six, eight, 14, 21, 28, 49, or 70 days. To render the animals cryptorchid, the rats were anesthetized with ether and the testes were translocated into the abdominal cavity. The inguinal canal was closed by ligation to prevent redescend of the testis into the scrotum.

*Efferent duct ligation for measurement of the testicular fluid production.* Unilateral ligation of efferent ducts of control and cryptorchid animals was carried out under ether anesthesia. The right or left testis, selected at random, was gently displaced from the scrotum in control rats or from the abdomen in cryptorchid rats, and the efferent ducts were then ligated. Care was taken not to disrupt blood supply to the testis. The testis was returned to the abdominal (cryptorchid) or scrotal (control) cavity and, 16 hours later, the animal was killed. The production rate of testicular fluid was measured in

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Reprint requests: Professor D. M. de Kretzer, Department of Anatomy, Monash University, Clayton, Victoria, Australia.

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both groups as the difference in weight between the ligated and nonligated testes.

#### Collection and Preparation of Tissue

Control and cryptorchid rats were killed by decapitation, and blood was collected. Testes and epididymides were rapidly dissected and weighed. Both ligated and nonligated testes were decapsulated and homogenized with 4 volumes of Tris-HCl buffer, pH 7.4, containing 1 mM EDTA, 2 mM mercaptoethanol, and 10% glycerol. Homogenates were centrifuged for 1 hour at  $105,000 \times g$  and the supernatants were stored at  $-20^\circ\text{C}$ . The pellets were resuspended in 1 ml of Tris-HCl buffer, pH 7.4, containing 10 mM  $\text{MgCl}_2$  and 0.1% bovine serum albumin (TMB buffer) and were stored at  $-20^\circ\text{C}$ .

#### ABP Assay

After homogenization of individual testes, 105,000 g cytosols were prepared. Equal aliquots of cytosols from four testes were pooled for ABP measurement using the steady state polyacrylamide gel electrophoresis method as previously described (Ritzen et al, 1974). The difference between the ABP content of ligated and unligated testes is equal to the production rate of ABP over 16 hours (Hagenas et al, 1978).

#### In Vitro Binding of $^{125}\text{I}$ -hCG and $^{125}\text{I}$ -FSH

Highly purified hCG (preparation CR 121, National Pituitary Agency) was iodinated using chloramine T (Greenwood et al, 1963) and purified on a prepacked Sephadex G25 column (1.5 ml, Pharmacia). The specific activity was  $38 \mu\text{Ci}/\mu\text{g}$ , as determined by the technique of Greenwood et al (1963). Pellets from the unligated testes were thawed, resuspended, and diluted to a concentration of 200 mg/ml. Aliquots of 100  $\mu\text{l}$  of testis homogenate were added to 100  $\mu\text{l}$   $^{125}\text{I}$ -hCG (100,000 cpm) and 50  $\mu\text{l}$  TMB buffer and incubated for 16 hours at  $22^\circ\text{C}$ . The nonspecific binding of  $^{125}\text{I}$ -hCG was determined by the addition of an excess of hCG (Pregnyl, Organon, 100 IU in 50  $\mu\text{l}$  TMB) instead of buffer alone. The incubation was terminated by the addition of 3 ml 0.9% saline and the tubes were centrifuged for 30 minutes. The supernatant was aspirated and the bound radioactivity was determined in a Packard gamma spectrometer. Specific binding to homogenates of control testes represented 15 to 22% of the added tracer.

Identical methodology was used to determine  $^{125}\text{I}$ -hFSH. Highly purified human FSH (Preparation hFSH-1, National Pituitary Agency) was labeled to a specific activity of  $49 \mu\text{Ci}/\mu\text{g}$  using lactoperoxidase (Miyachi et al, 1972) and purified as described for  $^{125}\text{I}$ -hCG. Aliquots of 200  $\mu\text{l}$  of testes homogenate (200 mg/ml) were added to 100  $\mu\text{l}$   $^{125}\text{I}$ -hFSH (50,000 cpm) and 50  $\mu\text{l}$  TMB buffer and incubated for 16 hours at  $22^\circ\text{C}$ . The nonspecific binding of  $^{125}\text{I}$ -hFSH was determined by the addition of an excess of hFSH (human pituitary gonadotrophin, Commonwealth Serum Laboratories, Australia: 40 IU in 50  $\mu\text{l}$  TMB), rather than buffer alone. Specific binding to homogenates of control testes represented 3 to 4% of the added tracer.

#### Radioimmunoassays

Serum was separated and stored at  $-20^\circ\text{C}$ . Testosterone was measured by radioimmunoassay according to a previously described method (Wang et al, 1974). The antiserum was raised against testosterone-3-carboxymethoxime coupled to porcine thyroglobulin. The intraassay coefficient of variation was 10.0% and the interassay coefficient of variation was 10.7%.

Levels of LH and FSH were measured by double antibody radioimmunoassays using methods described previously (Lee et al, 1975). The intraassay variations ranged from 7 to 9% for both assays and all samples were included in a single assay.

#### Statistical Analysis

Data were analyzed by Student's *t*-tests for comparison between groups, analysis of variance and Duncan's Multiple Range test, and the linear regression test.

## Results

#### Testicular and Epididymal Weights

No change in testicular weight was observed until four days after induction of cryptorchidism (Fig. 1). Between four days and two weeks, testis weight decreased and then reached a plateau representing approximately 35% of control.

Similarly, epididymal weight was not different from control between one and four days post-surgery (Fig. 2), but by six days a significant ( $P < 0.01$ ) and reproducible increase was found, which was followed by a subsequent rapid decrease by eight and 14 days. Epididymal weight then reached a plateau representing approximately 50% of control.

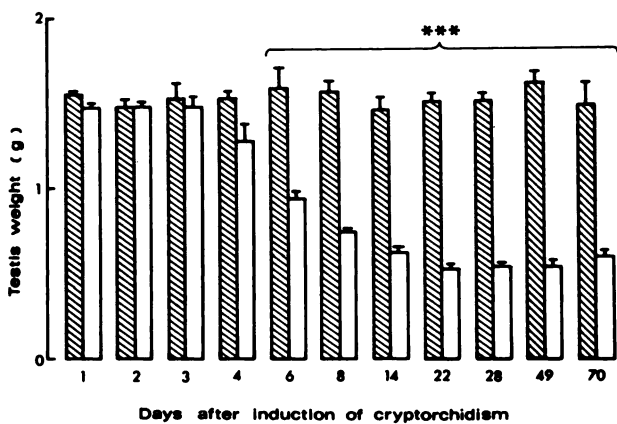


Fig. 1. Effect of cryptorchidism on testicular weights. The weights are expressed as mean  $\pm$  SEM ( $n = 5$ ).  $\blacksquare$  = control;  $\square$  = cryptorchid; \*\*\* $P < 0.001$ .

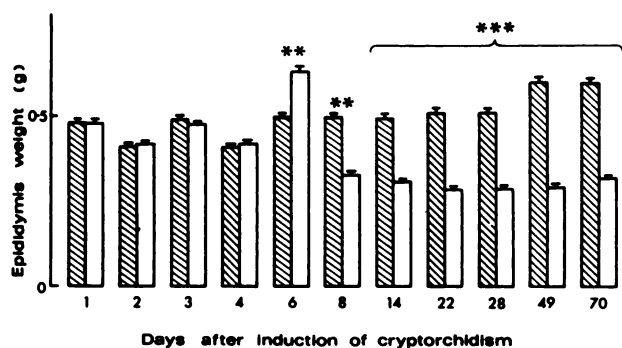


Fig. 2. Effect of cryptorchidism on epididymal weights. The weights are expressed as mean  $\pm$  SEM ( $n = 5$ ).  $\square$  = control;  $\square$  = cryptorchid; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

### Sertoli Cell Function

As early as two days after surgery, fluid production had decreased significantly ( $P < 0.01$ , Fig. 3). By six days, it has reached a plateau and remained consistently at 35% of control until the end of the experiment. The control values for fluid production did not change throughout the experiment.

As demonstrated by fluid production, the concentration of ABP measured as total amount for the testis in both ligated and nonligated sides fell by two days after the induction of cryptorchidism (Fig. 4). By 70 days after the induction of cryptorchidism, ABP was still produced by the testis and represented 28% and 17% of control for the nonligated and ligated testes, respectively.

The ABP production rate, measured as the difference in ABP content between the ligated and

the nonligated testes, decreased as early as the second day after the induction of cryptorchidism and became very low between the fourth and the end of the experiment.

### LH/hCG and FSH Receptors

A significant loss of testicular LH/hCG binding sites ( $P < 0.01$ ) was found by the sixth day after the induction of cryptorchidism (Fig. 5). This decrease was rapid until eight days and declined at a slower and linear rate between eight and 70 days postcryptorchidism.

In contrast, the number of FSH binding sites in the testis decreased significantly ( $P < 0.05$ ) as early as one day after the operation, then appeared to increase at three days, and subsequently declined rapidly between four and 14 days. The continued decrease was slower between 14 and 70 days (Fig. 6).

### Serum Testosterone, LH, and FSH Levels

Serum testosterone levels were significantly higher in control rats ( $P < 0.05$ ) on day 1 but did not differ significantly from cryptorchid rats at any other time (Fig. 7). Serum LH concentrations in cryptorchid rats (Fig. 7) were significantly lower than in controls ( $P < 0.05$ ) on the first day after the operation, but were significantly elevated on day 14 and remained elevated thereafter (Fig. 7). The serum FSH levels in the cryptorchid rats rose significantly by 14 days and remained elevated for the duration of the experiment.

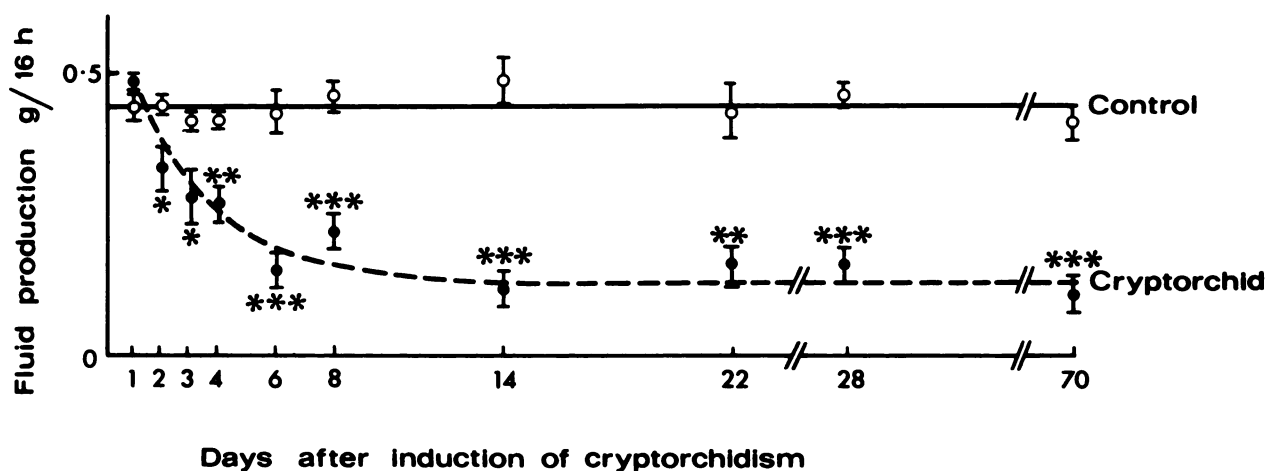


Fig. 3. Measurement of testicular fluid production by unilateral efferent duct ligation for 16 hours, after induction of cryptorchidism ( $n = 5$ ). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

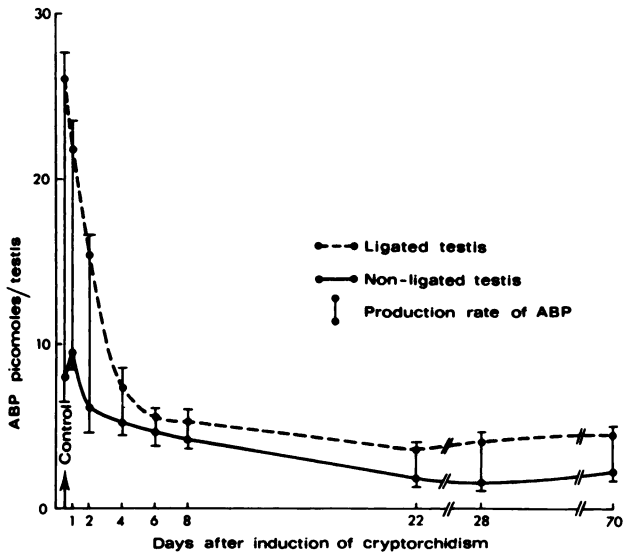


Fig. 4. Testicular ABP content after induction of cryptorchidism. ABP data are expressed as the mean value of duplicate estimations on cytosols pooled from four animals. Error estimates represent confidence levels established from duplicate assays. Unilateral efferent duct ligation was performed for 16 hours in order to measure the production rate of ABP, which is represented by the difference in ABP content between the ligated and nonligated testes (vertical lines).

Relationship Between Fluid Production, ABP, and Serum FSH and LH

A very high positive correlation was found between fluid production and ABP ( $P < 0.01$ ) as was a high negative correlation between the rise in FSH levels after the induction of cryptorchidism and the decrease in Sertoli cell activity as measured by fluid production and ABP ( $P < 0.01$ , Fig. 8). The relationship between Sertoli cell function and the rise in serum LH levels was also determined. The negative correlations between serum LH levels and both fluid production ( $r = -0.58$ ;  $P < 0.05$ ) and ABP ( $r = -0.66$ ;  $P < 0.05$ ) were lower than those with FSH.

Discussion

The degeneration of the seminiferous epithelium that is caused by experimental cryptorchidism has been recognized for a considerable length of time (Moore, 1924; Clegg, 1963). However, the loss of germ cells by the testis probably explains only partially the rapid decrease in testicular weight which occurred four days after induced cryptorchidism. The decrease in the production of testicular fluid reported in this paper

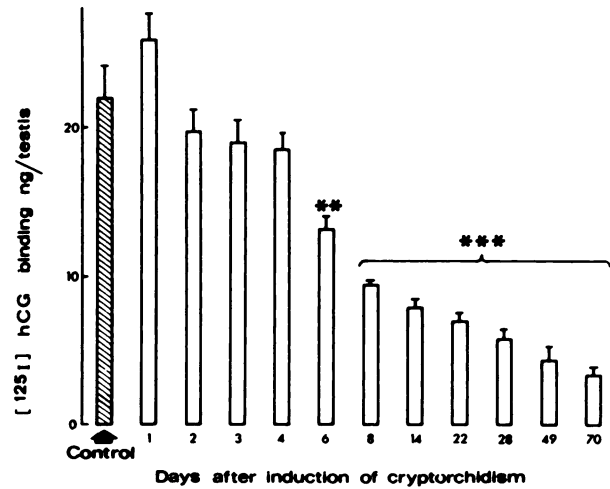


Fig. 5. Effect of cryptorchidism on the binding of  $^{125}\text{I}$ -hCG to testicular tissue *in vitro*. Each value represents the mean  $\pm$  SEM for four animals. \* $P < 0.05$ ; \*\*\* $P < 0.001$ .

probably contributed to the initial reduction testis weight.

The transitory hypertrophy of the epididymis observed at six days post-surgery has not been described previously and no explanation was apparent from the experiments reported herein. This may occur as a consequence of osmotic changes in the epididymis following the dramatic decrease in the entry of the efferent duct fluid after cryptorchidism or may represent the direct effects of increased abdominal temperature. The subsequent decline in epididymal weight observed from eight days to the end of the experiment is probably re-

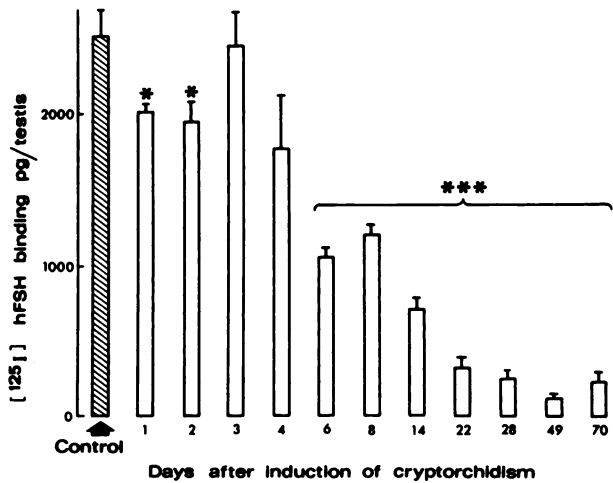


Fig. 6. Effect of cryptorchidism on the binding of  $^{125}\text{I}$ -FSH to testicular tissue. *in vitro*. Each value represents the mean  $\pm$  SEM for four animals. \* $P < 0.05$ ; \*\*\* $P < 0.001$ .

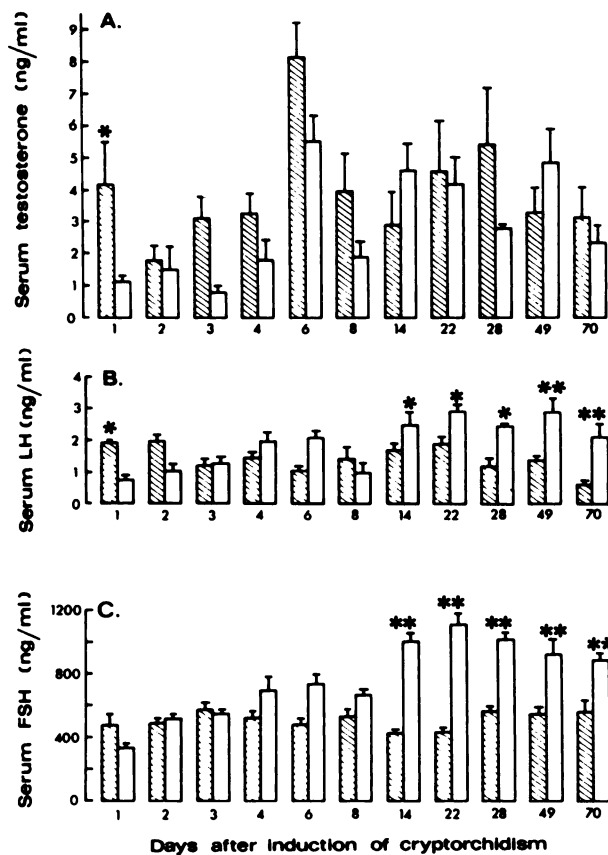


Fig. 7. Serum testosterone, LH, and FSH levels after varying durations of experimental cryptorchidism (n = 5). Statistical analysis by analysis of variance and Duncan's Multiple Range test. ▨ = control; □ = cryptorchid; \*P < 0.05; \*\*P < 0.01.

lated to decreased fluid and sperm production by the testis.

The present study also demonstrates that Sertoli cell function, as measured by ABP production and testicular fluid production, is rapidly impaired after the induction of cryptorchidism. Although Setchell (1970) claimed there was no early effect of cryptorchidism on fluid secretion, the present results show a significant decrease within two days. Our study highlights the rapidity with which this parameter of Sertoli cell function is impaired, since both ABP content and production were decreased two days after the induction of cryptorchidism, confirming the results of Hagenas et al (1978). The continued reduction in fluid production confirms the findings of Setchell (1970) and Hagenas and Ritzen (1976) in prolonged cryptorchidism in the rat, and of Barack (1968) who showed similar results in the long-term cryptorchid mouse testis. The decline in testicular and epididymal ABP content after testicular damage, cryptorchidism, Vitamin A deficiency, fetal irradiation, or hydroxyurea treatment (Hagenas and

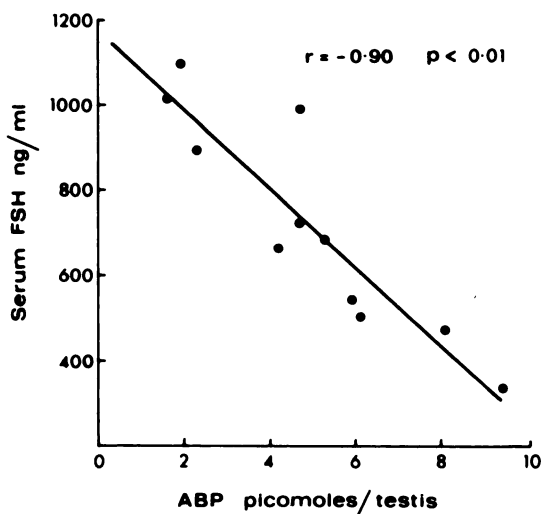
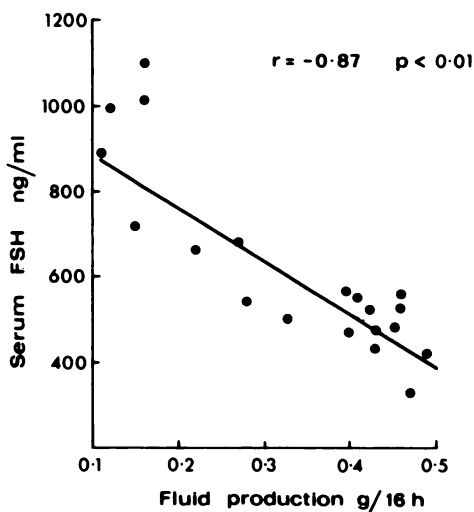
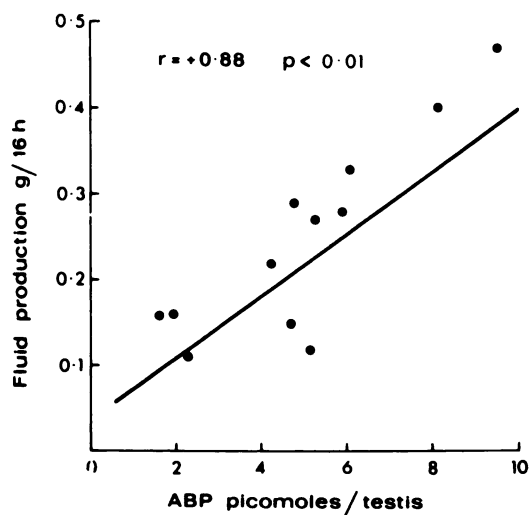


Fig. 8. The relationship between parameters of Sertoli cell function and FSH levels are illustrated using data from rats after differing lengths of exposure to cryptorchidism. The data represent the mean levels from the animals used to determine fluid production and ABP levels.

Ritzen, 1976; Rich and de Kretser, 1977) has demonstrated that Sertoli cell function is impaired in these states. The high correlation between the ABP and fluid production is evidence that both are effective markers of Sertoli cell function. Furthermore, their close correlation with serum FSH levels may be used as circumstantial evidence to link the impaired Sertoli cell function with the elevated FSH levels. Recent evidence has also shown that the Sertoli cell is the site of production of inhibin (Steinberger and Steinberger, 1976; Labrie et al, 1978; de Jong et al, 1978) and provides further evidence that the rise in FSH level is likely to be due to diminished inhibin production by the Sertoli cell.

A previous study (Hagenas et al, 1978) showed that the number of FSH receptors decreases after prolonged cryptorchidism, and it could be suggested that this represents down-regulation resulting from increased FSH levels. However, the present study has demonstrated a decline in the content of FSH receptors one to two days after cryptorchidism, prior to a detectable increase in serum FSH levels. Consequently, further studies are necessary to determine the cause of the receptor loss, which may be related to increased production of substances within the testes capable of preventing the binding of FSH to its receptor (Abou-Issa and Reichert, 1976; Reichert and Abou-Issa, 1977).

The biochemical evidence presented here shows that the Sertoli cell is dramatically and rapidly affected after cryptorchidism, and that the temporal relationships to the changes in Leydig cell function are of interest. This study confirms our earlier finding that Leydig cell function is altered after cryptorchidism (Kerr et al, 1979a; de Kretser et al, 1979; Risbridger et al, 1981). In this report, LH levels were elevated 14 days after cryptorchidism and the number of LH receptors declined after six days. Our previous studies have also shown significant Leydig cell hypertrophy seven days after this procedure (Risbridger et al, 1981). It is important to note that Sertoli cell function was impaired more rapidly than was Leydig cell function, supporting the postulate that Sertoli cell dysfunction may cause the changes in Leydig cells. This supports the evidence presented elsewhere that local factors are important in inducing the Leydig cell changes after cryptorchidism and that they are not related to the elevated LH levels (Risbridger et al, 1981). The postulate that the Sertoli cells normally exert a controlling influence on Leydig cells has received support from the investigations of

Sharpe et al (1982) who showed that media from cultures of Sertoli cells contain an LHRH-like material. Since LHRH and its agonists exert an inhibitory action on Leydig cell function, and since the rapid decline in Sertoli cell function would be expected to decrease the production of an LHRH-like material, the overall result would be consistent with removal of an inhibitory influence on the Leydig cells, thus allowing their hypertrophy. The failure of these hypertrophied Leydig cells to express their biologic capabilities *in vivo* is probably related to the decline in testicular blood flow that occurs after cryptorchidism (Damber et al, 1978), since they are able to maintain normal testosterone levels only in response to elevated LH levels. In contrast, *in vitro* stimulation of the cryptorchid testes results in greatly increased secretion of testosterone and related steroids (de Kretser et al, 1979).

The possible link between the function of these two cell types makes it vital that their capabilities be evaluated in various states of testicular dysfunction. While parameters for assessment of the Leydig cell are well known, this study establishes the excellent concordance between Sertoli cell function as evaluated by ABP and fluid production. The measurement of ABP is complex but the assessment of fluid secretion is simple, requiring careful ligation of the efferent ducts and evaluation of testis weight.

### Acknowledgments

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### International Symposium

Fundación Argentina de Endocrinología (FAE) is organizing the VIIth International Symposium. This meeting will discuss “Hormone and Cancer” and will take place May 9–13, 1983, in Buenos Aires, Argentina. The Chairman of the Symposium will be Dr. Erlio Gurpide. Main topics to be discussed will be: mechanism of action of hormones and antihormones; determination of hormone receptor; predictive value of diagnostic methods in hormone-dependent tumors; clinical use of biological tumor markers; and treatment of hormone-dependent cancer. Persons interested in receiving further information can write to:

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Fundación Argentina de Endocrinología  
Suipacha 1322—2° F  
1011—Buenos Aires  
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