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Pituitary and gonadal responses to hemicastration : A model for investigating the regulation of testicular function

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INTRODUCTION

The primary control of testicular function involves, to a large degree, interactions between pituitary hormones and the gonadally derived factors that control their release. Testicular factors known to modulate pituitary feedback inhibition, such as steroids and inhibin, provide the testis with mechanisms to control its own activity. Treatments or procedures that alter gonadal homeostasis can be used to investigate how various factors regulate testicular activity. For example, removal of one testis (hemicastration) results in a number of changes in the remaining gonad, including compensatory increases in testicular growth, daily sperm production and androgen secretion. The ability of one gonad to recognize the absence of the contralateral organ and respond with such dramatic changes has intrigued scientists for over a century. Yet, despite intensive research efforts, surprisingly little is known about what causes these compensatory responses. Presumably, the removal of testicular factors following hemicastration disrupts and alters the hypothalamic-pituitary-testicular axis, resulting in hormone-stimulated increases in both tubular and intertubular components of the remaining testis. However, characterizing a specific cascade of post-hemicastration events has proven difficult, especially since there appears to be species differences in many of the observed responses. In this review we attempt to summarize data on pituitary and gonadal responses to hemicastration in four species: the rat, ram, bull and boar. Because of the volume of literature on the subject, it is not possible to discuss all of the studies conducted in these species. Instead, our objective is to present a comparative overview of the responses observed among different species, including possible differences between seasonal and nonseasonal breeders. In addition, we also discuss the results of experiments designed to identify some of the mechanisms controlling compensatory hemicastration responses. Defining this model could potentially lead to a better understanding of the control of both spermatogenesis and steroidogenesis.

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GONADAL EFFECTS

Testicular growth

Compensatory hypertrophy of the remaining testis after hemicastration occurs in a number of adult and prepubertal seasonal breeders, including rams (Voglmayr and Mattner, 1968; Land and Carr, 1975; Johnson et al., 1971; de Reviers et al., 1980; Hochereau-de Reviers, 1975; Hochereau-de Reviers et al., 1976, 1980; Walton et al., 1980; Jenkins and Waites, 1983; Waites et al., 1983; Hoagland and Bolt, 1986; Brown et al., 1987; Mirando et al., 1989), stallions (Ott et al., 1984), rabbits (Ribbert, 1890), badgers (Canivenc and Relexans, 1967), and field voles (Delost, 1972; Martinet and Meunier, 1975). In contrast, compensatory hypertrophy in the rat (Lindgren et al., 1976; Moger, 1977; Selin and Moger, 1979a; Cunningham et al., 1978; Hochereau-de Reviers, 1975, Hochereau-de Reviers and Courot, 1978; Frankel and Wright, 1982; Putra and Blackshaw, 1982; Orth et al., 1984; Brown and Chakraborty, 1991; Brown et al., 1991a,b,c), bull (Hochereau-de Reviers, 1970; Barnes et al., 1980a; Sundby et al., 1981; Barnes et al., 1983; Leidl et al., 1980; Boockfor et al., 1983a; Schanbacher et al., 1987) and boar (Sundby et al., 1981; Putra and Blackshaw, 1985; Kosco et al., 1907; Minton and Wetteman, 1988) generally occurs only in prepubertal animals, and may be related to the absence of seasonal

variations in sexual activity in these species. An interesting exception is provided by Johnson and Neaves (1981) who observed an increase in the size of the remaining testis of aged (~2 years of age) but not young (6-10 months of age) adult rats. Testicular hypertrophy also has been observed in post-pubertal bulls (Johnson, 1978), although testicular development may not have been complete in those animals since they were only 2.5 years of age at the start of the study.

Few longitudinal studies have been conducted; however, it would appear that the magnitude and time required for onset and completion of hypertrophy varies considerably among species. In rams, significant compensatory hypertrophy is absent at 2 weeks (Brown et al., 1988, Brown et al., 1991d), but is generally observed by 3 to 4 weeks post-surgery (Jenkins and Waites, 1983; Waites et al., 1983; Hoagland and Bolt, 1986). In boars, a doubling of the mass of the remaining testis occurs within 2 to 4 weeks (Kittock et al. 1984; Kosco et al., 1987), whereas in bull calves hypertrophy onset is not observed until ~2 months post-surgery (Barnes et al., 1980a). A more rapid compensation is observed in rats, with hypertrophy occurring as early as 5 days after hemicastration (Cunningham et al., 1978; Brown and Chakraborty, 1991).

Evidence suggests that age affects gonadal sensitivity to the factors controlling compensatory hypertrophy. In rats, the degree of hypertrophy decreases as the age at hemicastration increases, and is non-existent after ~45 days of age (Hochereau-de Reviers, 1975; Putra and Blackshaw, 1982; Furuya et al., 1984). Brown et al. (1987) also found that the ~48% hypertrophy observed in 4-month-old ram lambs was not as great as the 80% or greater increase observed in animals altered at younger ages (Walton et al., 1978; 1980; Hochereau-de Reviers et al., 1980; Waites et al., 1983). The lower hypertrophy response in our study more closely resembles that observed in adult rams in which increases of 30-60% were reported (Johnson et al., 1971; Hochereau-de Reviers, 1975, Hochereau-de Reviers, et al., 1976; Hoagland and Bolt, 1986; Mirando et al., 1989). Similarly, unilateral gonadectomy of bulls at 1 week (Barnes et al., 1980a) or 3 months (Boockfor et al., 1983a) of age resulted in greater testicular hypertrophy (~60-70%) than in animals altered at 6 or 9 months of age (~12%) (Boockfor et al., 1983a) when all animals were examined 11-16 months post-surgery. Finally, in boars, the remaining testis hypertrophied ~100% in males hemicastrated between 1-2 months of age (Sundby et al., 1981; Putra and Blackshaw, 1985; Kosco et al., 1987), ~60% in 10-week-old males (Minton and Wettemann, 1988), but was unaffected in animals 3 months of age or older (Putra and Blackshaw, 1985).

In sheep, the time of year hemicastration is performed can affect compensatory responses. In the adult Il-de-France ram, hemicastration in the spring induces a true hypertrophy of the remaining testis in the autumn, whereas removal in the autumn merely prevents the normal seasonal decline (Hochereau-de Reviers et al., 1976). If animals are evaluated after one year, the remaining testis in the spring or autumn is equivalent to that of an animal hemicastrated 6 months earlier, indicating that rams hemicastrated in autumn only develop true hypertrophy after a delay of 6 months (Hochereau-de Reviers et al., 1976). However, inter-breed differences exist since true hypertrophy is observed year round in hemicastrated Préalpes sheep, a breed which shows little seasonal variation in testis weight (Hochereau-de Reviers et al., 1976). One study related breed differences in the testicular hypertrophy response to hemicastration to female prolificacy ranking. Increased testis growth following hemicastration at 20 to 25 weeks of age was greatest in Tasmanian Merino (72%), intermediate in Scottish Blackface (57%) and least in Finnish Landrace (42%) rams, and was inversely related to their breed ovulation rates (Land and Carr, 1975). It is also interesting that the degree of hypertrophy was inversely related to initial testis weight, with Finns possessing the largest testes and Merinos the smallest (Land and Carr, 1975). The authors speculated that the magnitude of hypertrophy might be associated more with the degree of gonadotropic stimulation than actual growth potential, which might in turn be related to differences in pituitary sensitivity to testicular negative feedback effects. However, to generate this information more comprehensive, comparative studies need to be conducted to explore the relationship between the secretory dynamics of pituitary-gonadal hormones and the hypertrophy response.

Testosterone

With few exceptions (see Moger, 1977, Furuya, 1990), unilateral castration of adult and prepubertal animals results in a rapid compensation in androgen production such that the testis of hemicastrates produces the same amount of testosterone as two testes of intact animals. This response is observed regardless of whether a concomitant increase in testis weight occurs or not. Androgen compensation is rapid and often subtle, and in studies where blood samples were taken infrequently (single point, weekly or monthly) or after a prolonged interval post-hemicastration, no differences in peripheral testosterone concentrations between hemicastrated and intact animals are detected (rat, Lindgren et al., 1976; Selin and Moger, 1979a; Frankel and Mock, 1982; Frankel and Wright, 1982; Ultee-van Gessel et al., 1985; Brown et al., 1991b,c: ram, Walton et al., 1978, 1980; Jenkins and Waites, 1983; Waites et al., 1983; Hoagland and Bolt, 1986; Mirando et al., 1989: bull, Johnson, 1978; Barnes et al., 1980c, 1981, 1983; Leidl et al., 1980; Sundby et al., 1981: boar, Sundby et al., 1981; Kosco et al., 1987; Minton and Wetteman, 1988; Kittock et al., 1984). More rigorous evaluations of blood samples collected soon after gonad

removal, however, do reveal an initial decrease in testosterone concentrations. For example, peripheral testosterone concentrations in hemicastrated rats were reduced at 4 hours but returned to intact levels by 8-24 hours post-surgery (Mock and Frankel, 1982; Frankel et al., 1984, 1989a,b; Agee et al., 1988; Brown et al., 1991a). In 4-month-old ram lambs, testosterone concentrations were reduced ~50% by 2 hours post-surgery, but then recovered to intact levels one week later (Johnson et al., 1971; Brown et al., 1987, 1988). Analysis of serial blood samples collected every 15 minutes for 10 hours revealed that both basal and pulsatile testosterone concentrations on Day 1 (4-14 hours post-surgery) were half those measured in intact animals; however, by Day 7 secretory patterns were similar between the two groups (Brown et al., 1988). Caution is advised, however, since some of the initial reduction in testosterone secretion may be due to factors other than removal of half the testicular mass. In both rats (Frankel and Wright, 1982; Mock and Frankel., 1982) and rams (Brown et al., 1988), initial reductions in testosterone have been observed in both hemicastrated and intact animals . These observations suggest surgery, handling and/or anesthesia stress might also affect this response, and emphasize the importance of properly designing studies to ensure accurate data interpretation.

The early recovery of peripheral testosterone concentrations to intact levels is not a function of increased testis size, but rather appears to be due to a dramatic increase in androgen output by the remaining testis. In adult hemicastrated rats, the normalization of peripheral testosterone concentrations is associated with a doubling of testosterone concentrations in the testicular vein (Mock and Frankel, 1982; Frankel et al., 1984, 1989a,b). However, this response is far from uniform, and in about 25% of the animals no androgen compensation is observed (Frankel et al., 1989b). Upon closer examination it was discovered that the magnitude of the testosterone response was related to which testis was removed. Specifically, increased testicular vein testosterone output was consistently greater in animals which had the left testis removed (2.82-fold increase) as compared to those which had the right testis removed (1.73-fold increase). There is no obvious explanation for why the hemicastration response is more robust when the right testis remains *in situ*; although, it is strikingly similar to that observed in hemiovariectomized rats where ovarian compensatory hypertrophy and numbers of ovulated ova are greater when the left ovary is removed (Chavez et al., 1987). This interesting observation in the rat clearly warrants further investigation to determine: 1) if similar asymmetry in testicular androgen compensation occurs in other species, or 2) if there is asymmetry with other hemicastration responses such as testicular hypertrophy and altered gonadotropin secretion.

In rams, compensatory androgen secretion is associated with a doubling of testosterone concentrations in the spermatic vein (Brown et al., unpublished observations), as well as an increase in the total amount of testosterone secreted into the rete testis fluid (Mirando et al., 1987b). An even more dramatic compensation in androgen secretion has been reported in bull calves hemicastrated at 1.5 months of age (Lindner and Rowson, 1961). In that study, mean testosterone output into the spermatic vein of the remaining testis was more than 7 times greater, and that of total androgens (testosterone plus androstenedione) about 5 times greater than the mean combined output of the paired testes of intact control animals when evaluated 5 months post-surgery. This marked increase in testosterone production by the remaining testis was related to an 11-fold increase in testicular androgen content. Other studies also report increased androgen content in the remaining testis of hemicastrates. Lindgren et al. (1976) found testicular testosterone concentrations doubled in adult hemicastrated rats. However, increased testicular testosterone in prepubertal rats appears to be related more to the degree of hypertrophy, since the content (ng/testis), but not concentration (ng/g), of testosterone was increased (Selin and Moger, 1979a; Brown et al., unpublished observations). This response is far from consistent, though, as several studies have failed to demonstrate increases in parenchymal testosterone concentrations or content in hemicastrates (prepubertal rats, Moger, 1977; Cunningham et al., 1978: adult rats, Brown et al., unpublished observations: rams, Mirando et al., 1989; Brown et al., unpublished observations).

Data regarding the testosterone response of the remaining testis to exogenous gonadotropin stimulation are conflicting. An increase in testicular responsiveness to luteinizing hormone (LH) was observed in prepubertal rats hemicastrated at 10 days of age and challenged 7 days later (Selin and Moger, 1979a). Since follicle-stimulating hormone (FSH) can increase LH-stimulated testosterone release (Odell et al., 1973; Ketelslegers et al., 1978), and an increase in FSH secretion is observed after hemicastration, it was hypothesized that FSH increased testicular responsiveness (Selin and Moger, 1979a). However, in that same study testicular responsiveness was not increased when LH was administered at 14 days post-surgery, despite the presence of elevated FSH in those animals as well. In a study of adult rats, Frankel and Wright (1982) reported testicular responsiveness to hCG was actually decreased, and they suggested that the remaining testis was already functioning at its maximal capacity to secrete testosterone. In still other studies, no changes in testicular responsiveness to gonadotropic stimulation have been observed (prepubertal rat, Moger, 1977: bull, Barnes et al., 1981). There are no obvious explanations for these disparate results in *in vivo* testicular responsiveness, and studies *in vitro* have done little to clarify them. Cultured Leydig cells from hemicastrated bulls (Boockfor et al., 1983b) and rats (Selin and Moger, 1979a) have been

shown to produce greater amounts of androgens (testosterone and 5α-androstane-3α,17β-diol, respectively) compared with cells from intact animals. In contrast, others report the testicular response to hemicastration cannot be maintained *in vitro* (rat, Ulteevan Gessel et al., 1985; Frankel et al., 1989a: ram, Jenkins and Waites, 1983; Mirando et al., 1987a). Perhaps Leydig cells from hemicastrates do produce more testosterone upon initial removal, but maintaining the compensatory state *in vitro* requires extra-testicular stimulation.

Convincing evidence that changes in pituitary gonadotropin secretion or concentrations of gonadal gonadotropin receptors mediate androgen compensation in hemicastrated animals has not been forthcoming (see below). However, studies in rats provide evidence for possible nervous system involvement. First, cutting the spermatic nerve by hemivasectomy partially blocked the recovery in testosterone secretion (Frankel et al., 1984). Second, local application of a β-receptor antagonist, propranolol, completely suppressed this response (Moger and Anakwe, 1986). Third, preliminary studies conducted by Frankel et al. (1989a) demonstrated that electrical stimulation of the superior spermatic bundle significantly increased testosterone concentrations in the testicular vein. These results provide a mechanism by which the remaining testis could be neurally stimulated, and that this stimulation is subsequently lost when it is transferred *in vitro* (Frankel et al., 1989a). However, not all data are supportive of this conclusion since bilateral denervation of the superior spermatic nerve alone had no effect on subsequent testosterone production (Frankel and Mock, 1982). The autonomic innervation of testicular blood vessels is thought to be primarily through the superior spermatic nerve, with the inferior spermatic nerve carrying parasympathetic nerves playing a minor role (Hodson, 1970). One explanation for the failure of the latter study to block the hemicastration response is that during the 12-week interval between denervation and hemicastration, testicular innervation may have functionally transferred from the superior spermatic to the inferior spermatic nerves (Frankel et al., 1984). Clearly, more studies are needed to determine whether the nervous system plays a role in maintaining testicular homeostasis, and if disrupting the neural link by unilateral castration somehow alerts the remaining gonad to the absence of the contralateral organ.

Testicular histology

Seminiferous epithelium

The increase in testis weight following hemicastration is principally due to enhanced activity of the tubular compartment. Since hypertrophy occurs in Sertoli cell-enriched testes devoid of germ cells, it is not likely to be due to proliferation of germinal components (Cunningham et al., 1978), but rather to increased activity of the Sertoli cells. That being established, the major disagreement becomes whether testicular hypertrophy is due to increased Sertoli cell number (hyperplasia), cell size (hypertrophy) or both. There are a number of factors which probably account for much of the confusion in the literature, including: 1) species differences; 2) age at the time of hemicastration; 3) time of evaluation relative to hemicastration; and 4) methodology. In rats, Sertoli cell division is maximal before birth and continues at progressively lower rates throughout the first two weeks of postnatal life (Orth, 1982). Hemicastration of 1-day-old (Hochereau-de Reviers, 1975) or 5-10-day-old (Hochereau-de Reviers and Courot, 1978) rats resulted in a significant increase in the total number of Sertoli cells per testis. When 3-day-old rats were hemicastrated, the percentage of Sertoli cells labelled with [^{3}H] thymidine in the remaining testis increased, suggesting that Sertoli cell proliferation was enhanced by hemicastration using this methodology (Orth et al., 1984). Cunningham et al. (1978) also suggested in rats hemicastrated at 5 days of age that hypertrophy was due to increased cell numbers based on significant increases in testicular protein and DNA concentrations in Sertoli cell-enriched testes. In contrast, when surgery was delayed until 10 days of age or later, no increase in Sertoli cell number was noted using morphometric techniques (Putra and Blackshaw, 1982; Hochereau-de Reviers and Courot, 1978). Instead, hypertrophy was related to increases in total seminiferous tubule length and a greater cross-sectional area (Putra and Blackshaw, 1982).

In ram lambs, Sertoli cell proliferation ceases at around 40 days of age (Hochereau-de Reviers et al., 1984). Therefore, if this species responds similar to the rat, increases in Sertoli cell number would not be expected in animals hemicastrated after that time. And, in fact, the data do generally support this hypothesis. In lambs hemicastrated at 1 week of age, seminiferous tubular mass was significantly increased at 8 and 12 weeks, and although Sertoli cell number was not specifically determined, the increased testis mass occupied by Sertoli cells, cytoplasm, and nuclei were suggestive of enhanced proliferation of this cell population (Waites et al., 1983). Following removal of one testis at 6 weeks of age, de Reviers et al. (1980) also reported an increase in the total number of Sertoli cells per testis. However, in another study of 6-week-old lambs, no significant changes in Sertoli cell numbers were observed (Hochereau-de Reviers et al., 1980). Instead, increased mean diameter (but not length) of the seminiferous tubules, and the nuclear cross-sectional area of Sertoli cells were primarily responsible for the hypertrophy. The difference in Sertoli cell responses between these two studies was not related to the time of year the remaining testis was removed; both studies compared hemicastrates to intact animals during the breeding season. In contrast, the length of time between hemicastration and evaluation

did differ. Increases in cell numbers reported by de Reviers et al. (1980) were observed 13 (20% increase) and 19 (52% increase) months after hemicastration, whereas only 5 months elapsed between surgery and slaughter in the study of Hochereau-de Reviers et al. (1980). Alternatively, it is possible that 6 weeks of age is a critical period relative to Sertoli cell development, and that differences of only a few days could determine whether proliferation occurs or not. By 6 months of age, changes evoked by hemicastration only involve increases in seminiferous tubule diameter and surface area (Hochereau-de Reviers et al., 1984). Furthermore, testicular hypertrophy in adult rams is not associated with increased Sertoli cell numbers (Hochereau-de Reviers, 1975; Mirando et al., 1989); however, there is disagreement as to whether it is due to an increase in total seminiferous tubule length (Hochereau-de Reviers et al., 1976) or tubule diameter (Mirando et al., 1989). Hemicastration of adult rams also increased the size of the Sertoli cell nucleus (Hochereau-de Reviers et al., 1976; Mirando et al., 1989), or at least prevented the decrease in Sertoli cell nuclear area and seminiferous tubule diameter which occurs during the non-breeding season (Hochereau-de Reviers and Pelletier, 1971; Hochereau-de Reviers, 1975; Hochereau-de Reviers et al., 1976).

Compensatory testicular hypertrophy in the bull apparently is not related to increases in Sertoli cell number, even when surgery is performed at a very young age (1 week); however, similar disagreements exist regarding whether hemicastration increases seminiferous tubule length (Leidl et al., 1980) or diameter (Johnson, 1978; Barnes et al., 1983; Boockfor et al., 1983a). None of these discrepancies can be related to differences in age at the time of hemicastration or to the elapsed time between surgery and evaluation. In prepubertal boars, hemicastration increased the total number of Sertoli cells per testis when surgery was performed at 1 month of age, but not at later ages (Putra and Blackshaw, 1985). Increases in seminiferous tubule length (Putra and Blackshaw, 1985; Kosco et al., 1989a), seminiferous tubule diameter (Putra and Blackshaw, 1985), and the relative mass of seminiferous tubules, parenchyma and Sertoli cells (Kosco et al., 1989a) also have been reported in the boar.

In conclusion, although the data conflict somewhat, the general consensus is that major changes in the seminiferous epithelium are responsible for hemicastration-induced testicular hypertrophy. It appears that once males reach the age at which Sertoli cell division ceases, no further increases in cell number are induced by hemicastration. Instead, alterations in tubule diameter or length account for the majority of the hypertrophy. Finally, when animals reach an age at which testicular hypertrophy is not longer observed, no changes in either seminiferous tubule diameter, length or Sertoli cell density are observed (Bergh et al., 1982; Putra and Blackshaw, 1982, 1985).

Interstitium

An enhanced activity of the interstitial compartment of the testis is credited for the rapid compensation in androgen secretion observed in hemicastrated animals. In fact, an early study by Liang and Liang (1970) even suggested that testicular hypertrophy in young pubertal rats was due to an increase in interstitial cells, rather than tubular count. Agee et al. (1988) also reported a slight increase in Leydig cell numbers in adult hemicastrated rats, as well as nuclear enlargement and chromatin condensation, increased cytoplasm granularity, more prominent nucleoli, and increased smooth endoplasmic reticulum, all of which indicate increased cellular activity. Most studies in the rat, however, do not suggest compensatory responses are due to Leydig cell hyperplasia (Lindgren et al., 1976; Bergh et al., 1982; Putra and Blackshaw, 1982; Cracchiolo and Hoagland, 1987; Furuya, 1990). Instead, alterations in the interstitium are primarily the result of Leydig cell hypertrophy (Bergh et al., 1982; Fuyura, 1990).

In lambs hemicastrated at 1 week of age, Waites et al. (1983) reported an increase in the relative mass of the interstitium, including an increase in interstitial cell mass, suggestive of cellular hypertrophy. Hochereau-de Reviers et al. (1980) also reported Leydig cell hypertrophy occurred in 6-week-old hemicastrates based on the finding of increased total volume of Leydig cells per testis, but not total volume of individual cells when compared to controls lambs. In contrast, no changes in the intertubular tissue, including volume of Leydig cells per testis, cross-sectional Leydig cell area and total Leydig cell numbers per testis were observed after hemicastration in 6-month-old lambs (Hochereau-de Reviers et al., 1984). Data on Leydig cell numbers in adult ram hemicastrates apparently are lacking; however, increases (Hochereau-de Reviers et al., 1976) and no changes (Mirando et al., 1989) in intertubular tissue volume have been reported. In prepubertal bulls, increases in total interstitial tissue volume were noted (Barnes et al., 1980b), whereas in prepubertal boars, marked increases in the mass of the interstitium, including the mass of Leydig cells and their nuclei and cytoplasm have been observed (Kosco et al., 1989b).

Spermatogenesis

Based on histological evaluations, enhanced spermatogenic activity in the remaining testis is observed following hemicastration. Specifically, increased numbers of germ cells per testis and Sertoli cell occupancy (number of germ cells/Sertoli cell) paralleled increases in the testicular hypertrophy response in rats hemicastrated between 10 and 50 days of age (Putra and Blackshaw, 1982). In 6-week-old ram lambs, hemicastration increased the total number of undifferentiated stem spermatogonia (A_o), and daily

production of leptotene primary spermatocytes and round spermatids, with no changes in renewing stem spermatogonia (A_1) (de Reviers et al., 1980; Hochereau-de Reviers et al., 1980). In older lambs (6 months), only daily production of leptotene primary spermatocytes and round spermatids were increased, with no changes observed in numbers of A_o spermatogonia, daily production of A_1 spermatogonia, or in the yields of primary spermatocytes/A_1 spermatogonium or meiotic prophase (% spermatids/leptotene gonia) (Hochereau-de Reviers et al., 1984). Taken together, data in the ram lamb suggest that increases in spermatogenesis in younger animals results, at least in part, from an increase in the stock of reserve stem cells (A_o spermatogonia), whereas in older lambs increased daily production of spermatids arises primarily from effects on the differentiation of A_1 spermatogonia (de Reviers et al., 1980; Hochereau-de Reviers et al., 1984). If the number of stem cells and their multiplication to A_1 spermatogonia is related to the number of Sertoli cells (Hochereau-de Reviers et al., 1984), then these age differences could be related to the increased Sertoli cell division that is observed in younger, but not older, ram lambs (de Reviers et al., 1980; Hochereau-de Reviers et al., 1984; Mirando et al., 1989). However, in adult rams, hemicastration affected both early and late stages of spermatogenesis, including increased daily production of round spermatids and total numbers of type A_o and A_1 spermtagonia (Hochereau-de Reviers et al., 1976; Mirando et al., 1989).

In bulls hemicastrated at 1 week of age, increased production of spermatozoa per testis (Barnes et al., 1980a) was not associated with increased survival of spermatids as indicated by the number of spermatids per A-type spermatogonia, but rather was related to greater numbers of spermatogonia per tubule cross-section and increased tubular volume (Barnes et al., 1980b). In adult and young pubertal bulls, increases in the relative numbers of germ cell nuclei present in tubules at stage VII of the cycle of the seminiferous epithelium were associated with increased Type A and B_2 spermatogonia, pachytene spermatocytes, and Step 7 spermatids (Johnson, 1978). Hemicastration of boars at 10 days of age resulted in a greater relative mass of spermatogenic cells (spermatocytes and spermatids) at 122 days of age, which was due to an earlier onset of spermatogenesis compared to intact controls (Kosco et al., 1989a). Putra and Blackshaw (1985) also reported an increase in the total number of germ cells per testis in younger (1-month-old), but not older (2-5-month-old), hemicasırates when evaluated 2 months post-surgery which was not related to increased Sertoli cell occupancy.

Another method of determining the sperm producing capacity of the testis involves counting the number of homogenization-resistant spermatids in unfixed testicular parenchyma. Dividing that number by a time divisor representative of the estimated life span of those spermatids (eg. 3.56 days for the ram; 6.3 days for the rat), then provides an estimate of daily sperm production (DSP) (Amann and Lambiase, 1969; Amann, 1970; Johnson and Neaves, 1981). Calculating the total number of spermatozoa or DSP per testis can be used to estimate total spermatogenic yield, whereas determining sperm numbers or DSP per gram of tissue provides an estimate of spermatogenic efficiency. In prepubertal rats, hemicastration at 2 weeks of age resulted in an increase in DSP of 29% at 3 months of age (23.6 ± 1.3 vs $18.3 \pm 1.1 \times 10^9$ DSP/testis); however, this increase was related primarily to the 27% increase in testis size (2.01 ± 0.06 vs 1.57 ± 0.09 g) since DSP/g testis (11.5 ± 1.2 vs $12.0 \pm 1.4 \times 10^6$) was not different between hemicastrated and intact rats, respectively (Brown and Chakraborty, unpublished data). In adult rats (10-12 months old), neither testis weight (1.9 ± 0.1 vs 1.7 ± 0.1 g), DSP/g (20.6 ± 1.2 vs $21.2 \pm 0.4 \times 10^6$) nor DSP/testis (38.3 ± 2.9 vs $36.6 \pm 2.1 \times 10^9$) were influenced by hemicastration (Brown and Chakraborty, unpublished data).

In prepubertal ram lambs, a 47% increase in DSP/testis was due entirely to the 48% increase in testis weight (Brown et al., 1987). Similarly, in bulls hemicastrated at 1 week (Barnes et al., 1980a) or 3 months (Barnes et al., 1983; Boockfor et al., 1983a) of age, and in prepubertal boars hemicastrated at 10 weeks of age (Minton and Wetteman, 1988) spermatogenic yield was increased, but only relative to the increase in testis weight spermatogenic efficiency was not altered. In the study of Boockfor et al. (1983a), when hemicastration was delayed until 6 or 9 months of age, neither testicular hypertrophy nor increases in spermatogenic yield were observed compared to the 3-month-old hemicastrates. In young pubertal bulls (~7 months and 2.5 years of age), slight but significant increases in testis weight (15-20%) were reported, however no accompanying increases in spermatogenic yield, as determined by total numbers of sperm/testis, were observed (Johnson, 1978).

Taken together, these reports suggest that increased spermatogenic yield by the remaining testis often is related more to the increase in testis size rather than to enhanced spermatogenic efficiency of the testicular tissue itself. There are some notable exceptions, however. In one study, Hochereau-de Reviers et al. (1976) reported the production of round spermatids/testis and testis weight of adult rams hemicastrated in the autumn and examined in the spring were both increased ~45%, suggesting only spermatogenic yield was enhanced. In contrast, when rams were hemicastrated in the spring and examined in the autumn, sperm production increased 70% while testicular weight increased only 32%, suggesting spermatogenic efficiency was also increased. In other studies, increases in spermatogenic efficiency of ~50% in adult hemicastrated rams maintained on a 12:12 hour light:dark schedule (Johnson et al., 1971), and in aged, but not in young, adult rats (Johnson and Neaves, 1981)

have been reported. These exceptions led us to speculate that perhaps compromised testes with suboptimal sperm production (eg., aged animals or seasonal animals during the non-breeding season) respond more robustly to hemicastration, with increases in spermatogenic efficiency, irrespective of increases in testicular size (Brown et al., 1987).

Enhanced spermatogenic capacity of the remaining testis has been shown to be associated with increases in the flow of rete testis fluid and total sperm output (Voglmayr and Mattner, 1968; Mirando et al., 1989), as well as an increase in epididymal sperm reserves (Smelser, 1933; Johnson et al., 1971; Barnes et al., 1980a; Minton and Wetteman, 1988). Although hemicastration is not believed to adversely affect fertility in any species, unilaterally castrated adult rams ejaculated a lower semen volume and tended to produce less sperm based on semen samples collected weekly for 13 weeks after surgery (although statistical differences in sperm production were only evident at Weeks 1, 6, 12 and 13) (Hoagland and Bolt, 1986). However, if data are expressed on a per testis basis, ejaculated sperm numbers in hemicastrates exceed those in intact animals (Barnes et al., 1980a).

Gonadotropin receptors

The growth, development and steroidogenic capacity of the mammalian testis is affected by temporal patterns of LH and FSH secretion, as well as its sensitivity to gonadotropic stimulation (via specific gonadal receptors). In an attempt to explain compensatory hypertrophy and androgen secretion, several studies have investigated the possibility that testicular LH or FSH receptor numbers might be increased by hemicastration. In general, however, unless testicular hypertrophy occurs no significant changes in gonadotropin receptor binding are observed. For example, in prepubertal rats increased LH and FSH receptor numbers were proportional to the increase in testis weight and only receptor content, not concentration, was increased (Brown and Chakraborty, 1991). Similar studies conducted in ram lambs also demonstrated no changes in LH or FSH receptor concentrations following hemicastration when animals were evaluated at 2, 7 or 14 days post-surgery (Brown et al., 1988). However, by 14 weeks post-surgery increased gonadotropin receptor content associated with testicular hypertrophy was observed (Brown et al., 1987). Finally, LH and FSH binding sites per testis in unilaterally hemicastrated bull calves were only increased relative to the increase in testis size (Schanbacher et al., 1987). Thus, with regards to testicular hypertrophy, increased gonadotropin receptor binding is most likely the result, rather than the cause, of the increase in testis size. Altered receptor numbers probably do not mediate androgen compensation either, since normalization of testosterone secretion occurs within hours (rats) or days (rams, bulls) of surgery, and increases in total receptor numbers/testis takes place over a much longer period of time.

In adult rats, any change in testicular sensitivity to gonadotropins would have to be related to an increase in receptor concentration since testis size is not affected by hemicastration. However, no changes in LH receptor concentrations were observed 24 hours after hemicastration (Frankel et al., 1984). Furthermore, we subsequently demonstrated that hemicastration does not stimulate acute (6, 12, 18, 24 or 96 hours post-surgery) or chronic (1, 2, 4, 8 or 12 weeks) changes in either LH or FSH receptor binding (Brown and Chakraborty, 1991).

PITUITARY EFFECTS

Follicle-Stimulating Hormone and Luteinizing Hormone

One consistent response to hemicastration is a significant increase in FSH secretion (rat, Moger, 1977; Cunningham et al., 1978; Selin and Moger, 1979a; Frankel and Wright, 1982; Orth et al., 1984; Rivier et al., 1989; Brown and Chakraborty, 1991; Brown et al., 1991a,b: ram, Walton et al., 1978, 1980; Jenkins and Waites, 1983; Waites et al., 1983; Brown et al., 1987, 1988, 1990: bull, Leidl et al., 1980; Barnes et al., 1981, 1983; Boockfor et al., 1983a; Schanbacher et al., 1987: boar, Kosco et al., 1987; Minton and Wetteman, 1988). The length of time FSH remains elevated above intact levels and the magnitude of the response appears to be species-specific and may also be related to age at the time of hemicastration. In our studies, there were fundamental differences between prepubertal (14 days old) and adult (10-12 months old) rats in the time course and magnitude of the hemicastration response. In prepubertal rats, the FSH rise occurred within 12 hours after surgery and concentrations remained elevated for 4 weeks (Brown and Chakraborty, 1991), whereas in adults, FSH was not increased until Day 2-3 and then remained significantly elevated for 8 weeks post-surgery (Frankel and Wright, 1982; Brown and Chakraborty, 1991). The magnitude of this rise was also consistently greater in prepubertal rats (1.5- to 6.5-fold increase) compared with adults (0.35- to 0.45-fold increase) (Cunningham et al., 1978; Frankel and Wright, 1982;.Orth et al., 1984; Rivier et al., 1989; Brown and Chakraborty, 1991; Brown et al., 1991a,b). Thus, there appears to be a temporal change in the ability of the pituitary gland to increase FSH synthesis following hemicastration in the rat, which is consistent with reports of an age-related decrease in the FSH response to bilateral castration (Hermans et al., 1980).

Unfortunately, comparing the time course of post-hemicastration FSH secretion among domestic livestock species is hindered by the infrequent bleeding regimens (weekly to monthly sampling) used by many studies. There are reports of increased FSH secretion by 1-4 weeks post-surgery in ram lambs (Walton et al., 1978;

Waites et al., 1983; Schanbacher, 1988), bulls (Leidl et al., 1980; Boockfor et al., 1983a; Schanbacher et al., 1987) and boars (Kosco et al., 1987). Upon closer examination we observed the FSH rise in ram lambs is extremely rapid, significantly increasing above intact levels by 6-8 hours post surgery (Brown et al., 1987, 1988). FSH remains elevated in hemicastrated rams for 4 to 13 weeks, apparently depending upon the age at the time of gonadal removal (Walton et al., 1980; Jenkins and Waites, 1983; Waites et al., 1983; Brown et al., 1987). When performed at an early age (~1 week old), FSH is elevated until 8-10 weeks of age after which concentrations fall dramatically (Walton et al., 1978, 190; Jenkins and Waites, 1983; Waites et al., 1983; Schanbacher, 1988). The abrupt decline of FSH in prepubertal animals has been speculated to be due to the development of a testicular feedback system, or to an increased sensitivity of the hypothalamo-pituitary axis to negative feedback (Jenkins and Waites, 1983). In older ram lambs (4 months of age), FSH was elevated for only 4 weeks post-surgery (Brown et al., 1987, 1991d). However, in adults (2 years of age) FSH was elevated throughout a 13-week experimental period (Hoagland and Bolt, 1986). Presumably, negative feedback systems are fully developed and operative in adult rams, so the extended period of hyper-FSH secretion in the latter study is difficult to explain. Nevertheless, it is clear that hemicastration-induced increases in FSH secretion occur in rams of any age, whereas the mechanisms responsible for restoring FSH to intact levels may be age-related.

Compared to either rats or rams, the duration of the FSH rise in bulls is substantially longer. Although blood sampling was not continued past 6 months post-surgery, concentrations were still significantly elevated in bulls hemicastrated at 3, 6 or 9 months of age (Boockfor et al., 1983a). No age-related differences in the magnitude of the FSH response were observed among the 3 age groups. In another study, hemicastration at 1 week of age elicited an increase in FSH that was maintained for the duration of a 26-week study period (Schanbacher et al., 1987). Leidl et al. (1980) further demonstrated that FSH concentrations in bulls hemicastrated at 10 months of age were elevated for 38 weeks before falling abruptly at 40 weeks post-surgery. In contrast, the FSH response to hemicastration in boars is comparatively short-lived, being elevated for only about 2 weeks (Kosco et al., 1987). Thus, there appear to be fundamental differences in the feedback regulation of the hemicastration response between species, with the bull requiring a substantially longer period of time before FSH is reduced to intact levels compared with either rats, rams or boars.

The exact mechanisms responsible for the dramatic rise in FSH following hemicastration are not known, but may be due to reduced testicular inhibin, a hormone implicated in regulating the secretion of this gonadotropin (Scott and Burger, 1981; de Jong and Robertson, 1985). In support of this hypothesis, an inverse relationship between serum FSH and inhibin concentrations following hemicastration has been demonstrated in prepubertal rats (Rivier et al., 1989; Brown and Chakraborty, 1991) and pre- and postpubertal rams (Schanbacher, 1988). However, this relationship was not observed in adult rats, suggesting inhibin may be a more important regulator of FSH secretion in the prepubertal animal (Rivier et al., 1989; Brown and Chakraborty, 1991). Indeed, although injections of inhibin-containing preparations reduce serum FSH in several species, they are more effective in younger animals (Hermans et al., 1980; Lorenzen et al., 1981). Also, removal of endogenous inhibin by passive immunization increases plasma FSH levels in male rats only during the first 21 days of age, and not at later ages (Rivier et al., 1989). In adult rats, testosterone has been suggested to be a more important regulator of FSH secretion than inhibin (Summerville and Schwartz, 1981). It has even been suggested that testosterone alone accounts for the testicular feedback signal in adult rats and that inhibin acts as a secondary modulator that is superimposed on the steroid feedback system (Jones et al., 1985). Rivier et al. (1989) demonstrated that exogenously administered testosterone can block the stimulatory effect of hemicastration on FSH secretion in both prepubertal and pubertal rats. However, testosterone may not be a major regulator in ram lambs because the magnitude of the FSH response at different ages shows no relationship to testosterone secretion (Walton et al., 1978, 1980; Jenkins and Waites, 1983). Furthermore, it seems unlikely that the short-term decrease in testosterone secretion observed after hemicastration is capable of inducing the extended rise in FSH levels observed in so many species, including the rat. Instead, alternative mechanisms need to be considered. For example, other factors present in gonadal tissues such as follistatin (Esch et al., 1987) and a Sertoli cell steroid, 3α-hydroxy-4-pregnen-20-one (Wood and Wiebe, 1989), have been shown to selectively suppress FSH secretion. Estradiol also inhibits FSH secretion in rats (Kalra et al., 1969; Tcholakian et al., 1978), rams (Schanbacher and Ford, 1977) and bulls (Schanbacher, 1984), and exogenous administration can suppress post-hemicastration increases in FSH and testicular hypertrophy in ram lambs (Jenkins and Waites, 1983) and bull calves (Schanbacher et al., 1987). Thus, removal of these or possibly other as yet unidentified factors may be more important regulators of FSH secretion following the removal of one testis in both adult and prepubertal animals. One area that deserves more attention involves the possibility that hemicastration disrupts a neural link between the testis and central nervous system, and induces changes in the hypothalamo-pituitary axis. For example, in one study of hemicastrated rats, GnRH concentrations were increased in the arcuate nucleus ipsilateral to the surgically removed testis, subsequently resulting in a significant increase in FSH, but not LH,

secretion (Mizunuma et al., 1983).

In our studies of prepubertal rats and sheep, we found that both synthesis and release of FSH is increased by hemicastration. In 14-day-old rats, serum FSH was elevated within 12 hours, with increases in pituitary concentrations observed by 2 days post-surgery (Brown and Chakraborty, 1991). Similarly, in 4-month-old ram lambs, the rise in serum FSH observed 6-8 hours post-hemicastration (Brown et al., 1987, 1988) was followed 7 days later by an increase in pituitary FSH concentrations (Brown et al., 1988). Interestingly, the increase in pituitary FSH occurred without a concomitant increase in pituitary GnRH receptor concentrations (sheep, Brown et al., 1988; rats, Brown et al., unpublished observations). Taken together, these data suggest the first response to hemicastration is an increase in FSH secretion, followed several days later by augmented synthesis that exceeds the rate of secretion. In contrast to prepubertal animals, coincident increases in pituitary FSH concentrations were not observed in adult rats, suggesting that synthesis was only increased to the extent that it could support the increase in secretion (Brown and Chakraborty, 1991). These latter data, coupled with the observation that hemicastration-induced increases in circulating FSH concentrations are attenuated in adult compared with prepubertal rats, further demonstrates the existence of age-related differences in pituitary responsiveness to gonadal manipulation.

Based on these observations of enhanced pituitary FSH production, we wondered whether the quality, or biological activity, of the secreted FSH would be the same as that in intact animals. This is an especially important question in light of the growing concern that analyses of immunoactivity may not reflect total biological activity *in vivo* (Hutchinson, 1988). Immunological activity of the gonadotropins is primarily dependent on amino acid sequences (Hutchinson, 1988; Sairam and Schiller, 1979), whereas biological activity is dependent on the nature of the carbohydrate residues present on the molecule (Sairam and Schiller, 1979; Sairam, 1989). Alterations in gonadotropin biopotency have been related to the ability of the pituitary gland to secrete multiple forms that vary in molecular weight, isoelectric properties, receptor-binding affinity and circulating half-life (Bogdanove et al., 1975; Sairam and Schiller, 1979; Robertson et al., 1982; Chappel and Ramaley, 1985; Keel and Grotjan, 1985; Dahl et al., 1988). Therefore, assessments of gonadotropin quality as well as quantity may be necessary to properly interpret data from experiments involving gonadal manipulation and/or treatment (Hutchinson, 1988). For example, alterations in gonadotropin biopotency (biological to immunological ratios; B/I ratios) have been reported as a result of aging (Tenover et al., 1987) or orchidectomy (Huhtaniemi et al., 1988), and in response to GnRH analog (Dahl et al., 1986, 1988; Huhtaniemi et al., 1988) or clomiphene (Tenover et al., 1987) treatment. We speculated that since FSH production was dramatically increased following hemicastration, its biopotency might be compromised. On the contrary, our results indicated that the biological activity of FSH was actually enhanced in hemicastrates. In sheep, increased bioactive FSH concentrations were observed within 6 hours post-surgery, similar to that observed for immunoactive FSH (Brown et al., 1990). And although the bioactivity was only increased for 7 days (compared to immunoactivity which was elevated for 28 days), the concentrations of bioactive FSH during that time period exceeded those of immunoactive FSH, resulting in an increase in the biopotency. Similarly, in hemicastrated adult rats, bioactive FSH concentrations increased 150% while immunoactive FSH increased only 34% (Brown et al., 1991b). In prepubertal rats, bioactive FSH only tended ($P=0.08$) to increase (Brown et al., 1991a), but this difference may have been related to when the analysis was conducted relative to surgery (adults = 4 days; prepubertal = 14 days). Since elevated bioactive FSH secretion was fairly short-lived in the ram lamb, it is possible that FSH bioactivity in prepubertal rats may have been significantly increased at times before the rats were examined. The factors responsible for altering the biopotency of FSH after hemicastration are unclear, but undoubtedly involve complex interactions within the hypothalamo-pituitary-testicular axis. The short-lived augmentation of FSH biopotency in the ram lamb occurred concomitant with the decrease in testosterone secretion. Since increases in FSH B/I ratios have been reported after complete castration in men (Huhtaniemi et al., 1988), it is tempting to speculate that testosterone may play a role in that response. In contrast, inhibin is decreased throughout the entire hemicastration FSH response (Schanbacher, 1988) and, therefore, may be a more important regulator of the quantitative, rather than qualitative, aspects of FSH secretion.

Hemicastration-induced testicular hypertrophy is presumably a result of Sertoli cell hypertrophy and/or hyperplasia. Since the growth and development of Sertoli cells is primarily under FSH control (Griswold et al., 1977; Orth, 1984), and hypertrophy does not occur in hypophysectomized rats unless they are supplemented with exogenous FSH (Brown et al., 1991c), the data suggest that increased FSH secretion following hemicastration stimulates the hypertrophy response. If this assumption is correct, then blocking the FSH rise should prevent hypertrophy from occurring. One method of altering FSH secretion involves administering follicular fluid which is known to contain non-steroidal FSH-suppressing factors (Lorenzen et al., 1981; Jones et al., 1985; Mason et al., 1985; Henderson et al., 1984, 1986; Findlay et al., 1985). Using this approach, twice daily injections of steroid-free follicular fluid to prepubertal rats (Selin and Moger, 1979b; Brown et al., 1991a) and rams (Brown et al., 1991d) blocked hemicastration-induced increases in FSH secretion and subsequently prevented

compensatory testicular growth. In rats, the inhibitory effect of follicular fluid on the testis was completely reversed by concomitant ovine FSH treatment, suggesting follicular fluid's actions were indirect through pituitary control of FSH secretion (Brown et al., 1991a). In ram lambs, follicular fluid treatment for 28 days prevented compensatory increases in FSH secretion and testicular growth, but only temporarily (Brown et al., 1991d). The suppression of FSH secretion was observed for about two weeks, after which FSH "escaped" from the inhibitory effects and increased to levels observed in saline-treated hemicastrates. This "escape" may have been the result of chronic exposure to foreign proteins in the follicular fluid which induced an immunologic response that curtailed its ability to suppress FSH (Channing et al., 1982). When measured on the last day of treatment, testicular hypertrophy in follicular fluid-treated rams was not observed; however, 2 months later significant hypertrophy had occurred. Therefore, it appears that once the inhibition of FSH secretion is released, compensatory responses proceed normally. The data also raise an interesting question regarding whether long-term suppression of FSH could completely abolish these compensatory events, or if they would only delay them until treatment was removed. Other approaches to inhibit post-hemicastration FSH secretion, such as estradiol (calf, Schanbacher et al., 1987) or testosterone (rat, Orth et al., 1984) administration, have also been shown to prevent testicular hypertrophy. These data provide strong support for a major role of FSH in mediating the compensatory testicular hypertrophy response. In related studies, follicular fluid treatment of hemicastrated gilts (Redmer et al., 1985) and heifers (Johnson et al., 1985) blocked the post-surgery rise in FSH secretion and prevented compensatory ovarian hypertrophy, suggesting that FSH may play a similar role in the female.

Exactly how FSH regulates Sertoli cell function in response to hemicastration is not completely understood, as very few studies have specifically addressed this question. Orth et al. (1984) demonstrated that hemicastration of immature rats increased the percentage of Sertoli cells labelled with [^{3}H]thymidine in the remaining testis. It was further determined that administration of exogenous FSH to cultures of immature rat testes significantly increased [^{3}H]thymidine labeling of Sertoli cells (Orth, 1984). If, as has been suggested for many species, Sertoli cell division ceases before puberty, and the ability of FSH to stimulate Sertoli cell proliferation also is age related, this may help explain why hemicastration-induced hypertrophy is more pronounced in prepubertal animals. In the study by Orth et al. (1984), the ability of Sertoli cells to incorporate [^{3}H]thymidine post-hemicastration had returned to normal within 3 weeks. Yet, we have shown that peripheral FSH concentrations are elevated for a longer period of time (Brown and Chakraborty, 1991). It would appear that normalization of Sertoli cell division in hemicastrates occurs because the cells become less responsive to the mitotic effects of FSH as the animal matures, rather than to a decline in actual hormone secretion (Orth et al., 1984). This is not unlike the observation that FSH-stimulated production of cAMP by rat Sertoli cells *in vitro* also decreases in progressively older rats (Steinberger et al., 1978).

The mechanism responsible for the rapid compensation in testosterone secretion following removal of one testis has baffled researchers for decades. And even today, no convincing explanation has been found. It has been shown that androgen compensation is prevented by hypophysectomy (Frankel et al., 1984). Yet, although LH is an important regulator of testosterone production, studies conducted in the rat (Hochereau-de Reviers, 1975; Selin and Moger, 1979a; Mock and Frankel, 1982; Frankel and Mock, 1982; Frankel and Wright, 1982; Orth et al., 1984; Rivier et al., 1989; Brown and Chakraborty, 1991; Brown et al., 1991a,b), bull (Haynes et al., 1976; Leidl et al., 1980; Barnes et al., 1983; Boockfor et al., 1983a) and boar (Kittock et al., 1984; Kosco et al., 1987) have failed to demonstrate that androgen compensation is the result of altered basal or pulsatile LH secretion. Studies conducted in rams are somewhat conflicting, however, with increases (Land and Carr, 1975; de Reviers et al., 1980; Hochereau-de Reviers, 1975; Hochereau de Reviers et al., 1976; 1980; Hoagland and Bolt, 1986) or no changes (Walton et al., 1978, 1980; Barnes et al., 1980c; Jenkins and Waites, 1983; Waites et al., 1983) in LH secretion being reported. Unfortunately, most of the studies conducted in sheep utilized infrequent blood sampling protocols. Since LH is secreted in a pulsatile manner, we designed studies to more precisely characterize the dynamics of LH secretion following hemicastration in the ram lamb. By collecting blood samples at 12- to 15-minute intervals for 10 hours on Days 1 and 7, and during the 4th, 8th and 12th month post-surgery, we did not detect any significant alterations in basal or pulsatile LH secretion in hemicastrates (Brown et al., 1987, 1988). We also did not observe any effect of hemicastration on the biological activity of the secreted LH (Brown et al., 1990). Finally, no discernible changes in pituitary LH content at 2, 7, or 14 days post-surgery were found (Brown et al., 1988). From our data, we concluded that altered LH secretion is not a consequence of hemicastration in the ram lamb. There is no obvious explanation as to why the studies conducted in sheep differ with regards to hemicastration-induced changes in LH secretion. The differences might be due to the different LH assays used (perhaps differences in the degree of FSH cross-reactivity); however, this explanation seems unlikely considering the rigorous validations that are conducted.

Since peripheral LH secretion apparently is unaltered by hemicastration, at least in the majority of studies, it has been speculated that increased gonadal sensitivity to LH might mediate compensatory androgen

secretion instead. However, as discussed above, corresponding increases in testis LH receptor concentrations are not a response to hemicastration. Instead, there is some evidence to suggest that FSH may influence Leydig cell function. In immature rats, exogenous FSH induces rapid hypertrophy and hyperplasia of interstitial cells which ultimately develop into mature Leydig cells (Kerr and Sharpe, 1985a,b). Exposure of rats to FSH also increases the responsiveness of the testis to LH-stimulated testosterone release *in vivo* and *in vitro* (Odell et al., 1973; Odell and Swerdloff, 1975; Chen et al., 1976, 1977; Selin and Moger, 1977). In addition, Ketelslegers et al. (1978) found a correlation between rising FSH levels and testosterone secretion in the developing rat. Finally, FSH stimulates the production of paracrine Sertoli cell factors which can, in turn, enhance Leydig cell testosterone production *in vitro* (Bergh, 1983; Trabone et al., 1984; Benahmed et al., 1987). Based on these observations we examined the role of FSH in modulating hemicastration-induced androgen compensation. However, our results determined that abolishing the FSH rise by follicular fluid treatment did not prevent compensatory testosterone secretion from occurring in either prepubertal or adult rats (Brown et al., 1991a,b) or in prepubertal ram lambs (Brown et al., 1991d). Clearly, more work is needed to elucidate the mechanisms responsible for compensatory androgen secretion, which undoubtedly will depend on a more thorough understanding of how various putative intratesticular agents or neural factors regulate testicular steroidogenesis.

Prolactin and Growth Hormone

Evidence that prolactin regulates some aspects of male reproductive function has led to speculation that it may be involved in the hemicastration response. Receptors for prolactin have been identified on Leydig cells (Aragona et al., 1977; Charreau et al., 1977; Costlow and McGuire, 1977), and a positive relationship between circulating prolactin concentrations, testicular LH receptor binding and testicular weight has been demonstrated in a number of species (mouse, Bohnet and Friesen, 1976: rat, Aragona et al., 1977: hamster, Bex and Bartke, 1977). In contrast, hypoprolactinemia induced by either chemical (CB-154), physical (decreased photoperiod, hypophysectomy) or genetic (*dw* and *df* dwarf mice) means results in testicular atrophy, reduced LH receptor binding and suppressed testosterone responses to LH (Gaston and Menaker, 1967; Boyns et al., 1972; Hafiez et al., 1972; Bohnet and Friesen, 1976; Aragona et al., 1977; Bex and Bartke, 1977). These studies suggest that prolactin may regulate the steroidogenic capacity of the testis, possibly through synergizing with LH. If that is true then it could explain how testosterone production by the remaining testis increases in the face of unchanged LH secretion. Unfortunately, the results to date do not substantiate such a role for prolactin (rat, Frankel and Wright, 1982: ram lamb, Waites et al., 1983; Brown et al., unpublished observations: boar, Kosco et al., 1987; Minton and Wettemann, 1988). To our knowledge, elevated prolactin secretion following unilateral castration has only been observed in one study involving 3-month-old bull calves (Barnes et al., 1983). In that study, prolactin was increased above intact bulls in serial samples collected at 1 and 6 weeks, but not at 12 or 24 weeks post-surgery.

A specific effect of growth hormone (GH) on testis function has not been firmly established, although it may affect Leydig cells by binding to prolactin receptors (Zipf et al., 1978). Data regarding changes in GH secretion in response to hemicastration are equivocal, although these differences may be species-related. Specifically, no changes in GH concentrations have been observed in hemicastrated prepubertal or adult rats (Brown et al., unpublished observations) or ram lambs (Waites et al., 1983; Brown et al., unpublished observations). In contrast, GH concentrations were elevated following hemicastration of prepubertal bulls (Al-Haboby et al., 1988) and boars (Kosco et al., 1987). In the boar, GH was elevated during the entire period compensatory hypertrophy was occurring, between 6 and 28 days post-surgery, compared to FSH which was only elevated during the latter half (between 14 and 28 days). Therefore, it was concluded that GH, rather than FSH, may mediate the hemicastration response in that species. In the bull, blood samples were collected every 15 days between 25 and 250 days post-surgery; GH was significantly elevated above intact controls on days 15 and 30, and between days 130 and 190. In both the bull and boar, increased GH secretion following hemicastration was associated with an acceleration of puberty compared with their intact counterparts. This response was compared to that observed in GH deficient patients with delayed puberty, where GH supplementation induced pubertal changes (Kusano et al., 1984; Al-Haboby et al., 1988). Unfortunately, too few studies have been conducted to conclusively determine GH's role in hemicastration responses, especially with regards to these apparent species differences. However, the comprehensive nature of the hemicastration response, involving both tubular and intertubular compartments, should provide an excellent model system for investigating how, or if, GH modulates testicular function.

CONCLUSIONS

Because of the dramatic changes to the hypothalamo-hypophyseal-gonadal axis which occur after unilateral castration, this technique has been used to study the regulation of testicular function in many mammalian species. However, despite decades of work and hundreds of studies, there are still many unanswered questions regarding how hemicastration

invokes these dramatic compensatory responses. It is generally agreed that FSH plays at least a partial role in compensatory hypertrophy through its actions on seminiferous tubule components, although there is much debate about whether its effects are mediated via cellular hyperplasia, hypertrophy or both. It is clear, however, that the influence of age, season and species must be carefully considered when evaluating the role of FSH in defining hemicastration-induced responses. Much less understood is the mechanism controlling enhanced androgen secretion. Unfortunately we know a lot more about what is not involved (i.e. altered gonadotropin secretion or testis receptor binding) than what is. Most likely, we will find that hemicastration-induced responses are not the result of changes in any one component, but rather involve a complex interplay between several factors that regulate the reproductive process.

Studies are now needed to investigate how the nervous system affects testicular function in general, and the hemicastration response in particular. As suggested above, hemicastration-induced perturbations in testicular innervation may announce the loss of one gonad, resulting in a cascade of events responsible for maintaining reproductive homeostasis. Additional studies are also needed to examine the involvement of paracrine and autocrine factors in mediating hemicastration responses. There is now a good body of evidence substantiating the existence of complex cell-cell interactions within the testis. These interactions could involve growth factors (eg. insulin-like growth factor-I and II, epidermal growth factor, transforming growth factor-α and β, fibroblast growth factor, nerve growth factor, interleukin-1) or other agents (eg. gonadotropin releasing hormone, arginine-like vasopressin, atrial natriuretic factor, inhibin/activin) implicated in the regulation of various aspects of testicular function (see reviews, Skinner, 1993; Sharpe, 1993; Spiteri-Grech and Nieschlag, 1993). In sum, continued investigations into the mechanisms controlling hemicastration responses are warranted, for they could ultimately provide information critical for developing better methods of treating human infertility or increasing the sperm producing capabilities of genetically and economically valuable males, or alternatively, for designing a successful male contraceptive.

REFERENCES

Agee, J., Parsa, C. & Huntrakoon, M. 1988. Arch. Androl. 20:1-9.

Al-Haboby, A.H., Loseth, K.L., Wheaton, J.E., Crabo, B.G. 1988. Dom. Anim. Endocrinol. 5:61-69.

Amann, R.P. 1970. In Johnson, A.D., Gomes, W.R. & Van-Demark, N.L. (Eds.) The Testis, vol. 1 Academic Press, New York, pp. 433-482.

Amann, R.P. & Lambiase Jr, J.T. 1969. J. Anim. Sci. 28:369-374.

Aragona, C., Bohnet, H.G. & Friesen, H.C. 1977. Acta Endocrinol. 84:402-409.

Barnes, M.A., Longnecker, J.V., Charter, R.C., Riesen, J.W. & Woody, C.O. 1980a. Theriogenology 14:49-57.

Barnes, M.A., Longnecker, J.V., Riesen, J.W. & Woody, C.O. 1980b. Theriogenology 14:58-66.

Barnes, M.A., Riesen, J.W. & Woody, C.O. 1980c. Theriogenology 14:67-81.

Barnes, M.A., Boockfor, F.R., Bierley, S.T., Kazmer, G.W., Halman, R.D. & Dickey, J.F. 1981. J. Anim. Sci. 53:1341-1350.

Barnes, M.A., Kazmer, G.W., Boockfor, F.R., Wade, R.J., Halman, R.D. & Dickey, J.F. 1983. Theriogenology 19:635-646.

Benahmed, M., Morera, A.M., Chauvin, M.A., Peretti, E.D. & Revol, A. 1987. Ann. NY Acad. Sci. 513:470-472.

Bergh, A. 1983. Int. J. Androl. 6:57-65.

Bergh, A., Damber, J.-E. & Lindgren, S. 1982. Experientia 38:597-598.

Bex, F.J. & Bartke, A. 1977. Endocrinology 100:1223-1226.

Bogdanove, E.M., Nolin, J.M. & Campbell, G.T. 1975. Rec. Prog. Horm. Res. 31:567-626.

Bohnet, H.C. & Friesen, H.G. 1977. J. Reprod. Fert. 48:307-311.

Boockfor, F.R., Barnes, M.A., Kazmer, G.W., Halman, R.D., Bierley, S.T. & Dickey, J.F. 1983a. J. Anim. Sci. 56:1376-1385.

Boockfor, F.R., Barnes, M.A. & Dickey, J.F. 1983b. J. Anim. Sci. 56:1386-1392.

Boyns, A.R., Cole, E.N., Golder, M.P., Danutra, V., Harper, M.E., Brownsey, B., Cowley, T., Jones, G.E. & Griffiths, K. 1972. Proceedings of the 4th Tenovus Workshop, pp. 207-216.

Brown, J.L., Stuart, L.D. & Chakraborty, P.K. 1987. J. Anim. Sci. 65:1563-1570.

Brown, J.L., Schoenemann, H.M., Stuart, L.D. & Chakraborty, P.K. 1988. Biol. Reprod. 39:845-861.

Brown, J.L., Schoenemann, H.M., Chakraborty, P.K., Stuart, L.D. & Dahl, K.D. 1990. Biol. Reprod. 43:548-553.

Brown, J.L. & Chakraborty, P.K. 1991. J. Androl. 12:119-125.

Brown, J.L., Dahl, K.D. & Chakraborty, P.K. 1991a. J. Endocrinol. 130:207-212.

Brown, J.L., Dahl, K.D. & Chakraborty, P.K. 1991b. J. Androl. 12:221-225.

Brown, J.L., Chakraborty, P.K., Mitchell, A.S. & Nelson, M.F. 1991c. Biol. Reprod. 44 (Suppl. 1):128 (Abstr.).

Brown, J.L., Schoenemann, H.M., Nelson, M.F., Mitchell, A.S. & Chakraborty, P.K. 1991d. J. Anim. Sci. 69 (Suppl. 1):426 (Abstr).

Canivenc, R. & Relexans, M.C. 1967. C.r. hebd. Séanc. Acad. Sci., Paris 264:2135-2140.

Channing, C.P., Tanabe, K., Turner, C.K. & Hodgen, G.D. 1982. J. clin. Endocrinol. Metab. 55:481-486.

Chappel, S.C. & Ramaley, J.A. 1985. Biol. Reprod. 32:567-573.

Charreau, E.H., Attramadal, A., Torjesen, P.A., Purvis, K., Calandra, R. & Hansson, V. 1977. Mol. Cell. Endocrinol. 6:303-307.

Chavez, R., Cruz, M.E. & Dominguez, R. 1987. J. Endocrinol. 113:397-401.

Chen, Y.D.I., Payne, A.P., Kelch, R.P. 1976. Proc. Soc. Exp. Biol. Med. 153:4730475.

Chen, Y.D.I., Shaw, M.J. & Payne, A.P. 1977. Mol. Cell. Endocrinol. 8:291-299.

Costlow, M.E. & McGuire, W.L. 1977. J. Endocrinol. 75:221-226.

Cracchiolo, S.A. & Hoagland, T.A. 1987. Annal. N.Y. Acad. Sci. 513:350-352.

Cunningham, G.R., Tindall, D.J., Huckins, C. & Means, A.R. 1978. Endocrinology 102:16-23.

Dahl, K.D., Biscak, T.A. & Hsueh, A.J.W. 1988. Science 239:72-74.

Dahl K.D., Pavlou, S.N., Kovacs, W.J. & Hsueh, A.J.W. 1986. J. Clin. Endocrinol. Metab. 63:792-794.

de Jong, F.H. & Robertson, D.M. 1985. Mol. Cell. Endocrinol. 42:95-103.

Delost, P. 1972. C. r. Séanc. Soc. Biol. 166:879-884.

de Reviers. M., Hochereau-de Reviers. M.T., Blanc, M.R., Brillard, J.P., Courot. M. & Pelletier, J. 1980. Reprod. Nutr. Dévelop. 20 (1B):241-249.

Esch, F.S., Shimasaki, S., Mercado, M., Cooksey, K., Ling, N., Ying, S., Ueno, N. & Guilleman, R. 1987. Mol. Cell. Endocrinol. 1:849-854.

Findlay, J.K., Gill, T.W. & Doughton, B.W. 1985. J. Reprod. Fert. 73:329-335.

Frankel, A.I. & Mock, E.J. 1982. J. Endocrinol. 92:225-229.

Frankel, A.I. & Wright, W.W. 1982. J. Endocrinol. 92:213-223.

Frankel, A.I., Mock, E.J. & Chapman, J.C. 1984. Biol. Reprod. 30:804-808.

Frankel, A.I., Chapman, J.C. & Cook, B. 1989a. J. Endocrinol. 121:43-48.

Frankel, A.I., Chapman, J.C. & Cook, B. 1989b. J. Endocrinol. 122:485-488.

Furuya, T. 1990. Biol. Reprod. 42:491-498.

Furuya, T., Kohno, J. & Hokano, M. 1984. Zool. Sci. (Tokyo) 1:977.

Gaston, S. & Menaker, M. 1967. Science 158:925-928.

Griswold, M.D., Solari, A., Tung, P.A. & Fritz, I.B. 1977. Mol. Cell. Endocrinol. 7:151-165.

Hafiez, A.A., Lloyd, C.W. & Bartke, A. .1972. J. Endocrinol. 52:327-332.

Haynes, N.B., Hafs, H.D., Purvis, K. & Manns, J.G. 1976. J. Reprod. Fert. 46:471-473.

Henderson, K.M., Franchimont, P., Charlet-Renard, C. & McNatty, K.P. 1984. J. Reprod. Fert. 72:1-8.

Henderson, K.M., Prisk, M.D., Hudson, N., Ball, K., McNatty, K.P., Lun, S., Heath, D., Kieboom, L.E. & McDiarmid, J. 1986. J. Reprod. Fert. 76:623-635.

Hermans, W.P., van Leeuwen, E.C.M., Debets, M.H.M. & de Jong, F.H. 1980. J. Endocrinol. 86:79-92.

Hoagland, T.A. & Bolt, D.J. 1986. Theriogenology 26:671-682.

Hochereau-de Reviers, M.-T. 1970. Morph. Aspects Androl. 1:43-46.

Hochereau-de Reviers, M.-T. 1975. Ann. Biol. anim. Biochem. Biophys. 15:621-631.

Hochereau-de Reviers, M.-T. & Pelletier, J. 1971. J. Reprod. Fert. 27:498 (Abstr).

Hochereau-de Reviers, M.-T. & Courot, M. 1978. Ann. Biol. anim. Biochem. Biophys. 18:573-583.

Hochereau-de Reviers, M.-T., Loir, M. & Pelletier, J. 1976. J. Reprod. Fert. 46:203-209.

Hochereau-de Reviers, M.-T., Blanc, M.R., Courot, M., Garnier, D.H., Pelletier, J. & Poirier, J.C. 1980. In Steinberger, A., Steinberger, E. (Eds.) Testicular Development, Structure, and Function. Raven Press, New York, pp. 237-247.

Hochereau-de Reviers, M.-T., Land, R.B., Perreau, C. & Thompson, R. 1984. J. Reprod. Fert. 70:157-163.

Hodson, N. 1970. In Johnson, A.D., Gomes, W.R. & Van-Demark, N.L. (Eds.) The Testis, vol. 1 Academic Press, New York, pp. 47-99.

Huhtaniemi, I.T., Dahl, K.D. & Hsueh, A.J.W. 1988. J. Clin. Endocrinol. Metab. 66:308-313.

Hutchinson, J.S.M. 1988. J. Endocrinol. 118:169-171.

Jenkins, N. & Waites, G.M.H. 1983. J. Reprod. Fert. 67: 325-334.

Johnson, B.H. 1978. Theriogenology 10:257-264.

Johnson, L. & Neaves, W.B. 1981. Biol. Reprod. 24 (Suppl. 1):36A.

Johnson, B.H., Desjardins, C. & Ewing, L.L. 1971. J. Anim. Sci. 33 (Suppl. 1):257.

Johnson, S.K., Smith, M.F. & Elmore, R.G. 1985. J. Anim. Sci. 60:1055-1060.

Jones, H.M., Wood, C.L. & Rush, M.E. 1985. Life Sci. 36:889-899.

Kalra, S.P., Prasad, M.R.N. & Uberoi, N.K. 1969. Fertil. Steril. 20:258-266.

Keel, B.A. & Grotjan, H.E. 1985. Endocrinology 117:354-360.

Kerr, J.B. & Sharpe, R.M. 1985a. Endocrinology 116:2592-2604.

Kerr, J.B. & Sharpe, R.M. 1985b. Cell Tiss. Res. 239:405-415.

Ketelslegers, J.M., Hetzel, W.D., Sherins, R.J. & Catt, K.J. 1978. Endocrinology 103:212-222.

Kittock, R.J., Kinder, J.E. & Johnson, R.K. 1984. J. Anim. Sci. 58:1271-1277.

Kosco, M.S., Bolt, D.J., Wheaton, J.E., Loseth, K.J. & Crabo, B.G. 1987. Biol. Reprod. 36:1177-1185.

Kosco, M.S., Loseth, K.J. & Crabo, B.G. 1989a. J. Reprod. Fert. 87:1-11.

Kosco, M.S., Loseth, K.J. & Crabo, B.G. 1989b. J. Reprod. Fert. 87:13-21.

Kusano, S., Shiki, Y., Ichimura, K., Amemiya, S., Noqaki, Y., Ohyana, K. & Kato, K. 1984. Excerpts Medica International Congress Series 652:861 (Abstr.).

Land, R.B. & Carr, W.R. 1975. J. Reprod. Fert. 45:495-501.

Leidl, W., Braun, U., Stolla, R. & Schams, D. 1980. Theriogenology 14:173-184.

Liang, D.S. & Liang, M.D. 1970. J. Reprod. Fert. 21:537-540.

Lindgren, S., Damber, J.-E. & Carstensen, H. 1976. Life Sci. 18:1203-1206.

Lindner, H.R. & Rowson, L.E.A. 1961. J. Endocrinol. 23:167-170.

Lorenzen, J.R., Dworkin, G.H. & Schwartz, N.B. 1981. Am. J. Phys. 240:E209-E215.

Martinet, L. & Meunier, M. 1975. Annls. Biol. anim. Biochem. Biophys. 15:607-609.

Mason, A.J., Hayflick, J.S., Ling, N., Esch, F., Ueno, N., Ying, S.-Y., Guilleman, R., Niall, H. & Seeburg, P.H. 1985. Nature 318:659-661.

Minton, J.E. & Wetteman, R.P. 1988. Dom. Anim. Endocrinol. 5:71-80.

Mirando, M.A., Cracchiolo, S.A., Graves-Hoagland, R.L., Perkins, C.R., Riesen, J.W. & Hoagland, T.A. 1987a. Annal. N.Y. Acad. Sci. 513:347-349.

Mirando, M.A., Frank, L.H., Hoagland, T.A., Woody, C.O. & Riesen, J.W. 1987b. Biol. Reprod. (Suppl. 1):36:159 (Abstr.).

Mirando, M.A., Hoagland, T.A., Woody, C.O. & Riesen, J.W. 1989. Biol. Reprod. 41:798-806.

Mizunuma, H., DePalatis, L.R. & McCann, S.M. 1983. Neuroendocrinology 37:291-296.

Mock, E.J. & Frankel, A.I. 1982. J. Endocrinol. 92:231-236.

Moger, W.H. 1977. Biol. Reprod. 17:661-667.

Moger, W.H. & Anakwe, O.O. 1986. J. Reprod. Fert. 76:251-256.

Odell, W.D. & Swerdloff, R.S. 1975. J. Steroid Biochem.6:853-857.

Odell, W.D., Swerdloff, R.S., Jacobs, H.S. & Hescox, M.A. 1973. Endocrinology 92:160-166.

Orth, J.M., Higginbotham, C.A. & Salisbury, R.L. 1984. Biol. Reprod. 30:263-270.

Orth, J.M. 1982. Ant. Rec.203:485-492.

Orth, J.M. 1984. Endocrinology 115:1248-1255.

Ortavant, R. 1959. Ann. Zootech. 8:183-321.

Ott, K.M., Mannen, K.A., Dinger, J.E., Hoagland, T.A., Woody, C.O. & Riesen, J.W. 1984. J. Anim. Sci. 59 (Suppl. 1): Abstr. 512.

Putra, D.K.H. & Blackshaw, A.W. 1982. Aust. J. Biol. Sci. 35:287-293.

Putra, D.K.H. & Blackshaw, A.W. 1985. Aust. J. Biol. Sci. 38:429-434.

Redmer, D.A., Christenson, R.K., Ford, J.J., Day, B.N. 1985. Biol. Reprod. 32:111-119.

Ribbert, H. 1890. Virchows Arch. Path. Anat. Physiol. 120: 247-272.

Rivier, C., Meunier, H., Roberts, V. & Vale, W. 1989. Biol. Reprod. 41:967-981.

Robertson, D.M., Foulds, L.M. & Ellis, S. 1982. Endocrinology 111:385-391.

Sairam, M.R. 1989. FASEB J. 3:1915-1925.

Sairam, M.R. & Schiller, P.W. 1979. Arch. Biochem. Biophys. 197:294-301.

Schanbacher, B.D. 1984. J. Anim. Sci. 58:943-948.

Schanbacher, B.D. 1988. Endocrinology 123:2323-2330.

Schanbacher, B.D. & Ford, J.J. 1977. Endocrinology 100:387-393.

Schanbacher, B.D., Fletcher, P.W. & Reichert, L.E. 1987. Biol. Reprod. 36:1142-1148.

Scott, R.S. & Burger, H.G. 1981. Biol. Reprod. 24:541-550.

Selin, L. & Moger, W.H. 1977. Endocrine Res. Comm. 4:171-182.

Selin, L. & Moger, W.H. 1979a. J. Endocrinol. 82:95-104.

Selin, L. & Moger, W.H. 1979b. Fertil. Steril. 32:248.

Sharpe, R. 1993. In Russell, L.D & Griswold, M.D. (Eds.) The Sertoli Cell. Cache River Press, Clearwater, Florida, pp. 391-418.

Skinner MK (1993) In Russell, L.D & Griswold, M.D. (Eds.) The Sertoli Cell. Cache River Press, Clearwater, Florida, pp. 237-247.

Smelser, G.K. 1933. Anat. Rec. 57 (Suppl.):28.

Spiteri-Grech, J. & Nieschlag, E. 1993. J. Reprod. Fert. 98:1-14.

Steinberger, A., Huntz, M. & Heindel, J.J. 1978. Biol. Reprod. 19:566-572.

Summerville, J.W. & Schwartz, N.B. 1981. Endocrinology 109:1142-1150.

Sundby, A., Andersen, O. & Standal, N. 1981. Theriogenology 16:249-257.

Tcholakian, R.K., Chowdhury, M. & Chowdhury, A.K. 1978. Biol. Reprod. 19:431-438.

Tenover, J.S., Dahl, K.D., Hsueh, A.J.W., Lim, P., Matsumoto, A.M. & Bremner, W.J. 1987. J. Clin. Endocrinol. Metab. 64:1103-1108.

Trabone, E., Behahmed, M., Reventos, J. & Saez, J.M. 1984. Cell Tissue Res. 237:257-262.

Ultee-van Gessel, A.M., Leemborg, F.G., de Jong, F.H. & van der Molen, H.J. 1985. J. Endocrinol. 106:259-265.

Voglmayr, J.K. & Mattner, P.E. 1968. J. Reprod. Fert. 17:179-181.

Waites, G.M.H., Wenstrom, J.C., Crabo, B.G. &

Hamilton, D.W. 1983. Endocrinology 112:2159-2167.

Walton, J.S., Evins, J.D., Hillard, M.A. & Waites, G.M.H. 1980. J. Endocrinol. 84:141-152.

Walton, J.S., Evins, J.D. &Waites, G.M.H. 1978. J. Endocrinol. 77:75-84.

Wood, P.H. & Wiebe, J.P. 1989. Endocrinology 125:41-48.

Zipf, W.B., Payne, A.H. & Kelch, R.P. 1978. Endocrinology 103:595-600.