Endocrine Diagnostics for Stallion Infertility

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Introduction

Infertility in breeding stallions contributes to low conception rates (60 - 65%) and substantial financial loss in the equine industry. There are a number of conditions associated with poor fertility such as mismanagement, anabolic steroid treatment, infection, fever, tumors, injury, disease, behavior or endocrine disorders [1-10]. A number of these problems can be diagnosed and treated appropriately. However, for stallions with poor sperm quality and endocrine disorders not associated with hypogonadotropic hypogonadism (abnormally low GnRH, LH, FSH and testosterone), the exact nature of their condition and the hormonal factors involved has not yet been elucidated. Previous studies in our laboratory have identified endocrine factors and mechanisms important for normal reproductive function and have demonstrated specific alterations in pituitary and testicular function between fertile, subfertile and infertile stallions [11-25].

A significant percentage of idiopathic subfertile/infertile stallions have been diagnosed with primary testicular degeneration marked by progressive softening and/or reduction of the testes, endocrine imbalance and poor seminal parameters [1,2,21]. These stallions make up a heterogeneous population of poor breeding performers with unknown pathophysiology. Recent evidence indicates that local regulatory factors such as inhibin and insulin-like growth factor-1 (IGF-1] (paracrine/autocrine factors) are important modulators of steroidogenesis and spermatogenesis [26-28]. Data generated in our laboratory support the hypothesis that the primary testicular disorder in these stallions may be, in part, an initial failure of the paracrine/autocrine system which is then followed by abnormal spermatogenesis and a hormonal imbalance [24]. If this is the case then assessing testicular function by carrying out a number of different endocrine/paracrine/autocrine tests may assist the researcher and clinician to identify the underlying cause of the problem. The tests would involve the following:

1. Measurement of circulating concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estrogen and inhibin to assess the hypothalamic-pituitary-testicular axis,
2. GnRH stimulation tests to assess pituitary and subsequent testicular responsiveness,
3. a human chorionic gonadotropin (hCG) stimulation test to directly assess testicular responsiveness and,
4. a testicular biopsy to measure local paracrine/autocrine factors such as inhibin and IGF-1 or evaluate histopathology.

Endocrine Approach: Monitoring Specific Alterations in the Hypothalamic-Pituitary - Testicular Axis

Previous studies on the endocrine regulation of reproductive function in the stallion have demonstrated disparities in pituitary and testicular output among fertile, subfertile (idiopathic oligospermia) and infertile (idiopathic azoospermia) animals [1,7,8,14,15,18,29-31]. Low levels of testosterone have been found by some investigators in azoospermic stallions [29]. In contrast, testosterone levels in normal and impotent stallions showed no significant differences while the serum concentrations of estradiol-17β and LH were low [8]. In contrast, Irvine and coworkers [31] refuted these results demonstrating that serum levels of LH, estradiol and testosterone (T) were not predictive or causally related to abnormalities in sexual behavior. Burns and Douglas [7] made the first association between elevated plasma concentrations of FSH and subfertility in stallions. Three recent studies suggest that circulating plasma levels of FSH and estrogens, and not LH and T, may be good markers to predict future changes in fertility [1,14,15], whereas another group of investigators suggests that measurement of serum levels of estrogens and testosterone may help in the diagnosis of infertile stallions when other methods are not available [30].

In addition to measuring basal levels of reproductive hormones in the male, several investigators have used the GnRH stimulation test whereby a bolus or intermittent infusion doses of synthetic GnRH are given to assess pituitary and testicular responsiveness [32,33]. The GnRH stimulation test appears to be of some value in revealing intrinsic differences in acute gonadotroph and Leydig cell stimulation between normal men and men with reproductive disorders [34-40]. When stallions were challenged with exogenous pulses of GnRH, investigators observed different pituitary and testicular responses between fertile and subfertile stallions, which appeared to be dependent on season and gonadal status [11,14]. The pituitary and testes
of fertile stallions appeared to be more responsive to pulsatile administration of GnRH in the non-breeding season than the breeding season [11]. In contrast, the pituitary responsiveness to repetitive pulses of GnRH in subfertile stallions was significantly reduced during the non-breeding season and not affected by seasonal changes [14].

When stallions were subjected to discrete, non-pulsatile, doses of GnRH in a subsequent study, responsiveness was observed to be normal [15]. In this study, the capacity of Leydig cells to respond to discrete doses of GnRH (via endogenous LH) was monitored and shown to be significantly reduced. Further studies on a subpopulation of these subfertile stallions demonstrated the presence of a possible circulating LH isoform(s) with reduced bioactivity [13]. Taken together, the results of these studies suggest that the low testicular response in subfertile stallions may be due to the following pathological conditions: 1) a hypothalamic-pituitary dysfunction in gonadotropin production or release and/or 2) a primary testicular disorder in production or release in steroid hormones.

A Hypothalamic-Pituitary Disorder or a Primary Testicular Disorder in Idiopathic Subfertile/Infertile Stallions?
As discussed above, previous studies have demonstrated that idiopathic subfertility/infertility may be associated with normal to high plasma gonadotropin levels (particularly FSH), a circulating form of a biologically inactive LH isoform, decreased pituitary and testicular responsiveness to GnRH and low plasma estrogen levels in the presence of normal concentrations of plasma testosterone [13-15]. Although these observations suggest both a pituitary and testicular dysfunction, it is not apparent which reproductive organ has a primary defect or how one organ might affect the demise of the other. Recent studies in our laboratory, however, point to a primary testicular problem. In one study, a group of subfertile stallions had a significantly lower testosterone response to a challenge of hCG (10,000 IU) than did the fertile group [21]. In another study, gonadotropin levels in subfertile and infertile stallions declined from elevated levels to normal castrated levels by one year post-castration [23]. Taken together, these studies suggest that the primary problem may not be at the level of the hypothalamus or pituitary but at the level of the testes.

To further explore testicular function in idiopathic subfertile and infertile stallions, two recent studies were carried out whereby testicular tissue from fertile, subfertile and infertile stallions was assessed for LH receptor binding activity and inhibin concentrations. The first study demonstrated that the number of LH receptors and receptor affinity did not change with fertility status suggesting that a testicular disorder is most likely present at the post-receptor level [22]. The second study indicated that in stallions with poor fertility, testicular inhibin concentrations declined early on, before changes in testicular steroid hormones were observed, suggesting that an early paracrine/autocrine dysfunction may occur at the level of the Sertoli cell [24]. If inhibin acts as a paracrine factor influencing Leydig cell steroidogenesis, as suggested in the rat literature [41,42], then an increase or decrease in testicular inhibin may directly affect steroid production and eventually spermatogenesis. We propose that the cause of idiopathic subfertility/infertility in stallions may initially involve testicular disorders associated with changes in paracrine/autocrine factors that are necessary for local interactions among Sertoli, Leydig and germ cells. A testicular biopsy method would be useful in measuring paracrine/autocrine factors in testicular tissue.

Paracrine/Autocrine Approach: Testicular Biopsy
Little research has been done in the stallion to identify paracrine/autocrine factors as modulators of testicular function. However, work in other species suggests that these factors may be important for initiation and maintenance of steroidogenesis and spermatogenesis [3]. Paracrine factors are those that are secreted by one cell type and act upon another cell type to initiate a response. An autocrine factor is secreted from one cell type and acts upon the same cell type. Although testosterone and estrogen have been identified as part of the endocrine system where hormones are secreted from one gonad, enter the circulation and act upon another gonad, both appear to also play a role as paracrine/autocrine modulators. Reports in the literature clearly indicate that testosterone secreted by Leydig cells acts upon Sertoli and germ cells to maintain and restore spermatogenesis in the adult testis, whereas estrogen may be secreted from either the Sertoli or Leydig cell and act upon the same cell to regulate steroidogenesis [43-45]. Recent evidence in our laboratory suggests that both inhibin and IGF-1 may play a role in local regulation of testicular function [24,46]. As discussed above, it has been reported by others that inhibin and IGF-1 appear to modulate Leydig cell steroidogenesis and germ cell development [26-28]. The involvement of paracrine and autocrine modulation of steroidogenesis and spermatogenesis not only suggests a more complicated story but also supports the conceptual argument that not one hormone or factor alone is sufficient to do the job efficiently and effectively.

To identify and monitor testicular paracrine/autocrine factors, it will be extremely important to develop a safe and efficient method of obtaining testicular tissue via testicular biopsy. The use of a testicular biopsy procedure has been controversial. Recently however, it appears that by using a "Biopty" instrument (C.R. Bard, Inc, Covington, GA), one could remove tissue and analyze local testicular factors [47]. Although the Biopty instrument provides a relatively simple approach to removing tissue, there are risks involved such as hemorrhage, inflammation and infection. Further research to identify paracrine/autocrine factors as indicators of declining fertility is warranted.
Conclusion
In summary, endocrine, paracrine and autocrine diagnostics will be very helpful in identifying certain reproductive problems in stallions involving the hypothalamic-pituitary-testicular axis. Evidence suggests that many of these stallions have a primary testicular disorder. The testicular dysfunction may involve a decline in testicular factors necessary for local regulation followed by abnormal sperm production and loss of feedback control of the hypothalamic-pituitary axis. To diagnose this problem and others, the following tests below may be helpful. It is important to note that these tests are only useful if baseline values in fertile stallions have been established in the laboratories running the tests. The lab tests for endocrine, paracrine or autocrine factors involve validated radioimmunoassays or enzyme immunoassays.

1. **Measurement of serum/plasma concentrations of FSH, LH, testosterone, estrogen and inhibin** to assess the hypothalamic-pituitary-testicular axis - to obtain accurate baseline levels of plasma or serum hormones. Daily blood samples for a minimum of three days should be drawn at the same time each day (preferably between 9 - 10 AM). High plasma concentrations of FSH followed by declining estrogens and inhibin, high LH and finally low testosterone are the sequence of endocrine events most often observed in stallions with progressive testicular dysfunction. In cases of hypogonadotrophic hypogonadism, very low LH, FSH and testosterone levels will be observed.

2. **A single pulse GnRH stimulation test** - to assess pituitary and testicular responsiveness. A single dose of 25 µg of GnRH should be given IV at 9 AM. Blood samples should be taken at -30 min and 0 min prior to treatment and every 30 minutes thereafter up to 120 min. Plasma or serum samples should be tested for LH and testosterone. An abnormally low response to a single challenge may help in the diagnosis of 1) a pituitary disorder in stallions with tumors or hypogonadotropic hypogonadism or 2) a testicular disorder in stallions with testicular dysfunction.

3. **A three pulse GnRH stimulation test** - to assess pituitary responsiveness. Three small doses should be given intravenously (5 µg/dose) one hour apart in the non-breeding season. Blood samples should be collected every 30 minutes for a minimum of 1 hour before and 6 hours after the start of treatment. Plasma samples should be analyzed for LH. Compared to fertile stallions, subfertile/infertile stallions have a significantly lower response to the second and third injection of GnRH, indicative of a pituitary problem.

4. **An hCG stimulation test** - to assess testicular responsiveness. An injection of 10,000 IU of hCG given IV. Blood samples should be collected every 30 min for a minimum of 1 hour before and 6 hours after treatment and analyzed for testosterone and estrogen. Compared to fertile stallions, infertile stallions have a significantly lower testosterone and estrogen response to hCG.

5. **Testicular biopsy** - to provide a direct measurement of paracrine/autocrine factors in testicular tissue along with a histological report. A biopsy approach can result in hemorrhage, inflammation and infection and should be done with some caution. Under mild sedation, using stocks to secure the stallion, a small incision is made in the scrotum in the center of the cranio-lateral quarter of the most affected testis using care to identify and avoid the caput epididymis whose position may overlie this area in some stallions. The Biopsy instrument attached to a 14 gauge split needle is placed through the incision against the tunica vaginalis, subsequently fired with the needle projecting into the testicular parenchyma and then removed. Two subsequent samples are collected through the same incision but at slightly different angles. Two punches are each placed in 1.2 ml of PBS and snap frozen in dry ice and alcohol or liquid nitrogen and stored frozen until assayed for paracrine/autocrine factors. The assay is not available commercially but can be run on a case by case basis in Dr. Jan Roser’s lab. The third sample is placed in Bouin's solution for 6 hours, transferred to 50 % alcohol and submitted for histological examination.

References


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