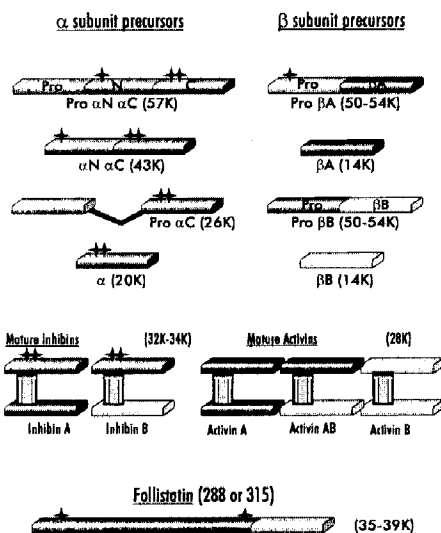


*Classically, inhibins and activins are defined by their ability to modulate the secretion of pituitary follicle-stimulating hormone (FSH). Inhibins suppress the release of FSH, while activins stimulate its release.*

Inhibins and activins are polypeptide hormones which belong to the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily. Inhibins are heterodimers consisting of a common  $\alpha$ -subunit linked to either a  $\beta_A$  subunit (Inhibin A) or  $\beta_B$  subunit (Inhibin B) by disulfide bridges. Activins are homo- or heterodimers of the  $\beta_A$  or  $\beta_B$  subunits producing Activin A, B, and AB. The subunits of the inhibins and activins are differentially processed, giving rise to various molecular forms (see Figure 1). The monomeric  $\alpha$  subunit is found free in the circulation at molar levels significantly greater than the dimers, but only the dimeric forms are physiologically active. Activins are bound with high affinity to follistatin with a possible regulatory role, while both inhibins and activins are found to bind reversibly with  $\alpha_2$ -macroglobulin.

Figure 1. Various molecular forms of inhibins and activins



+ = Glycosylation sites

Adapted from Pangas A. and Woodruff T., *Trends in Endocrinology & Metabolism*, 11, 309-314 (2000).

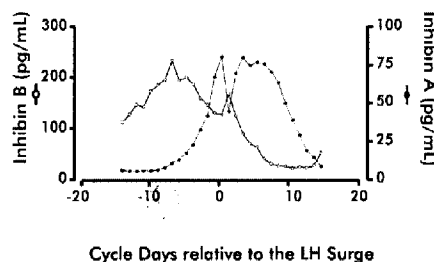
Inhibins are secreted by granulosa cells of the ovary in the female and Sertoli cells of the testis in the male. Both Inhibin A and Inhibin B are produced in females, but in males Inhibin B is the predominant circulating inhibin. The primary endocrine role of Inhibin B appears to be the regulation of gametogenesis via a negative

feedback mechanism on the production of FSH by the pituitary gland. Inhibins also have a local paracrine action in the gonads.

In women of reproductive age, circulating Inhibin A and B have different temporal profiles during the menstrual cycle. As shown in Figure 2, Inhibin A rises in the late follicular phase to a maximum in the luteal phase with an intermediate peak at ovulation. By contrast, Inhibin B levels rise to a mid-follicular maximum with a secondary peak at ovulation before falling to basal levels in the luteal phase. It has been reported that Inhibin A is produced largely by the dominant follicle/corpus luteum, whereas Inhibin B is produced by the smaller developing antral follicles.

During pregnancy, the level of Inhibin A is maintained by its secretion from the fetoplacental unit. Circulating Inhibin A levels reach an initial peak at 8-10 weeks, decline until approximately 20 weeks gestation, and then rise gradually until term. In postmenopausal women, circulating Inhibin A and B may fall to very low levels reflecting the absence of follicular activity.

Figure 2. Serum Inhibin A & Inhibin B profiles during the human menstrual cycle. LH = Luteinizing hormone



In males, Inhibin B is the predominant circulating inhibin produced by the Sertoli cells of the testis in support of spermatogenesis. The concentration of Inhibin B in the circulation of normal males is usually <480 pg/mL, and in contrast to the female, levels are essentially constant. However, in pathological situations such as infertility, levels of Inhibin B may be diminished. Inhibin A does not appear to have a significant role in the male as levels in the circulation are minimal, of the order measured in the circulation of postmenopausal women.

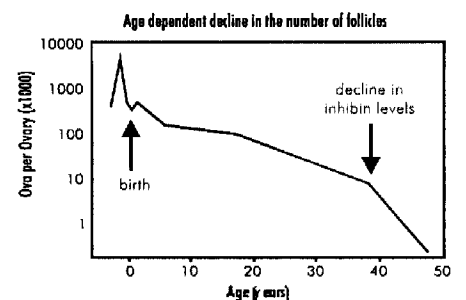
## Clinical Research Applications

### Disorders of Ovulation

#### Ovarian Reserve and Assisted Reproductive Technology (ART)

Ovarian reserve is a representation of the number of follicles remaining in a woman's ovary and is a useful measure of fertility status. During fetal life, the full complement of oocytes is deposited in the ovary, and following birth the number declines. As women age, there is a demonstrable reduction in follicle number, especially evident towards the age of 40 years (see Figure 3). The decline in fertility during aging is due mainly from the loss of oocytes and follicles in the ovary.

Figure 3. Age dependent decline in the number of follicles



Adapted from Conway et al (1997) *Current opinion in Obstetrics and Gynaecology*, 9: 202-206

Inhibin B is secreted directly by the granulosa cells of the small, developing follicles of the ovary, in contrast to FSH, which is secreted by the anterior pituitary gland. For this reason, Inhibin B serves as a direct, more sensitive and earlier marker of ovarian follicle number. As shown in Figure 3, there is a rapid decline in circulating Inhibin B levels at approximately age 40 years at the time of accelerated decline in follicle number. Low circulating levels of Inhibin B are potentially indicative of low ovarian reserve and the perimenopausal transition.

There is an increasing trend in developed countries around the world to defer child-rearing until later in life. An increasing number of women have found that their chances of a successful conception and pregnancy have been compromised by an earlier than expected

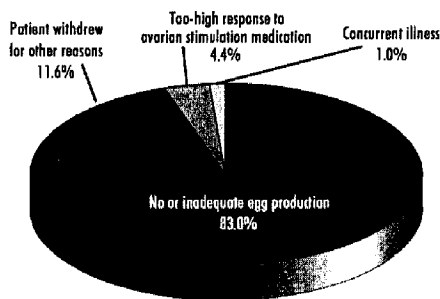
**"A young woman (20-30 years) with "normal" ovarian reserve should have a mid-follicular Inhibin B level of 125-150 pg/mL. We are concerned if the level is below 100 pg/mL, especially in the absence of a "normal" FSH. With levels below 80 pg/mL we are extremely pessimistic about the response of the ovaries to stimulation."**

**Dr. Gillian Lockwood**  
**Medical Director, Midland Fertility Services Aldridge, UK**

decline in ovarian reserve. In the US alone, infertility affects 6.1 million American women per annum, representing approximately 10% of reproductive age women. This condition is often addressed by the use of artificial reproductive technologies (ART), which involves the pharmacological stimulation of folliculogenesis to provide oocytes for collection for in vitro fertilization (IVF) and subsequent embryo transfer (ET) to the uterus. This is an invasive process requiring professional intervention and expensive pharmaceutical regimes.

In 1999, the US CDC reported 86,822 attempted cycles of which 14% were discontinued prior to egg retrieval. Of these, 83% were cancelled because of inadequate follicular development (see Figure 4). This number may be reduced by prior assessment of ovarian reserve.

**Figure 4.** Reasons for discontinuation of ART cycles.

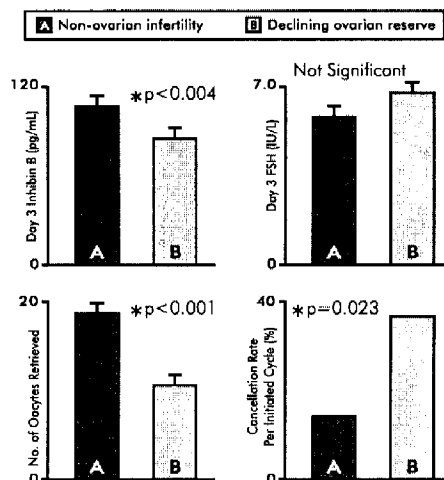


Adapted from the 1999 Report from the CDC

Current assessment methods for ovarian function include the measurement of circulating FSH, estradiol, and the clomiphene challenge test. Inhibin B is produced by the developing follicles of the ovary and is thus a direct marker of ovarian reserve. Serum measurement of Inhibin B on day 3-5 of the cycle is used increasingly as part of the assessment of ovarian reserve and oocyte retrieval outcome. Combining these measurements with other clinical criteria allows proper prediction of the success of an ART intervention (see Figure 5).

Prior knowledge of the likelihood of successful oocyte retrieval before the commencement of expensive and invasive IVF procedures allows informed decision making and appropriate expectation of success on behalf of the patient and the physician. A low

**Figure 5.** Cycle day 3 serum Inhibin B & FSH, number of oocytes retrieved and cancellation rate per initiated cycle from two patient groups:  
 A= non-ovarian infertility (tubal or male factor)  
 B= Declining ovarian reserve ( $E_2 < 500$  pg/mL or  $< 4$  mature follicles in previous ART cycles)



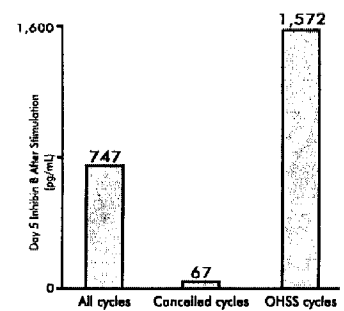
Adapted from Seifer et al., Fertil. & Steril., 72: 63-65 (1999)

Inhibin B measurement prior to ART will identify those women entering ART programs with potentially insufficient ovarian reserve to support successful oocyte retrieval. Such patients can be counseled by the physician and given a more informed indication of their fertility status. The patient can then decide whether she wishes to take the financial and emotional risk and proceed with IVF using her own oocytes, or consider the alternative possibility of using donor eggs. Following such an informed decision, patient anxiety and distress is minimized, and the doctor-patient relationship is protected from unnecessary misunderstanding and frustration. Financially, cancelled IVF cycles in the USA represents many millions of dollars, and substantially more on a global basis.

Knowledge of a woman's ovarian reserve prior to and during ART is also useful in assessing the level of pharmacological stimulation required in the IVF cycle. Too little stimulation can fail to produce enough oocytes for collection and over-stimulation can increase the risk of Ovarian Hyperstimulation Syndrome (OHSS). As an elevated number of follicles predisposes to OHSS, an elevated level of circulating Inhibin B will indicate the increased risk of OHSS prior to IVF treatment (see Figure 6). At risk patients can

therefore be managed appropriately, avoiding this potentially life-threatening IVF complication.

**Figure 6.** Inhibin B levels in all cycles, cancelled and OHSS cycles.

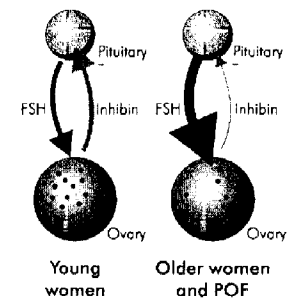


Adapted from Fawzy et al, Human Reprod., 17:1535-1543 (2002).

**Perimenopausal Transition & Premature Ovarian Failure**

During early perimenopause or transition, follicular phase levels of Inhibin B decline substantially, while Inhibin A and estradiol remain within normal ranges until menopause is more advanced. As circulating Inhibin B levels fall, the suppressive effect on pituitary FSH secretion declines leading to an elevation in circulating FSH. Elevated FSH levels then accelerate follicular recruitment and an overall decline in ovarian reserve as menopause approaches (see Figure 7).

**Figure 7.** Endocrine regulation of FSH and Inhibin B in younger & older women & in patients with Premature Ovarian Failure (POF).



The onset of menopause before the age of 40 is defined as premature ovarian failure (POF), a condition which affects about 1% of women. The resulting infertility is not problematic for women who have completed their child-bearing, but devastating for those who have deferred their families due to career or other socioeconomic factors. In addition to infertility, POF also poses

other health risks such as osteoporosis and conditions associated with the menopausal state. It has been reported that women who develop POF at an earlier age have a 1.6-2.0 times higher risk of all-cause mortality compared to women who undergo normal menopause. Early diagnosis of POF is important for patient management to reduce the risk of potential health consequences.

*"Osteoporosis is a disabling condition resulting in more than 1.5 million fractures each year. The estimated national direct expenditure for osteoporosis and related fractures is \$14 billion each year."*

CDC

Regular screening is useful for women with a family history of early onset of menopause and for women hoping to defer pregnancy towards the age of 40 or older. In addition to the need for an early diagnosis of POF, a proper understanding of a woman's biological clock can allow planning for an unassisted natural pregnancy. Traditionally, the physician has relied upon FSH and estradiol measurements to determine ovarian age and fertility status. Pituitary FSH offers limited value in some cases as it is an indirect marker of ovarian activity which fluctuates considerably from cycle to cycle during the perimenopausal phase. In contrast, follicular phase Inhibin B measurements may prove to be superior for POF diagnosis since Inhibin B is a direct marker of ovarian activity, and is more consistent from cycle to cycle. A combined measurement of Inhibin B and FSH may also prove to be more effective in the diagnosis of this condition than FSH alone.

### Inhibins and Activins in Pregnancy

#### Prenatal screening for Trisomy 21

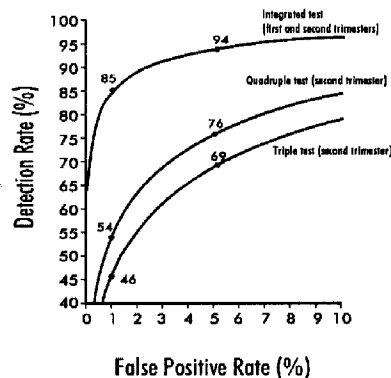
Prenatal screening for Trisomy 21 (Down Syndrome) and other chromosomal abnormalities involves the estimation of risk of having an affected pregnancy on the basis of factors such as maternal age, maternal serum concentrations of various analytes and ultrasound measurements. Women with a risk above a specified level are classified as positive on screening and are offered a definitive diagnostic test - either amniocentesis or chorionic-villus sampling. The use of these

biochemical tests can reduce or eliminate the risk of other forms of fetal testing.

Over the last decade, numerous studies have demonstrated the increased discrimination of new analytes for prenatal screening in both the first and second trimester. The triple test, performed in the early second trimester utilizes maternal serum alpha-fetoprotein (MSAFP), unconjugated estriol ( $uE_3$ ) and human chorionic gonadotropin (hCG) and has been used most extensively. Recent research clearly now includes the use of Inhibin A as a fourth marker in this panel, and the quadruple test improves the detection rate from 69% to 76% without changing the false positive of 5%.

The utility of Inhibin A has led to the development of a new integrated test, which increases the detection rate to 94% without changing the false positive rate. The integrated test also incorporates measurements of serum pregnancy-associated plasma protein-A (PAPP-A) and nuchal translucency in the first trimester and MSAFP,  $uE_3$ , hCG and Inhibin A in the second trimester (see Figure 8).

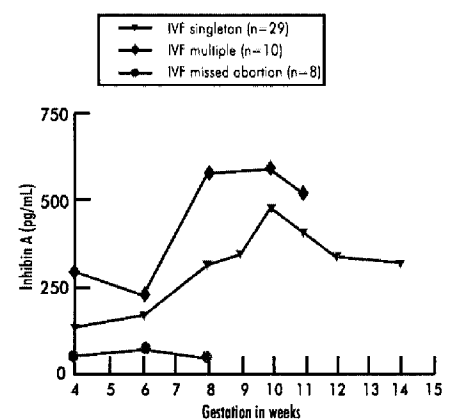
**Figure 8.** Rates of Detection of Down Syndrome and False Positive Rates for Various Screening Tests. The triple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin in the second trimester. The quadruple test includes measurements of serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and Inhibin A in the second trimester. The integrated test includes measurements of serum pregnancy-associated plasma protein A and nuchal translucency in the first trimester and measurements of serum alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and Inhibin A in the second trimester.



### Early detection of Viable Pregnancy in IVF Patients

Following an IVF cycle, both patient and physician are anxious to know if the embryo has implanted successfully. The usual marker of pregnancy is a raised level of human chorionic gonadotropin (hCG) in the blood or urine. In IVF cases, hCG is a less reliable marker as it may be artificially raised if exogenous hCG was administered to the patient to trigger ovulation. Inhibin A may prove to be a more reliable indicator of pregnancy as it is produced by the fetoplacental unit and is not directly affected by IVF pharmacological agents. Furthermore, Inhibin A also reflects the numbers of embryos successfully implanted as circulating levels are further elevated with multiple pregnancies (see Figure 9).

**Figure 9.** Inhibin A in IVF pregnancies



Adapted from Lackwood et al., *Biol. Reprod.*, 57: 1490-1494 (1997)

### Pre-eclampsia

This condition occurs in 5% of all pregnancies and is characterized by high blood pressure with proteinuria and a marked increased risk of perinatal mortality. In extreme cases, patient management requires the delivery of the pregnancy before term which leads to a series of infant health problems associated with premature birth. The condition is believed to result from the failure of the myometrial spiral arteries of the trophoblast to develop thin, non-muscular walls effectively reducing the efficiency of the placenta.

**"We conclude that Inhibin B is the best available endocrine marker of spermatogenesis in subfertile men."**

Pierik et al., JCEM, 83:3110-3114 (1998)

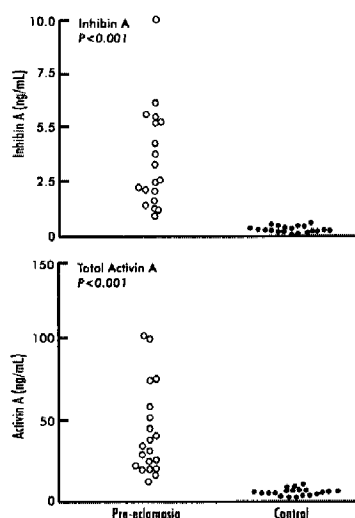
*"Pre-eclampsia and other hypertensive disorders of pregnancy are a leading global cause of maternal and infant illnesses and death. By conservative estimates, these disorders are responsible for 76,000 deaths each year."*

Pre-eclampsia Foundation

Currently, medical intervention cannot offer a cure for this condition, but can arrest and manage the symptoms temporarily by the use of therapeutic agents and careful patient management. Early diagnosis or early warning of pre-eclampsia allows early medical intervention, thereby delaying the severity of the condition and date for induction of pregnancy. Any delay in delivery is highly significant in the health and management of the premature infant.

Inhibin A and Activin A have the potential to be used as early markers in screening for pre-eclampsia. As shown in Figure 10, Inhibin A and Total Activin A serum concentrations are significantly raised in pre-eclampsia (~30 weeks gestation) compared to gestational age-matched controls. Measurements of Inhibin A at 15-20 weeks show that the increase in serum level can be predictive of the later onset of pre-eclampsia, especially those requiring delivery before 30 weeks.

**Figure 10.** Inhibin A and Total Activin A serum concentrations are significantly raised in pre-eclampsia (~30 weeks gestation) compared to gestational age-matched controls.



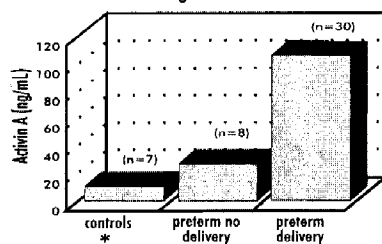
Adapted from Mutukrishna et al., Lancet, 349: 1285-1288 (1997)

### Preterm Labor

Preterm labor is the leading cause of perinatal morbidity and mortality in the United States. It usually results in preterm birth, a complication that affects 8 to 10 percent of births in the United States each year. Management of preterm labor and preterm birth account for health care expenditures of over \$3 billion per year. Despite extensive clinical research, the rate of premature births has not changed, and some data indicate the rate may be worsening. There are a multitude of risk factors that increase the chance of preterm labor, which include multiple pregnancies, maternal medical or behavioral complications, uterine or fetal causes, infective causes, and abnormal placentation.

Early prediction of a premature delivery is important as there are possible interventions to improve the chance of fetal survival, such as accelerating pulmonary development with steroids. Diagnosing early preterm labor is difficult and has a high false-positive rate. False diagnoses of preterm labor have resulted in unnecessary and potentially hazardous treatment for thousands of women. Improved methods of early diagnosis would be a significant advance in the treatment of women at risk of preterm labor. It has been reported that serum Activin A levels are elevated in the maternal circulation in patients who developed preterm labor compared to the control group (see Figure 11). Further studies are required to investigate the usefulness of Activin A as a diagnostic marker of preterm labor to improve early detection of the condition in clinical practice.

**Figure 11.** Maternal serum Activin A (mean  $\pm$  SD) in gestational age-matched normal subjects, patients with preterm labor who responded to tocolytic treatment, and in patients with preterm labor who delivered within 48 hrs from the diagnosis.



\* = P < 0.01 compared to other groups

Adapted from Petroglio et al., JCEM, 80: 558-561 (1995)

### Recurrent Miscarriage

It has recently been reported that measurements of circulating Inhibin A and hCG in pregnant women from 6 weeks gestation is predictive of recurrent miscarriage. Women who suffered recurrent miscarriage were found to have lower circulating levels of Inhibin A and hCG than gestational age matched controls with pregnancies that progressed to term.

### Male Infertility

Of the numerous couples facing infertility concerns, up to 40% of the cases are due to male factor problems. Causes of male infertility can be broken into three major categories:

1. Failure, due to hormonal abnormalities, to produce an adequate number of sperm;
2. Failure, due to testicular abnormalities, to produce an adequate number of normally functioning sperm;
3. Production of sperm that cannot be deposited in the female genital tract because of ductal obstruction or abnormal sexual function.

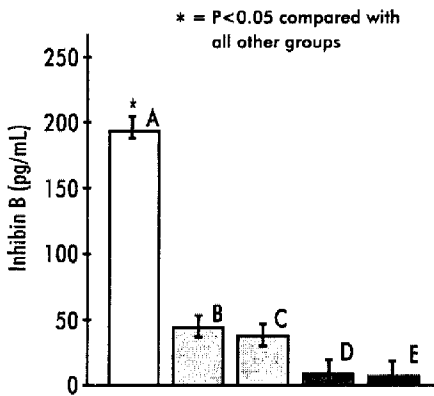
With the development of intracytoplasmic sperm injection (ICSI) in IVF, the surgical extraction of sperm has brought successful pregnancy to many couples with male factor problems. In cases where the sperm count in the ejaculate is zero or inadequate to effect conception naturally, sperm may be surgically extracted from either the epididymis or testis. Extraction of sperm from the epididymis is possible in men with obstructive causes. However, in cases of non-obstructive azoospermia, sperm production by the Sertoli cells of the testis is lower than the threshold required for ejaculation of sperm, and direct testicular sperm extraction (TESE) is required to obtain a viable sperm for ICSI. Sperm extraction is expensive and invasive for the patient, and so it is important to evaluate the degree of testicular function and probability of a successful outcome prior to the procedure.

At present, the likelihood of finding a viable sperm during a TESE-ICSI attempt is determined by quantitative testicular needle biopsy, a test which is also an invasive, traumatic procedure for the patient. A simple blood test to determine a man's circulating Inhibin B level may prove to be a relatively non-invasive test of testicular function, thus reducing the need for testicular biopsy. Inhibin B has been shown to be a direct marker of Sertoli cell function and spermatogenesis in adult males, and levels have

been correlated with testicular volume and sperm density. Very low levels of Inhibin B indicate inadequate sperm production by the testis, suggesting the lower probability of success with TESE procedures. Figure 12 shows Inhibin B levels in normal males and in males with various disturbances of the hypothalamic-pituitary-testicular axis.

Figure 12. Inhibin B levels in normal males and in men with various disturbances of the hypothalamic-pituitary-testicular axis.

- A = Normal Males (n=16)
- B = Males with Kallman's Syndrome (n=7)
- C = Infertile males with elevated FSH (n=29)
- D = Males with Klinefelter's Syndrome (n=9)
- E = Males with bilateral orchidectomy (n=10)



Adapted from Anelwalt et al., JCEM, 81:3341-3345 (1996)

#### Puberty and Gonadal Function: Boys

As Inhibin B is a product of the Sertoli cells of the testis, serum levels in prepubertal males may serve to predict abnormal testicular function and detect early Sertoli cell development.

The traditional test for testicular function in prepubertal boys is the hCG challenge test, which involves the measurement of testosterone response following injection of hCG. This procedure is time consuming and requires repeated blood sampling over a time course following hCG injection. A simple blood test to determine the level of Inhibin B has been shown to correlate with hCG stimulation results, and thus the Inhibin B test may be used in the near future to replace the time-consuming hCG test.

#### Puberty and Gonadal Function: Girls

Both Inhibin A and B show an initial peak at birth which falls to a minimum until the prepubertal years. While Inhibin A is undetectable before the onset of puberty, Inhibin B remains low but measurable and is presumably produced by the preantral ovarian follicles. Inhibin B rises to a peak early in puberty before settling to a steady level during early adulthood. This surge in Inhibin B could relate to the commencement of follicular activity at the onset of puberty, and therefore it is likely that Inhibin B may serve to be a useful marker of pubertal development in girls.

The undetectable level of Inhibin A in the prepubertal years followed by a peak later in puberty supports the proposal that it is produced mainly by the corpus luteum rather than the small ovarian follicles.

#### Cancer Research

##### Ovarian Cancer

Ovarian cancer is the 5<sup>th</sup> most common cause of cancer deaths, the high mortality rates being attributed to the late detection of the disease. Effective treatment options are available during the early stages of the disease prior to metastatic spread, and thus early detection should greatly reduce the mortality rates.

Granulosa cell tumors of the ovary constitute about 5% of the total number of ovarian cancers. The most common ovarian cancer marker, CA-125 is effective in detecting the majority of epithelial cancers, but is less effective in detecting granulosa cell tumors. Inhibin levels are found to be significantly elevated in women with granulosa cell tumors, and therefore a combination of Inhibin and CA-125 measurement may detect a greater percentage of ovarian cancers. A study conducted recently has shown that the combined measurement of serum CA-125 with Total Inhibin detects up to 95% of ovarian tumors (see Figure 13).

Measurement of serum Total Inhibin levels should also be useful in the post-operative follow-up of patients to detect further recurrence of the disease (see Figure 14).

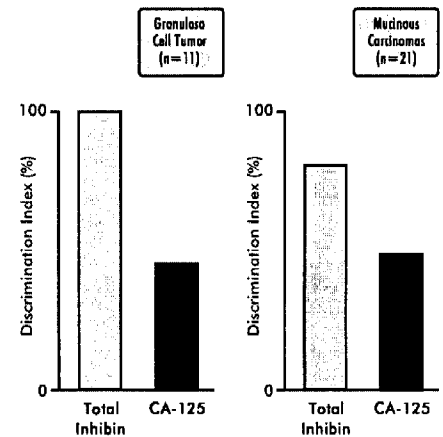
##### Other cancers

Current literature on prostate cancer research implicates a role for inhibins and activins in the pathogenesis of this disease. Moreover, recent

studies have shown that Activin A is significantly elevated in the tissue and serum of postmenopausal women with breast cancer.

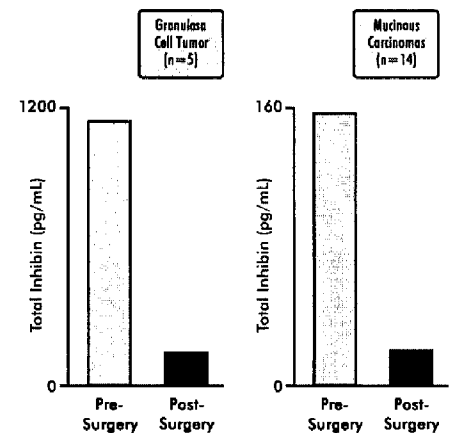
It is likely that more data will accumulate to support the measurement of serum levels of inhibins and activins in various oncological conditions.

Figure 13. Total Inhibin assay discriminates mucinous & granulosa cell tumors better than CA-125



Adapted from Robertson et al., Clin. Chem, 45:651-658 (1999)

Figure 14. Pre- and post-surgical levels of total Inhibin in mucinous and granulosa cell tumors.



Adapted from Robertson et al., JCEM, 87:816-824 (2002)

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