LEPTIN ACTION ON OVULATION AND LEPTIN RECEPTORS ACROSS THE RAT OESTROUS CYCLE.

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Abstract

Leptin, a hormonal product of the *Lep* or *OB* gene, is expressed by adipocytes and circulates in relation to adiposity. Leptin is thought to play a role in regulating food intake and maintaining body weight. Although six isoforms of the leptin receptor exist, leptin exerts its effects by interacting with the long form of the leptin receptor, *OB-RB*. The leptin protein and leptin receptors have been localised in many reproductive tissues, including the ovary. Several publications indicate that the ovary is directly affected by leptin, in particular leptin appears to be able to inhibit ovarian cell steroid production. The high levels of leptin in obese women, and in animal models of obesity, implicate a link between elevated leptin and infertility.

The purpose of this project was to examine the effects of acute leptin treatment on ovulation and other important ovulatory mediators, *in vivo* and *in vitro*. Establishing the relationship between acute leptin concentrations and ovulation may provide insight into the link between the high leptin levels in obese women and infertility. It was also desired that the pattern of leptin receptor expression across the rat oestrous cycle be investigated. The pattern of leptin release in women throughout the menstrual cycle indicates that modulating leptin levels may be a mechanism of altering the sensitivity of leptin across the cycle. Understanding the relationship between leptin and ovarian leptin receptor expression will assist in comprehending the importance of leptin sensitivity in the oestrous cycle of the rat.

The effect of systemic leptin administration on ovulation in the rat ovary, both *in vivo* and *in vitro*, was investigated. Immature gonadotropin-primed rats, injected with leptin

experienced a loss in body weight, food intake and a decline in ovulation *in vivo* and ovaries perfused with leptin also ovulated significantly less. Plasma progesterone and oestradiol levels were unaffected in either model. However, feed restriction alone did not inhibit ovulation.

To investigate the mechanism of leptin-induced inhibition in ovulation, the numbers of follicles entering the pre-ovulatory pool following leptin treatment were established. Leptin treatment did not affect the recruitment of pre-ovulatory follicles from the antral follicle pool. The importance of ovarian leukocytes in ovulation prompted an investigation into the effect of leptin on two leukocytes important in ovulation. A decrease in food intake, either as a result of leptin-treatment or feed restriction, specifically reduced the numbers of neutrophils and monocytes/macrophages infiltrating the theca interna of pre-ovulatory follicles without affecting the numbers found in the stroma. However, this reduction was not solely responsible for the leptin-induced inhibition in ovulation.

In vitro ovarian follicular culture (4 h and 12 h) was used as a tool to investigate if high leptin concentrations could inhibit other factors important to ovulation, such as meiotic competence of oocytes, granulosa cell proliferation, steroid or prostaglandin E_2 synthesis and interleukin-Iβ production. High concentrations of leptin in follicle culture do not inhibit meiotic maturation or steroid synthesis, while an effect on prostaglandin E_2 synthesis may exist. Granulosa cell proliferation was not inhibited by leptin in FSH and IGF-I supplemented culture media, while leptin was able to inhibit the stimulatory effects of IGF-I on FSH-stimulated rat granulosa cell oestradiol production without affecting progesterone production, as previously reported. Leptin did not appear to have

an adverse effect on the components of ovulation tested and therefore impacts the ovulatory cascade in a way that remains to be defined.

Finally, the expression of two isoforms of the leptin receptor (*OB-RB* and *OB-RA*) were investigated throughout the oestrous cycle in order to assess whether ovarian sensitivity to leptin varied throughout the cycle. The isoforms of the leptin receptor were lower in the pro-oestrus and di-oestrus stages than the met-oestrus stages of the rat oestrous cycle in order to modulate leptin sensitivity across the cycle. The fluctuations in the leptin receptors may be a response to the levels of circulating steroid hormones and leptin.