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EXPRESSION OF THE pS2 PEPTIDE IN NORMAL HUMAN GLANDULAR ENDOMETRIUM DURING THE MENSTRUAL CYCLE: A POSSIBLE FUNCTION

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Since the identification of pS2 mRNA in the MCF-7 human breast cancer cell line (Masiakowski *et al.* 1982), and its demonstrated response to oestrogen stimulation (Kida *et al.* 1989; Brown *et al.* 1984), a great deal of interest has been focused on this small polypeptide. Although a number of studies have demonstrated pS2 expression in the breast, few studies have documented pS2 expression in other tissues. Glandular epithelium of the endometrium, like the breast, is subject to hormonal fluctuations directly related to the organ-specific function. Those studies that have included normal endometrial epithelium, did not detect the pS2 peptide (Rio *et al.* 1988; Chambon *et al.* 1984; Piggot *et al.* 1991). In endometrial adenocarcinoma, pS2 has been detected but its expression was notably variable (Henry *et al.* 1991), or very weak (Wysocki *et al.* 1990). Hormone regulated proteins specifically expressed by glandular endometrium during the menstrual cycle may be of value in the treatment of infertility, acting as markers for the functional readiness of endometrium for implantation. A previous study, evaluating the expression of a number of breast-associated markers in endometrial tissues, showed there to be menstrual cycle phase-specific patterns of immunostaining implicating hormonal regulatory mechanisms (Rye *et al.* 1993). In view of this, and the reported oestrogen responsiveness of pS2, we have assessed its expression in endometrial tissues from different stages of the menstrual cycle to determine if there is oestrogen regulation of pS2 in this tissue. If so, pS2 could be of value as a marker of functioning endometrium in the menstrual cycle.

In this study 21 tissues were obtained from dilatation and curettage (D and C) cases, e.g. investigation for dysmenorrhoea, association with laporascopic sterilization, and infertility investigation. Cases were selected on the grounds of having no apparent endocrinological problems and no local organic pathologies. All cases were obtained from patients in the 20 to 30-year-old age group, with a regular cycle history. Tissues were fixed in formaldehyde and paraffin-wax embedded. Endometrial specimens were dated from the last menstrual period and were only used in the study if there was corroboration by independent histological dating which followed standard histological criteria (Hendrickson and Kempson, 1980). The

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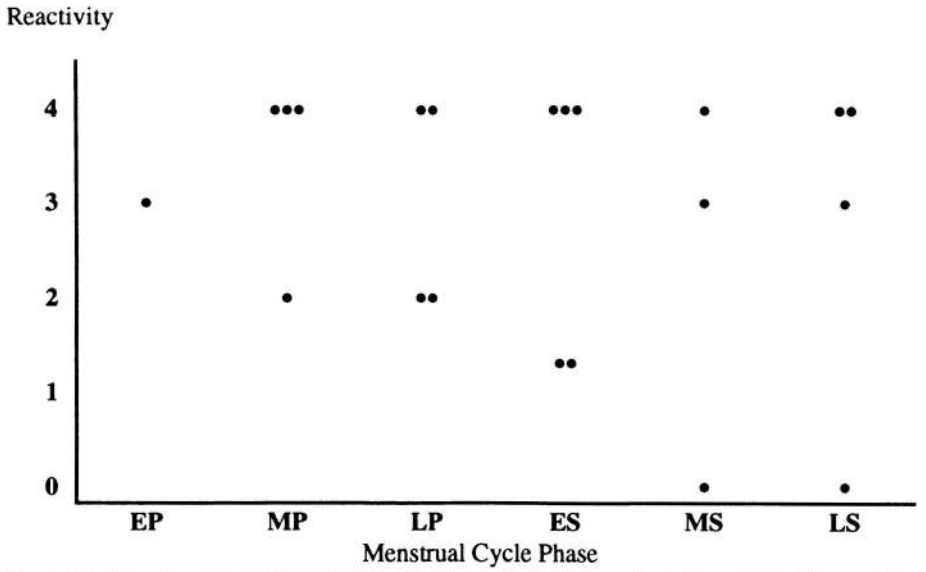

Figure 1. Schematic representation of pS2 antibody reactivity in the endometrium during the menstrual cycle. The degree of change in intensity and numbers of cells staining was graded according to a 5-point scale (see text); ●, number of cases

expression of pS2 was determined by an indirect streptavidin:biotin immunohistochemical technique using a second generation murine monoclonal antibody raised against affinity purified pS2 peptide (CIS-UK). Positive staining was graded according to a 5-point scale, taking into account the intensity of glandular staining and the proportion of stained cells. A score of 4 indicates intense staining of more than 95 per cent of glands, compared to a score of 1 which is indicative of occasional and/or weak glandular epithelial staining.

In contrast to the previous reports of pS2 expression in endometrium we have demonstrated pS2 in 19/21 (90 per cent) of cases with binding to glandular epithelium of the endometrium. The distribution of pS2 did not appear to show cycle phase specific expression (Figure 1). Indeed intense staining was observed in endometrial tissues from all stages of the menstrual cycle. Staining was localized at the luminal membrane of the glandular epithelium (Figure 2), with cytoplasmic reactivity limited to those areas adjacent to the gland luminal membrane. No stromal reactivity was observed at any stage of the cycle.

Our results of pS2 expression in normal endometrium are contrary to the findings of other studies using antibodies (Piggot *et al.* 1991) and mRNA approaches (Rio *et al.* 1988; Chambon *et al.* 1984). The inability to detect pS2 mRNA may have been due to a low glandular/stromal cell ratio in the tissues analysed. Moreover, the immunohistochemical approach of Piggot *et al.* (1991) used antibodies raised against different parts of the pS2 peptide. The immunohistochemical distribution of pS2 detected by these antibodies were notably different from our findings: only diffuse cytoplasmic staining was observed with no membrane localization.

The expression of pS2 in the endometrium, as identified in this study, does not show a menstrual cycle phase-specific pattern concordant with that of an oestrogen-regulated peptide. These findings suggest that the oestrogen-induced pS2 peptide as

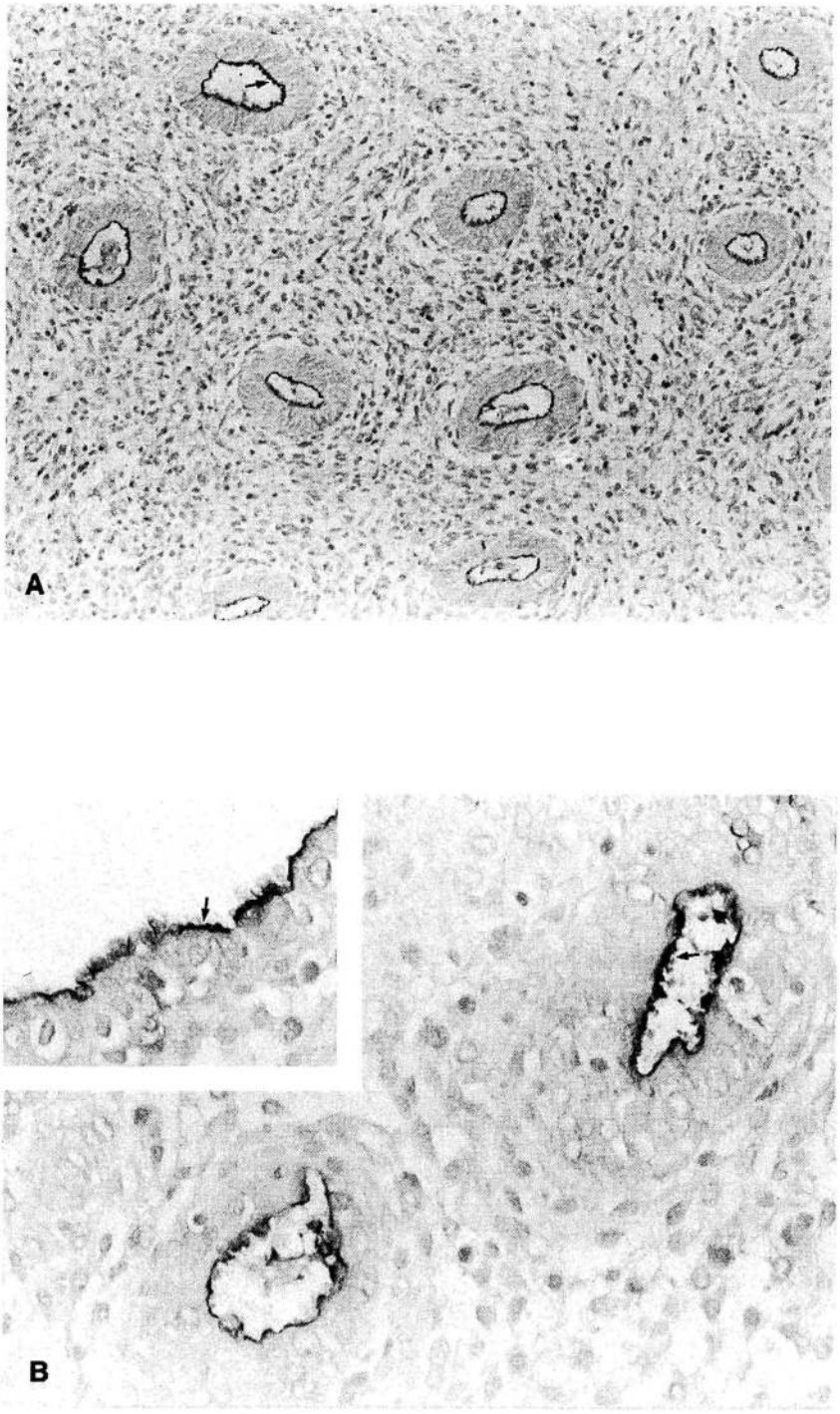

Figure 2. Photomicrographs of glandular endometrium from the proliferative (A) and secretory (B) phases of the menstrual cycle showing strong reactivity of the pS2 antibody localized at the glandular luminal membranes (arrowed). Antibody reactivity was also localized at surface endometrium (inset)

expressed in normal endometrium is dependent on other additional factors. Indeed this supports other recent studies in breast which have demonstrated that DNA enhancer elements flanking the pS2 gene are responsive not only to oestrogen but also the epidermal growth factor (EGF), a tumour promoter (TPA), and the c-Ha-*ras* and c-*jun* oncoproteins (Nunez *et al.* 1989). In gastric mucosa EGF has also been shown to directly induce pS2 secretion (Wright *et al.* 1991). Recent reports indicate that this oestrogen-independent expression of pS2 is the result of a shared signal transduction pathway linking different stimulatory agents to elevated pS2 gene expression (Wysocki *et al.* 1990). Indeed, perhaps the induction of pS2 occurs by a mechanism similar to that involved in the spatial summation of action potentials in the transmission of nerve impulses.

Since the expression of pS2 does not follow a controlled pattern during the secretory phase it is unlikely to be of value as a marker of the functional state of endometrium in its readiness for implantation. Although pS2 does not appear to be of use in this respect, its expression in endometrium does shed some light on its possible function as a regulator of cell proliferation: the expression of pS2 in normal endometrial tissue may serve to modulate proliferation in the menstrual cycle. The high level of expression observed throughout the cycle in 90 per cent of those cases studied, and the absence or weak expression of pS2 detected in endometrial adenocarcinomas (Henry *et al.* 1991; Wysocki *et al.* 1990), would appear to support this theory. Although earlier reports concerning the induction of the pS2 peptide by oestrogen found that it was not involved in the growth-stimulating effect of oestrogen in the MCF-7 breast cancer cell line (Davidson *et al.* 1986; Kida *et al.* 1989), this does not preclude the possible role of pS2 in modulating proliferation by an oestrogen-independent pathway. This theory of pS2's functional role is further supported by its structural similarity with several peptide growth factors such as insulin-like growth factor-I and porcine spasmolytic polypeptide (PSP) (Rio *et al.* 1988; Baker, 1988; Mori *et al.* 1988). Indeed it has been suggested that pS2 belongs to a new family of growth factors (Thim, 1989).

The localization of pS2 at the apical membrane of normal glandular endometrium would suggest that it may function by a paracrine mechanism. A diagrammatic summary of the known and postulated interactions and mechanisms of pS2 induction is shown in Figure 3.

The pS2 peptide may also act in a protective role by modulating the expression of mucins. The structural similarity of pS2 with EGF, PSP/hSP, and carbohydrate catabolic enzymes (Tomasetto *et al.* 1990) indicates some involvement in this function. Moreover, recent studies have detected pS2 in mucous secreting cells of the normal gastric mucosa (Piggot *et al.* 1991), gastric carcinomas (Henry *et al.* 1991), and in mucinous gynaecological adenocarcinomas (Wysocki *et al.* 1990). The detection of pS2 in normal endometrium identified by us further supports this functional role.

We suggest that in human endometrial tissue during the menstrual cycle the pS2 peptide may serve in a protective and/or proliferative role through its modulation of mucin/glycoconjugate expression. In addition we suggest that the expression of pS2 in this tissue reflects the complex multifunctional actions of the agents involved in its induction. Further study involving the interaction of these agents in their role of pS2 induction is required to fully understand the functional nature of the pS2 peptide.

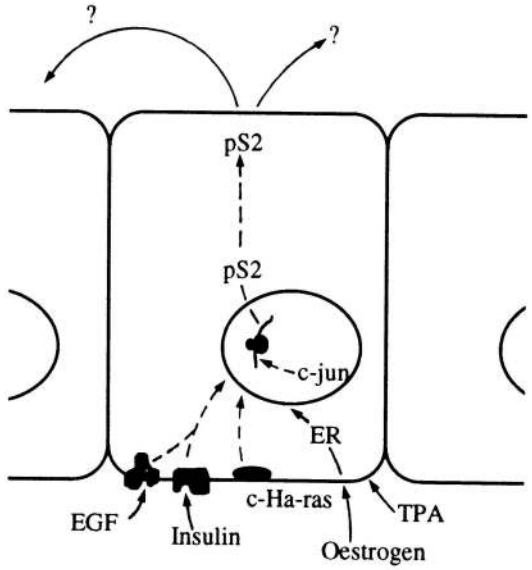

Figure 3. Diagrammatic summary of the interactions and mechanisms of pS2 induction in relation to its proposed function

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