Implantation of the primate embryo

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Blastocyst implantation is a critical step in the establishment of pregnancy. It requires maternal recognition of the preimplantation embryo and mutual sensitization of blastocyst and uterus. This involves a series of morphological, biochemical, immunological and vascular changes in the uterine bed. Implantation begins with apposition and attachment of blastocyst on uterine epithelium, followed by penetration of epithelial layer by embryonic trophoblast cells, associated with endometrial vascular changes and decidualization and finally the tapping of maternal blood vessels by trophoblast cells and initiation of placentation. A significant degree of diversity is seen in the process of implantation among the lower mammalian species and domestic animals that have been studied, and thus it is not feasible to extrapolate information obtained from these species to the higher primates including man. The present review aims at focussing the physiological aspects of blastocyst implantation in the non-human primate using the rhesus monkey (*Macaca mulatta*) as the experimental animal model. It is now apparent that in the primate, endometrial differentiation and secretory type of activity under progesterone dominance is *sine qua non* to support implantation, but no well-defined implantation window nor any marker of endometrial receptivity could be detected. Embryonic viability, on the contrary, appears to be a major determinant factor towards successful implantation and pregnancy.

FROM the view-point of evolution, the process of blastocyst implantation is an effective adaptation towards successful sexual reproduction in mammals. Our knowledge about this process is very thin for obvious reasons. Mammals include a variety of animal species sharing some common biological features but displaying a wide array of behaviourisms in the process of survival and continuing their respective progenies. In fact, not all mammals exhibit blastocyst implantation and placentation, nor are these features only found in mammals. Furthermore, the modes, mechanisms and patterns, as well as physiologic correlates of blastocyst implantation are so widely variable among different species belonging to *eutherian* (placental) mammals that one may fail to find any general evolutionary scheme except the commonality in the purpose of this process. Since blastocyst implantation is a critical step in the process of establishment of pregnancy, the understanding of this process is potentially significant for the control of fertility, treatment of infertility and of early embryo loss in the human. However, practical and ethical constraints limit the use of human material for such studies. As a consequence, attention has been given to different non-human primates.

The higher primates (*anthropoids*) show a significant degree of commonality in their reproductive characteristics. Among the higher primates, three main groups are distinguished: the New World monkeys, the Old World monkeys and the apes. Undoubtedly, the apes (*Hominoidae*) which now include 11–12 living species, like gibbons, pans and gorillas are very close to the human regarding their reproductive characteristics. However, ethical limitations clamp their use in biomedical investigations. The next choice is the Old World monkeys (*Cercopithecidae*), like macaques which are abundant in this subcontinent, and are quite close, though not identical, to the reproductive characteristics in the human. As a result, macaques have been extensively used in studies of human reproduction.

Our aim is to review different physiological aspects of blastocyst implantation especially in the rhesus monkey (*Macaca mulatta*) and to extrapolate this knowledge, whenever possible, to blastocyst implantation in the human. Synchronous changes in the endocrine milieu, uterus and embryo are considered as *sine qua non* for successful blastocyst implantation. It is generally believed that endocrine synchrony is best reflected in uterine receptivity. Additionally, embryo growth and endometrial maturation may involve essential local interactions. These issues are primarily addressed in this review.

Embryo development

Ovulation generally occurs within 24–36 h after the preovulatory oestrogen surge both in the human and in the rhesus monkey[1, 2]. It has been observed that the rate of embryo transport in the genital tract is a species-specific event and is regulated by the circulatory levels of oestrogen and progesterone. In rodents and lagomorphs, the uterine luminal environment may suggestively contain one or more factors which are hostile to the growth and development of early stage embryos[3–7]. However, it is now clearly evident that patterns of embryo transit and of embryo development are highly characteristic in primates (*primate pattern*), which are

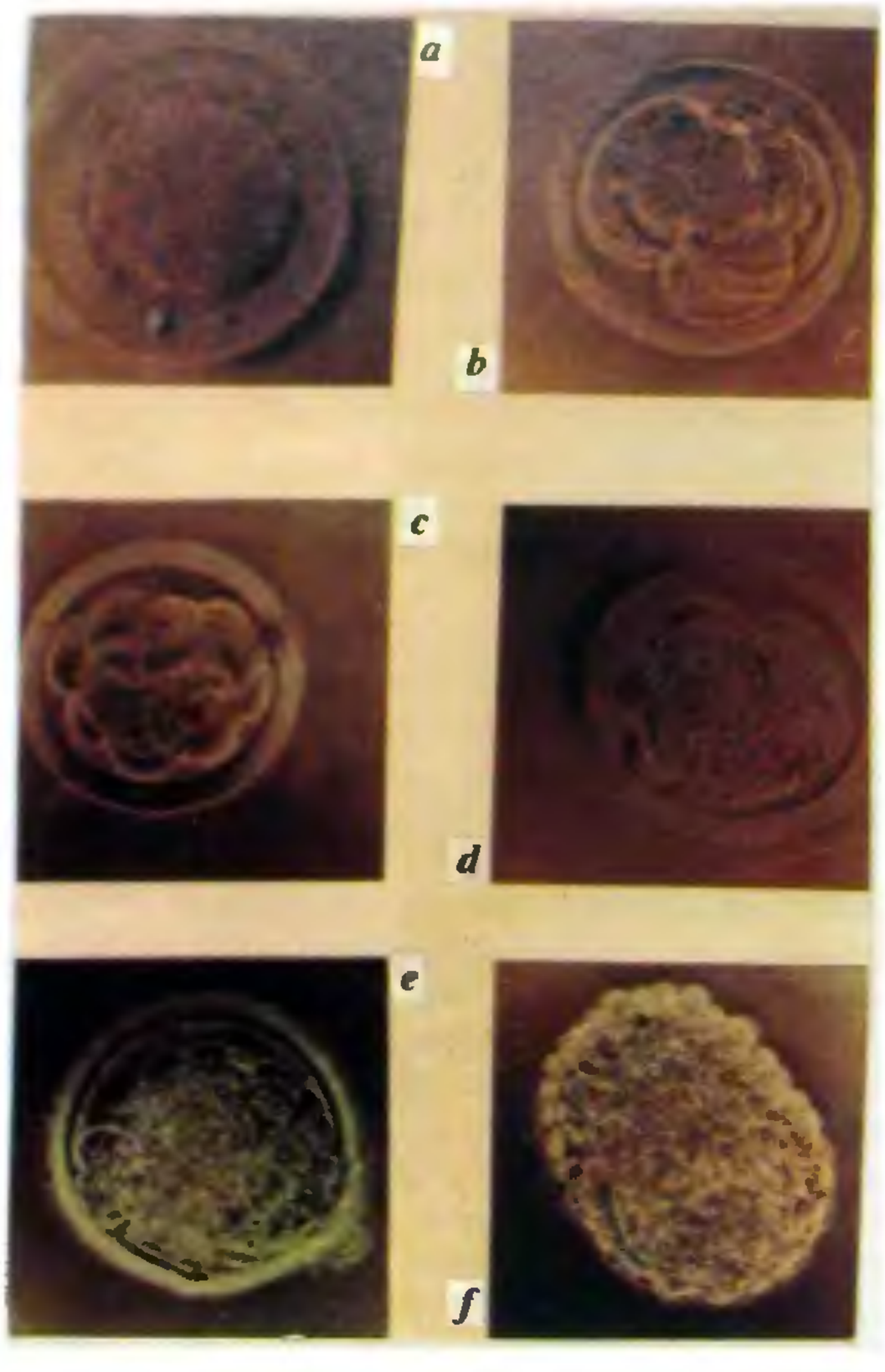

Figure 1. Stages of development of rhesus preimplantation embryos recovered by flushing of the reproductive tract and viewed in living state under phase contrast microscope. *a*, one cell collected on day 1; *b*, six cell on day 2; *c*, morula on day 3; *d*, early blastocyst on day 4; *e*, advanced blastocyst on day 5; *f*, zona-free blastocyst on day 6, of gestation.

not comparable with those in mouse, rat and rabbit. The environmental milieu of uterine lumen after ovulation does not exhibit hostility, and indeed can support normal development of all preimplantation stages of embryo till implantation in monkeys and women. Marston and his colleagues[8, 9] recovered three tubal stage (8 cell) embryos from rhesus monkeys and immediately transferred them to the uteri of the same animals. Three pregnancies resulted, of which two matured to term and one ended in early resorption following its implantation; transfer of 2 cell stage, but not 4 cell stage embryos to uterine cavity resulted in implantation, pregnancy and delivery of normal pups. Krietmann and Hodgen[10] also obtained pregnancies in 5 out of 31 attempts in monkeys with transfer of oocytes recovered by follicular aspiration to isthmus region of oviduct of same animal in which coital insemination had been performed. With the introduction of *in vitro fertilization* (IVF) and *embryo transfer* (ET) programmes in the human, it is now well established that successful pregnancies occur with transfer of 2 cell and 4 to 8 cell embryos to uterine lumen, and indeed the maximum rate of pregnancy is obtained when 2 to 4 cell stages are transferred 33–37 h after insemination[11, 12].

We have evidence to suggest that embryos enter into the uterus in the pre-morula stages in monkeys. The frequency of obtaining 8 to 16 cell pre-morula stage embryos from uterine flushings is highest on day 3 of gestation[13]. Similar observations have been reported for the human and the monkey by others[14, 15]. An embryo after 16 cell stage appears as a morula along with characteristic compaction. As shown in Figure 1, a temporal association exists between embryo stage of development and gestational age in both human and monkeys[13, 16]. However, the rates of embryo development for human and monkey reported in the literature are widely variable (Figure 2). Preimplantation embryos can be classified into two types, based on their rate of development: rapidly cleaving embryos and slow cleaving embryos. According to the general consensus, it is presumed that rapidly cleaving embryos are potentially more viable leading to successful implantation. On the other hand, slow cleaving embryos are highly liable to fall back in time with endometrial maturation, thereby causing pre- and peri-implantation embryo loss[12, 17].

Generally, anthropoids exhibit a higher degree of preimplantation embryo loss. Though no true estimate of early embryo loss in primates is available, it has been estimated that there is about 20–30% preimplantation embryo loss in several non-human primates, while in human it could be as high as 50% (ref. 18). We have observed that abnormality in embryo development in rhesus monkeys kept in semi-natural captivity shows a pattern: abnormality is high during the transition months of the breeding season, i.e., October and March[19]. During the months of November to February, the rate of abnormal embryo development was consistently low (Figure 3). It should be mentioned that rhesus monkeys are seasonal breeders in their natural habitat and in semi-natural captivity, with highest reproductive activities being seen from October to March. In a human study, it was observed that occurrence of births with Down syndrome (chiefly from nondysjunction in the meiotic division of oocyte) was higher among those conceptions which occurred during the annual transitional months of spring and autumn[20].

Our knowledge about activation and regulation of preimplantation embryo development in primates is inadequate. There is now evidence to suggest that activation of human embryonic genome occurs at 4 cell stage[21]. However, the biological significance of this observation remains to be explored[22]. We have shown histochemically that 4 cell stage onwards, preimplantation rhesus embryos exhibited steroid dehydrogenase activity, with highest activity in morulae and blastocysts, maximal activity being present in zona-free

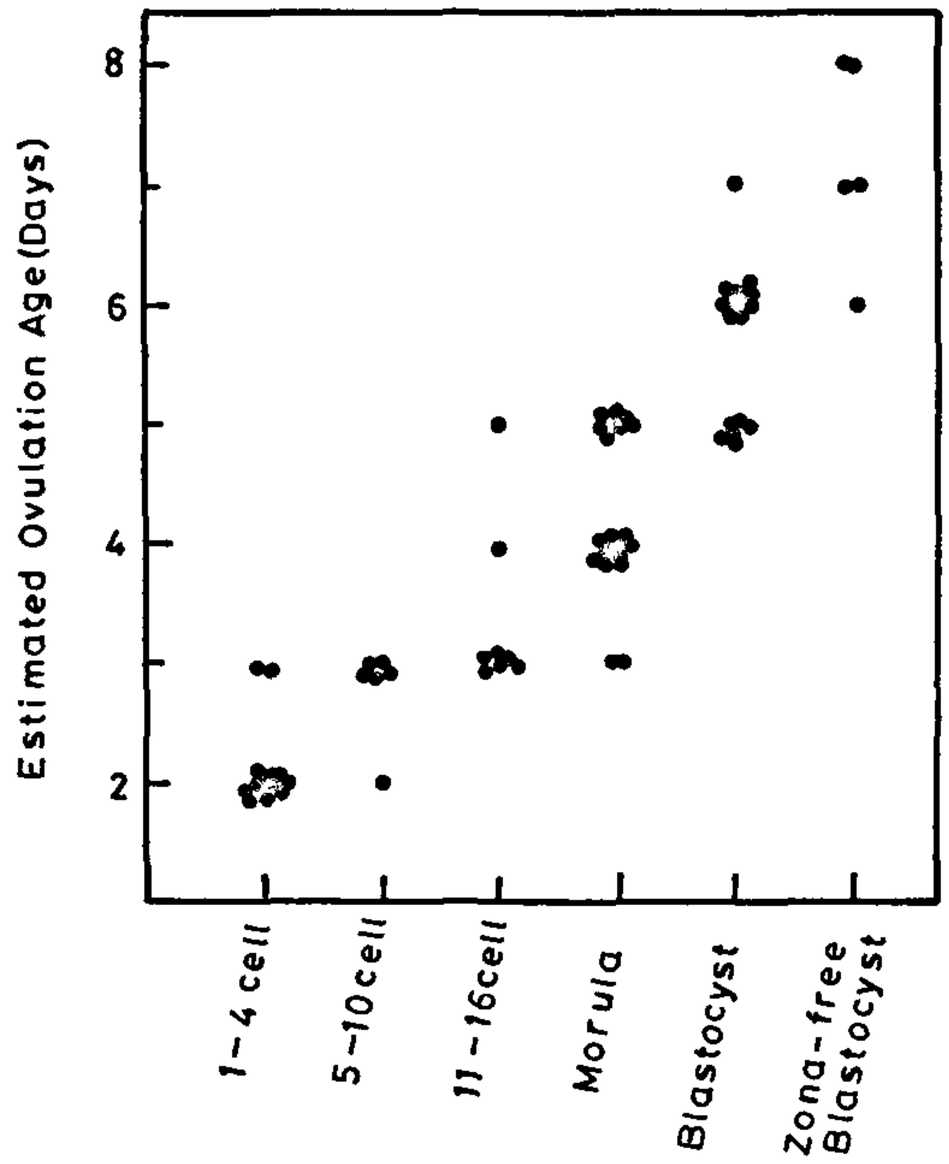

Figure 2. Correlation between estimated ovulation age and stages of preimplantation embryos recovered from rhesus monkeys The ovulation ages of embryos were estimated on the basis of the day of ovulation. day 0 being 24 h after peak level of oestrogen in peripheral circulation. Embryos judged to be slow growing or abnormal have not been included. Data obtained from 69 embryos. From one female monkey, around 3–6 preimplantation stage embryos can be recovered by flushing the reproductive tract during two successive breeding seasons[13].

blastocysts[13]. Almost similar profiles were seen in the polypeptide secretory activity and epidermal growth factor binding activity (unpublished data). Thus, we presume that preimplantation embryos of the rhesus monkey start giving clear functional manifestation around 16 cell to morula stages, at a time when embryos generally enter into uterine lumen.

Endometrial correlates of receptivity

The issues of endometrial receptivity and implantation window in primates are largely unattended. According to a hypothetical and statistical two independent parameters model for blastocyst implantation proposed by Spiers *et al.*[23], the probability of pregnancy from a single embryo transfer is represented by U. E, where U is the probability of uterine receptivity and E is the probability of embryonic survival. This model is simple and linear, and does not consider the probability of intersection between U and E. Blastocyst implantation is, however, a complex, non-linear and interactive process involving a viable embryo and differentiating endometrium. As a result, a wide range of predictive values for U and E have been yielded from different laboratories[24, 25]: U = 0.31–0.64, and E = 0.21–0.43. It is however, apparent from studies in human and non-human primates that the implantation window is not very restricted in these species. We have reported[26] that an asynchrony of 2–3 days between endometrial secretory maturation and embryonic development is not incompatible with implantation and establishment of pregnancy. Thus, the suggestion put forward by Bergh and Navot[27] that an endometrium undergoing typical secretory maturation during the mid-luteal phase may be optimal to support implantation and that developmental viability of blastocyst is a primary determinant of successful implantation appears highly realistic.

The next issue is the identification of markers for uterine receptivity in women and other non-human primates. Though there is no such available marker in the literature, the presence of pinopods, absence of long microvilli and overall smoothening of the surface contour of endometrium during the peri-implantation period are suggestively indicative of endometrial receptivity[28]. Given the fact that endometrial epithelium exhibits a high degree of mosaicism in its hormone responsiveness and cellular expression[29, 30], there is no common consensus about such morphological features as the index of endometrial receptivity.

Endometrial sensitization during implantation is primarily regulated by the hormonal milieu within the tissue bed. Nonetheless, we postulate that functional differentiation of endometrium in the primate in the presence of a preimplantation stage blastocyst is not identical to that occurring at a comparable time of the

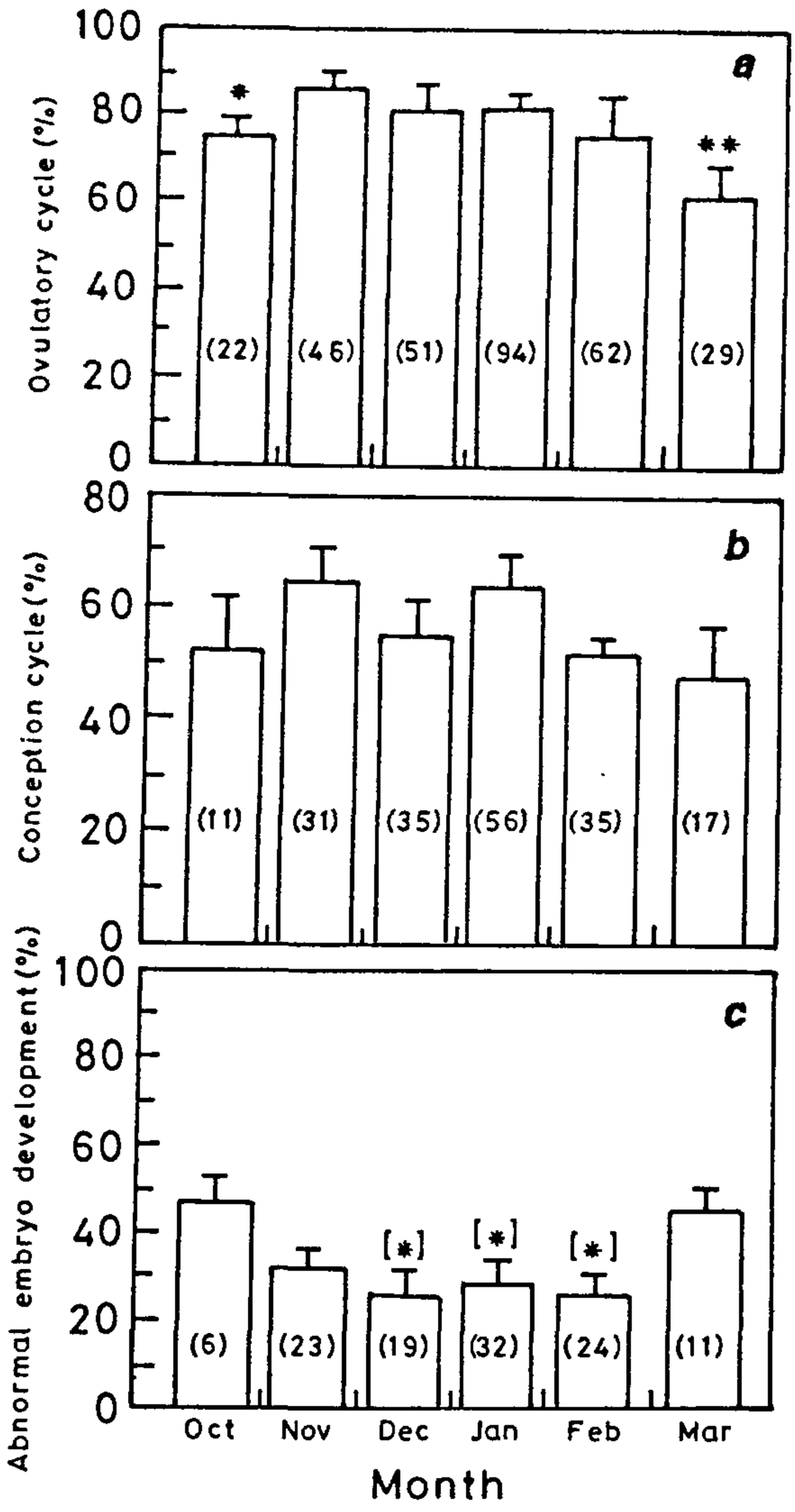

Figure 3. Month-wise distribution of percentage of ovulatory cycles (*a*), conception cycles (*b*) and of abnormal embryo development (*c*) in female rhesus monkeys during the breeding season (October to March) Values are ± SEm from data collected over five years and 304 cycles studied Figures in parentheses are the number of menstrual cycles (*a*), number of ovulatory cycles (*b*) and number of fecund cycles (*c*), respectively, studied in each month $*p < 0.03$ and $p < 0\ 02$ compared with data of November, December and January (*a*) and [*] $p < 0\ 02$ in within group comparison (*c*) as revealed from psd test. Data taken from Ghosh and Sengupta[19].

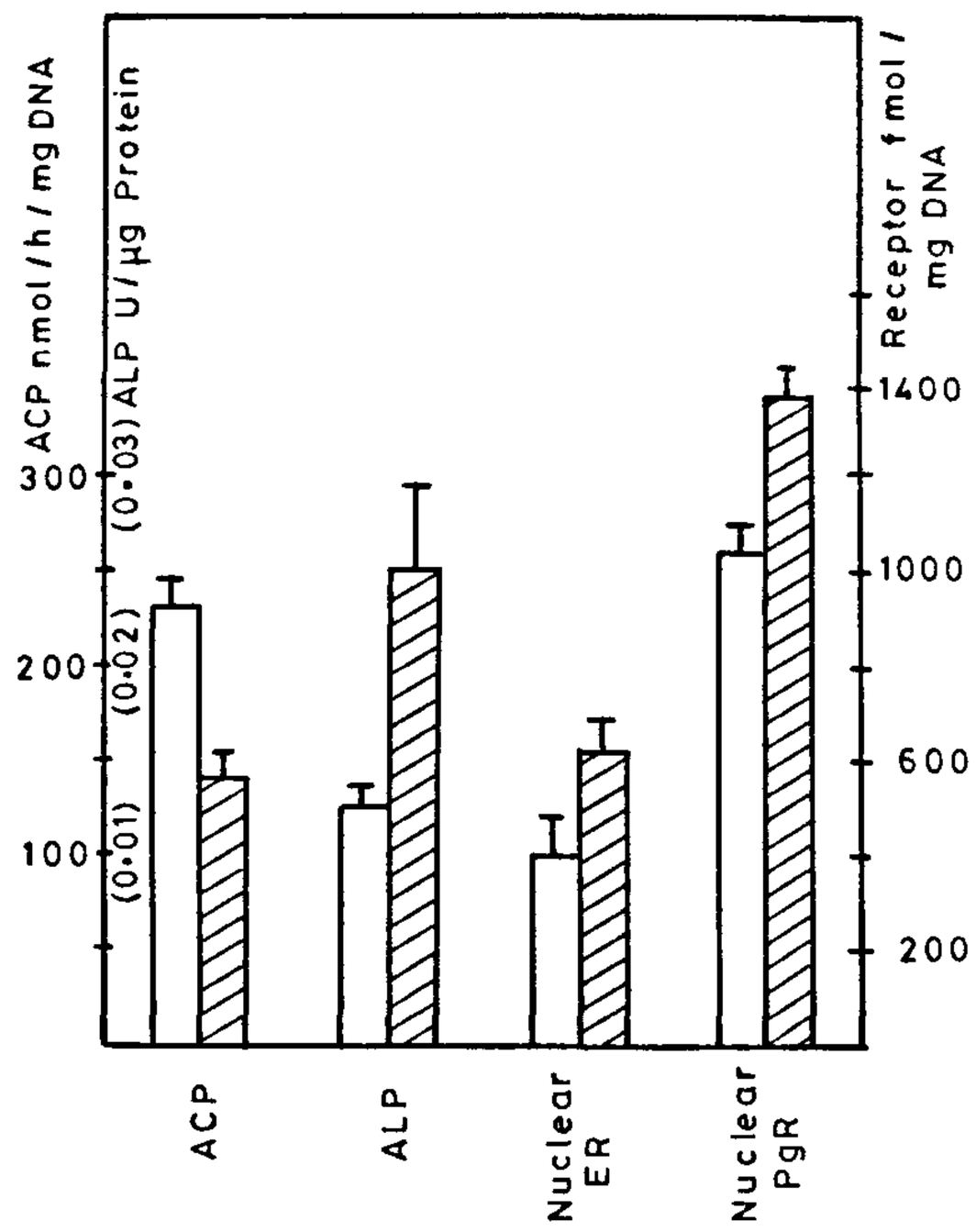

Figure 4. Data shown for total acid phosphatase (ACP) total alkaline phosphatase (ALP), nuclear oestrogen receptors (ER) and nuclear progesterone receptors (PgR) in endometria obtained on day 6 post-ovulation in non-mated, menstrual cycles [□] and conception cycles [▨] of rhesus monkeys[31–33].

luteal phase of a non-conception cycle. Indeed, there are biochemically distinguishable features in preimplantation stage endometrium of the rhesus monkey. These include suppression of acid hydrolytic enzymes[31], enhancement of alkaline phosphatase[32] and higher retention of nuclear receptors for oestrogen and progesterone[33] in preimplantation stage endometrium compared with secretory, post-ovulatory stage endometrium at a corresponding stage of the luteal phase from non-fecund, menstrual cycle (Figure 4). Our observations suggest that preimplantation phase rhesus endometrium responds to the presence of an embryo in the uterine lumen with an arrest of premenstrual involution as a maternal response to implanting blastocyst[34]. Though the tentative functional relevance of the above-mentioned changes in prenidatory endometrium appeared logical, sufficient morphological associations were not available in the literature.

We have now recently shown that monkey endometrium during the preimplantation period displays subtle but discernible changes when compared with luteal phase endometrium from the non-fecund, ovulatory cycles[35]. Foss *et al.*[36] and Hertig[37] had earlier indicated that vascular prominence together with increased blood flow and associated oedema were important features distinguishing endometrium of a conception cycle in the human. The occurrence of increased oedema (Figure 5) with extravasation of radiolabelled albumin in preimplantation endometrium has been confirmed in our study using the rhesus monkey[35]. It appears that enhanced vascular permeability is a likely cause of increased oedema in prenidatory endometrium, with no significant change in tissue blood volume[35].

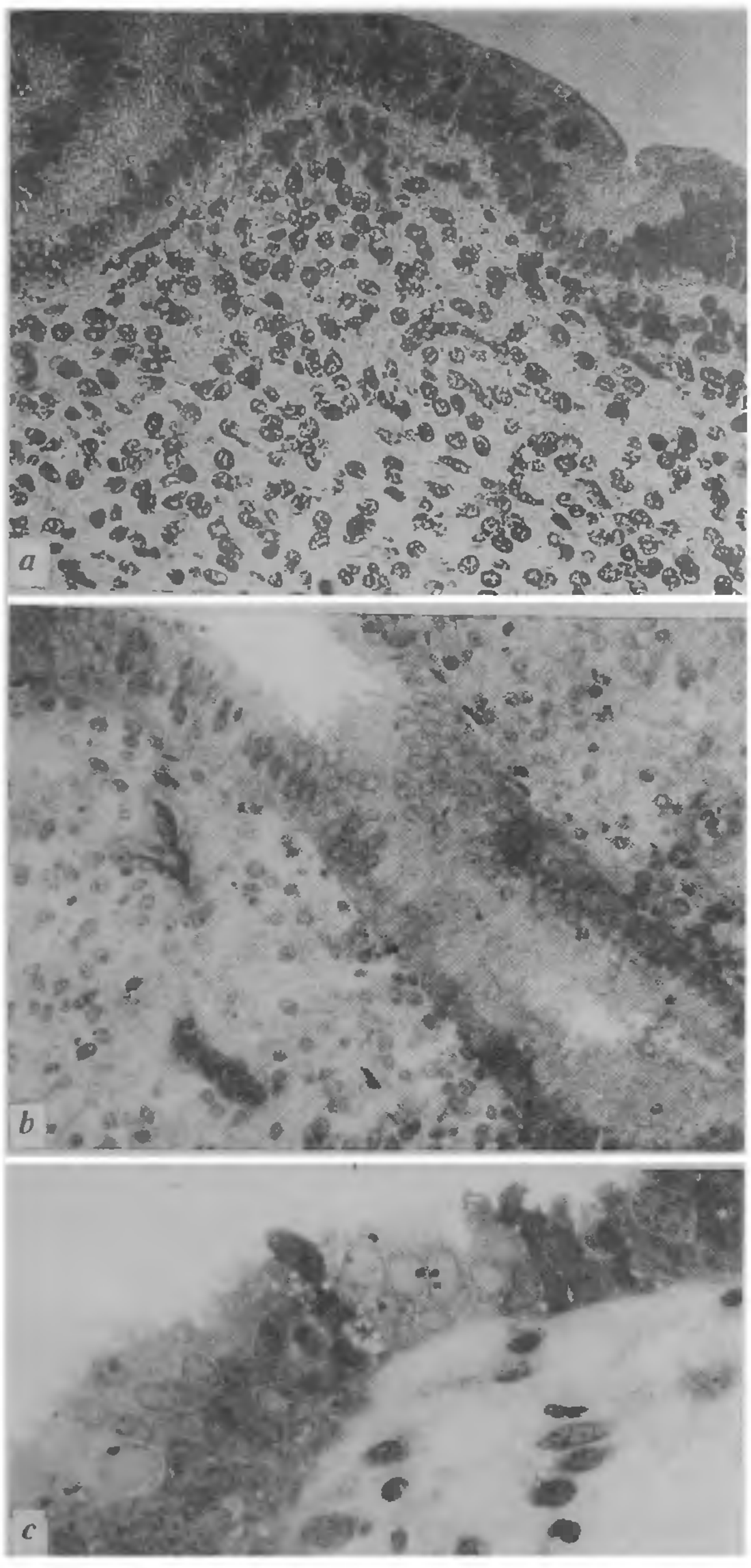

Figure 5. Surface epithelia with glandular invaginations from the superficial zones of endometrial samples obtained on day 6 after ovulation in non-mated, menstrual (*a*) and conception (*b*), (*c*) cycles of rhesus monkeys. Conception cycle endometria showed conspicuous presence of small pinopods on apical surfaces, intraepithelial lymphocytes and marked vascular congestion and oedema[35]

Interesting changes are seen in the expression of von Willebrand (vW) factor in the capillary network. Though there was no change in the numerical densities of vW factor positive capillaries in both types of secretory endometrial samples, the degree of expression was less in prenidatory endometrium.

Table 1. Morphological features of conception cycle endometrium showing increases when compared to non-fecund, menstrual cycle endometrium collected from rhesus monkeys on day 6 after ovulation

Index	Probability of significant difference between groups
Maximum gland cell height (μm)	$P < 0.05$
Volume fraction of gland occupied by gland cell (%)	$P < 0.03$
Mitosis/1000 gland cells	$P < 0.01$
Number of supranuclear vacuoles/100 gland cells	$P < 0.05$
Degree of pseudo-stratification of gland cells	$P < 0.02$
Amount of oedema	$P < 0.02$
Extravascular albumin volume	$P < 0.02$

Data taken from Ghosh *et al* [35]

Increase in oedema in preimplantation stage endometrium in the rhesus monkey could be a direct or indirect consequence of a blastocyst-derived factor[38]. We have earlier reported that the steroid hormonal milieu in the endometrial tissue bed is marginally altered on day 6 of gestation, compared with day 6 luteal phase in non-fecund cycles[39]. It is not known, however, if these changes are causally associated. Given the fact that endometrial oedema in any case increases rapidly during days 5 to 8 after ovulation during normal menstrual cycle[37, 38], the reported increase in oedema in prenidatory endometrium may not have high potential value for routine diagnosis. Nevertheless, it may be a physiologically significant endometrial reaction required for blastocyst implantation in the primate.

Based on semi-quantitative analysis, Hertig[37] also showed that glandular changes in the endometrium are the early distinguishing events in a human conception cycle. However, one may fail to detect these changes in biopsy samples[40]. We have observed distinctive changes in glandular epithelium in conception cycles of rhesus monkeys[35]. Cellularity of endometrial glands was enhanced, in association with increased number of mitoses, higher gland cell volume to total gland volume, and increased pseudostratification of glandular epithelium. At the same time, there were marginal increases in both gland cell height and percentage of glandular epithelium showing apical vacuoles (Table 1). However, no significant change could be detected either in the degree of glandular secretions or in lumen density.

It is being increasingly recognized that immunomodulation at the local level of endometrium may operate to support blastocyst implantation and placentation. Although endometrium is strategically supplied

Table 2. Implantation in the Old World (*Catarrhini*) anthropoidea primates

Species	Day of implantation	Nature of trophoblast invasion	Type of implantation	Nature of placentation
Rhesus monkey	8–9	Intrusive	Eccentric	Bidiscoid, villous, hemomonochorial
Baboon	8–9	Intrusive	Eccentric	Discoid, villous, hemomonochorial
Chimpanzee	6	Intrusive	Partially interstitial	Discoid, villous, hemomonochorial
Human	6	Intrusive	Interstitial	Discoid, villous, hemomonochorial

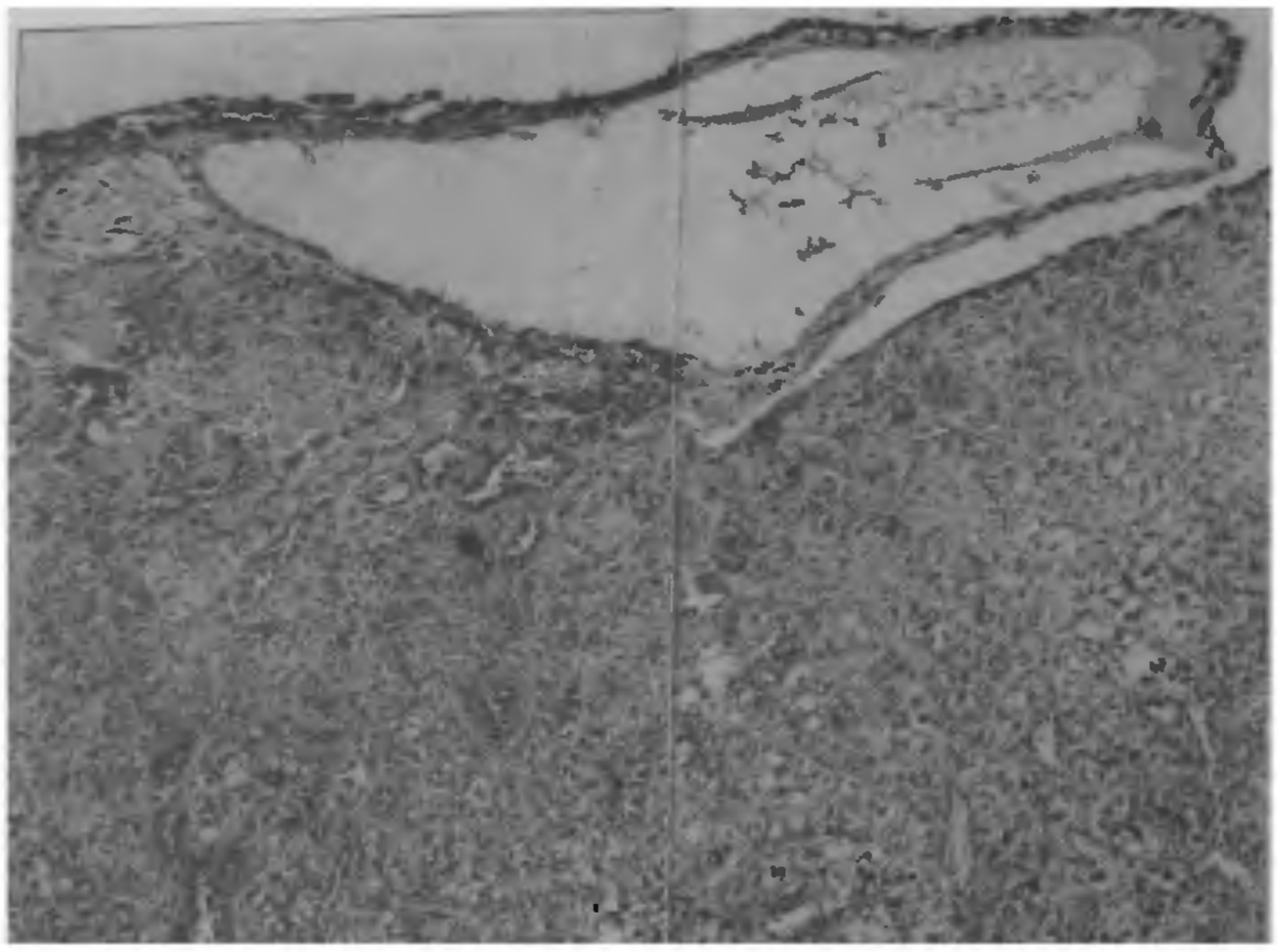

Figure 6. A survey montage micrograph of an implantation site in rhesus monkey obtained during the immediate post-implantation period of pregnancy Epithelial plaque acini are scattered in the stroma.

with afferent and efferent limbs of systemic immune traffic, innate immunomodulatory functions of endometrium may play a major role in the process of successful and evolutive implantation[41, 42]. It is now evident that immuno-competent antigens are expressed not only in stromal reticulolymphomyeloid cells, but also in epithelial compartment[43, 44]. This tempts us to believe that immuno-modulatory antigens may be involved in the process of blastocyst attachment and implantation. For example, we have observed that the epithelial expression of LNF-III (CD 15) is highest during the secretory period. An adhesion epitope like LNF-III in epithelium may be involved in cell–cell adhesion, and embryo–endometrial attachment[45]. In fact, an involvement of LNF-I has been suggestively implicated in the process of implantation in the mouse[46]. Similarly, the expression of a secretory sialokeratan sulphate has been found to occur in human endometrial epithelium at a time which coincides with the implantation window (see Aplin[47]). It is possible that such specific expressions in epithelium at luminal site may play a cardinal role in the acceptance of the blastocyst in a temporo-spatial manner. However, no substantial knowledge is yet available in this regard.

Blastocyst implantation and endometrial changes

Substantial studies have been done to describe the anatomical, histological and ultrastructural bases of blastocyst implantation and associated changes in maternal endometrium in many species including primates[18, 37, 38, 48–50]. Table 2 provides cardinal information relating to blastocyst implantation in a few primate species including the human. The zona-free denuded blastocyst apposes, attaches, adheres and penetrates through the endometrial surface epithelium. It appears that zona pellucida is actually shed, and does not undergo lysis. Interestingly, unlike in non-primate

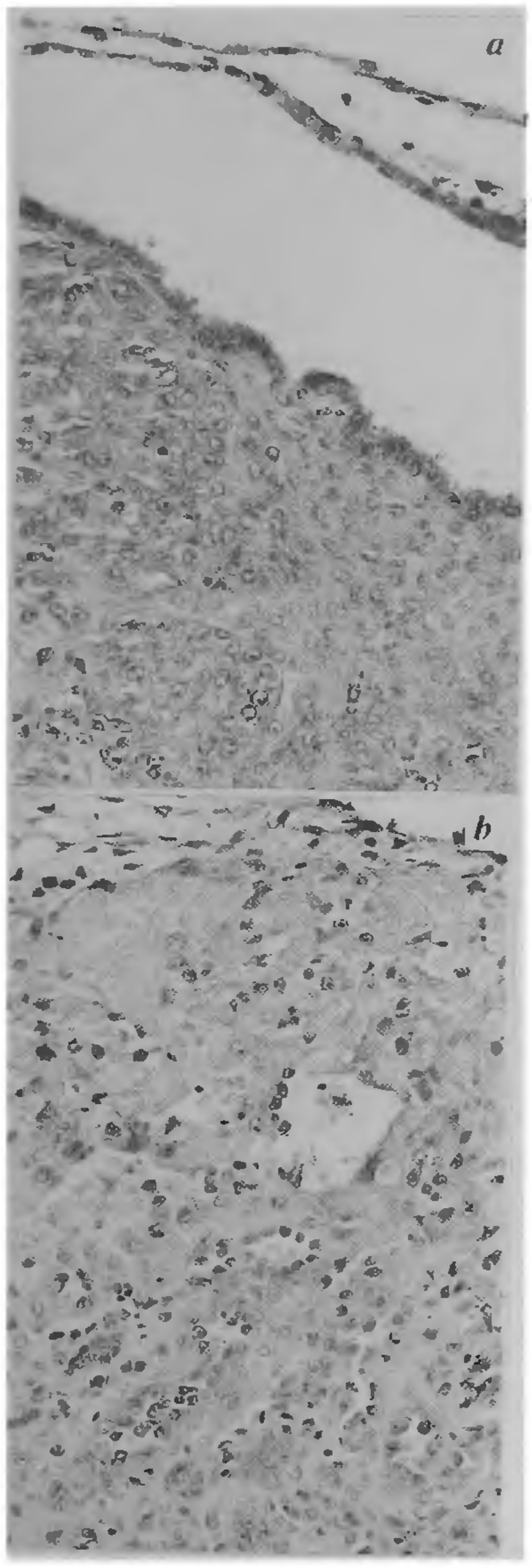

Figure 7. ırvey micrographs of implantation zone in the immediate post-implantation period of pregnancy in the rhesus monkey. Well-formed plaque acini are seen within an oedematous matrix peripheral to (*a*), and immediately below the site of embryo attachment (*b*).

laboratory mammals, blastocysts of anthropoids attach to the uterine surface epithelium at embryonic poles[51]. Three characteristic endometrial changes appear as consistent features during blastocyst implantation in primates – 1) Vascular reaction which includes oedema, vascular hypertrophy and congestion; 2) Glandular hyperplasia; and 3) Decidual transformation of stromal cells.

In macaques, an additional cellular response is seen in the form of cellular plaques immediately after the induction of oedema following blastocyst attachment and implantation (Figures 6, 7). The plaques are epithelial in origin because these cells appear as the acinar budding from the base and neck of glands, and show strong immunopositive precipitation of cytokeratins and do not express vimentin, although only a marginal expression of epithelial membrane antigen could be detected in these cells (Figure 8). Initially, epithelial plaques are seen at sites lateral to the primary implantation site, but soon more cells are recruited into plaque reaction with resultant increase in their numbers and the dimension of plaques. Reportedly, plaque cell reaction is also seen in the baboon but to a lesser extent, however, these are seen in New World monkeys and in some varieties of shrews[50, 52]. The epithelial plaques are transient in nature and undergo degenerative changes around two weeks of gestation when stromal decidualization also becomes distinctive.

Our knowledge about hormonal and paracrinal regulation, as well as the functions of endometrial epithelial plaque and stromal decidual cells is very limited. We presume that epithelial plaques subserve two functions. On the one hand, these cells display secretory activity, possibly towards angiogenesis, vascular hypertrophy and stromal decidualization. On the other hand, plaques also limit and direct trophoblastic growth. However, such putative functions of epithelial plaques remain to be tested. It will be interesting to examine the underlying cytokine network operative in the process of epithelial plaque formation, its degeneration and decidual transformation of stroma. To this end, we have established an experimental system using rhesus monkeys, in which bilateral ovariectomized animals are hormone primed, and artificial deciduogenic stimuli are applied to induce plaque and decidual cell reactions[53]. Interestingly, the temporo-spatial characteristics and fine structural features of endometrial responses in such an experimental system are highly similar to those seen in the pregnancy (Figure 9)[49, 53, 54]. Table 3 shows a comparison between endometrial changes induced during pregnancy-associated decidualization and those induced by the application of artificial deciduogenic stimulation to hormone primed uterus in rhesus monkeys. Using this model, we have shown that an antiprogestin like RU486 could inhibit epithelial plaque cell reaction[55], and that the induction of plaque degeneration also involves expression of IGF-BP in surrounding stromal cells (unpublished data). There is evidence to suggest that decidual cells in human and monkeys synthesize and secrete insulin-like growth factor binding protein (IGF-BP)[56], and its expression is regulated by oestrogen and progesterone (unpublished data).

Hormonal requirements

Progesterone is essential for blastocyst implantation in all primates hitherto studied. Progesterone insufficiency

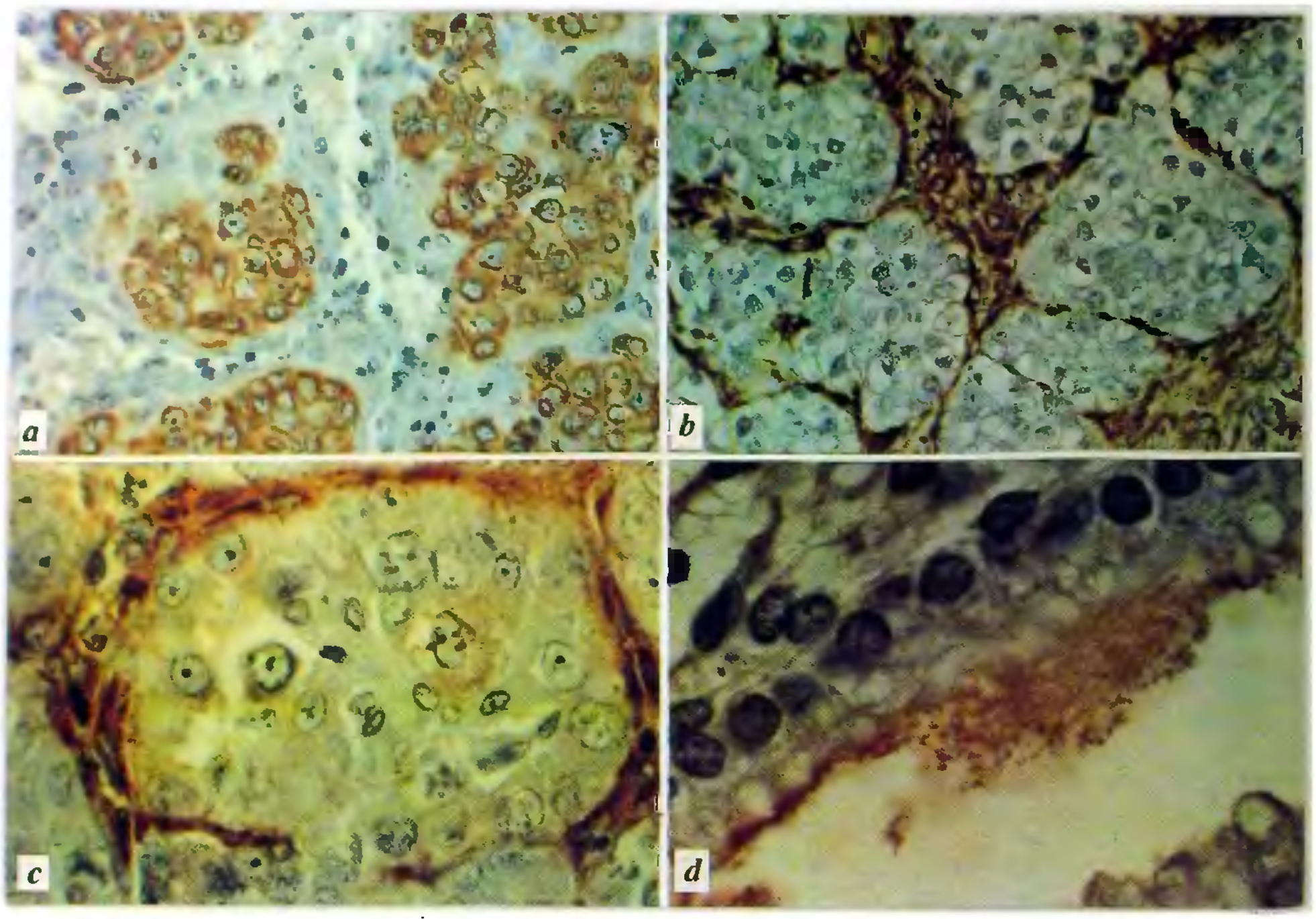

Figure 8. Endometrial plaque cells express strong immunopositivity for cytokeratin antibodies (*a*) with conspicuous absence of vimentin, which, however, is strongly expressed by stromal cells (*b*). Plaque acini are enclosed by basement membrane-like structure which contains immunopositive collagen IV (*c*). However, plaque cells express minimal level of epithelial membrane antigen which is occasionally expressed by glandular epithelial cells (*d*).

Table 3. Endometrial responses in the rhesus monkey to blastocyst implantation during pregnancy, and deciduogenic stimulation applied to ovariectomized, oestrogen-progesterone primed uterus (artificial decidualization model)*

	Pregnancy associated decidualization		Artificial decidualization	
Endometrial response	Day of onset	Features	Day of onset	Features
Endometrial plaque	10	By basal cells of luminal epithelium and neck of glands	9	Epithelial hyperplasia and formation of plaque acini at neck of glands
Oedema	10	Moderate to high peripheral subepithelial oedema	9	Very high subepithelial oedema
Decidual cells	13	Enlargement of fibroblasts to form decidual cells	13	Fibroblasts show swelling with rounding-up and enlargement of nuclei
Endometrial granulated lymphocytes	24	Increased numbers of cells showing eccentrically placed spherical nucleus and membrane-bound granules with marginal vesiculation	17	Increased numbers of cells around blood vessels and near glands showing eccentrically placed spherical nucleus and membrane-bound granules bearing marginal vesicles; these cells are phloxine positive

*24 h after oestrogen peak designated as day 0 of pregnancy cycle or artificial decidualization cycle; data for pregnancy cycle obtained from Enders *et al.*[49]; data for artificial decidualization cycle obtained from Ghosh and Sengupta[53], and Sengupta *et al.*[54]

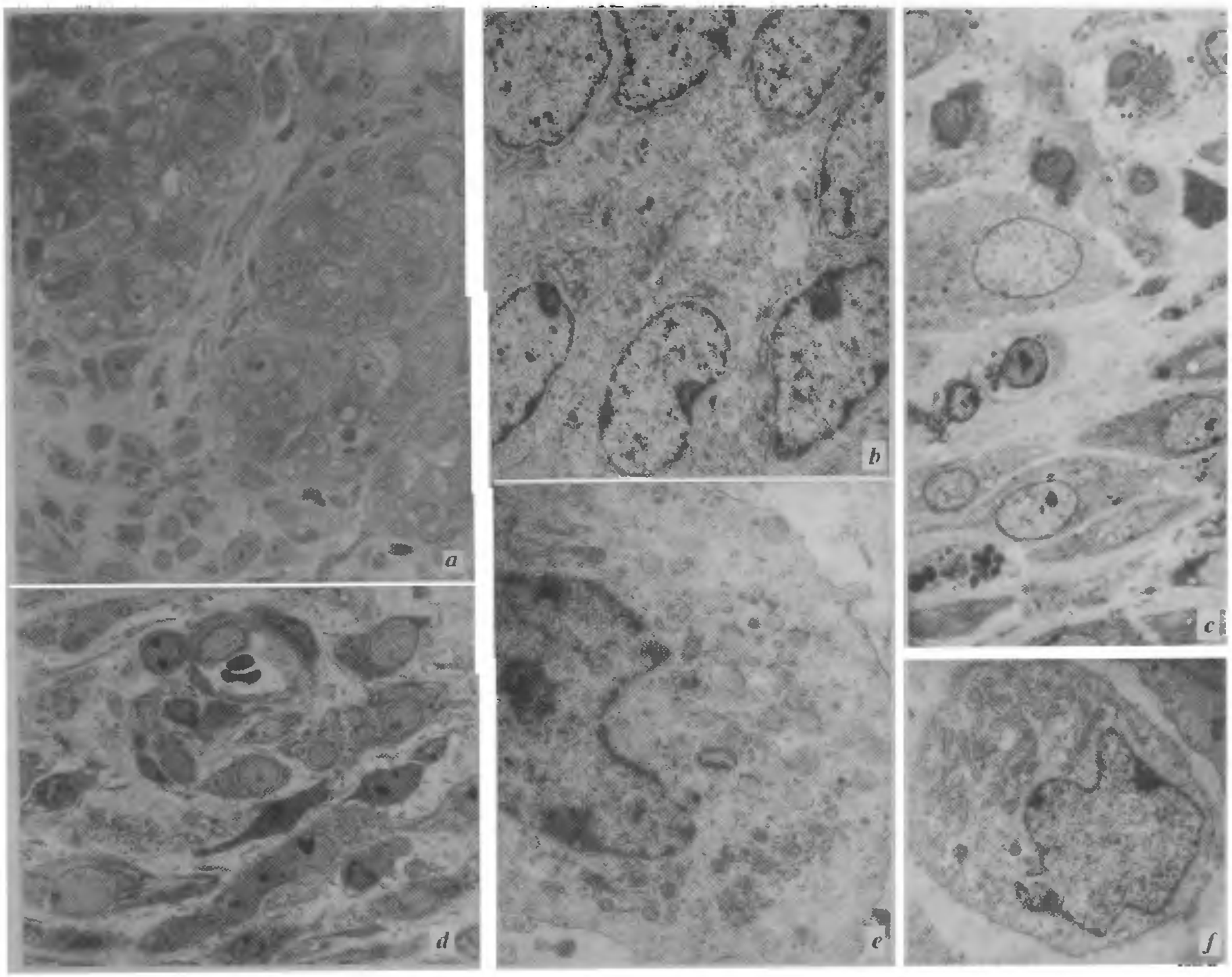

Figure 9. Light and electron microphotographs of plaque, decidual and endometrial granulated lymphocyte cells in endometria during artificially induced plaque-decidual cell reaction in the rhesus monkey. Well-formed plaque acini (*a*), survey electron micrograph of plaque cells showing extensive RER (*b*), decidual cell with round large nucleus and granular cells in endometrial matrix (*c*), typical stromal cell decidualization around a spiral arteriole (*d*), electron micrograph of endometrial granulated lymphocyte showing eccentrically located kidney-shaped nucleus, numerous membrane-bound granules with dense core, a few showing marginal vesiculation (*e*), electron micrograph of a decidual cell showing large nucleus with prominent skein-like nucleolus, engorged RER and 'lamina externa' or an extracellular membrane-like structure is found associated with the decidual cell (*f*)

causing inadequate endometrial secretory differentiation remains as a well known cause of infertility in women[57]. Studies have revealed that suppression of progesterone action at the endometrial level by the administration of high affinity antiprogestin like RU486 causes a delay in endometrial maturation in the human[58, 59] and inhibits blastocyst implantation in the monkey[60–62].

However, the role of ovarian oestrogen in the process of blastocyst implantation in primates is not clear. Though it has been shown that follicular phase oestrogen profiles may be indicative of the prospect of pregnancy[63], it may only be reflective of follicle development and oocyte quality, and probably has nothing to do with endometrial receptivity. Supportive evidence comes from human IVF-ET laboratories showing that a constant dose of oestradiol priming may lead to implantation without any peri-ovulatory type oestrogen surge[64].

The issue of requirement of luteal phase ovarian oestrogen is polemical. Generally, there are marked increases in concentrations of both progesterone and oestradiol in peripheral circulation during implantation in primates[65]. Meyer *et al*[66] showed in rhesus monkeys that ovariectomy performed during the preimplantation period of gestation followed by only progesterone supplementation could lead to implantation. On the contrary, Bosu and Johansson[67] observed that some amount of oestrogen is required, besides progesterone for blastocyst implantation in rhesus monkeys. Furthermore, there is also evidence in the literature that administration of anti-oestrogen during the peri-implantation period may inhibit the establishment of pregnancy in human[68] and in bonnet monkeys[69]. The absence of luteal phase oestrogen failed to inhibit the secretory maturation of endometrium in human[70]. We have

reported that ovariectomized, hormone-primed rhesus monkeys treated with progesterone alone could induce sufficient secretory maturation (albeit a lag of 2 days in the scale of the standard dating criteria of Noyes *et al* [71] could be seen), and such endometrium could support embryo implantation and live birth of babies following embryo transfer. While these results indicate that luteal phase oestrogen of *ovarian* origin is not obligatory, when sufficient progesterone is given, to induce blastocyst implantation, the possibility that oestrogen may be available at the site of implantation still remains to be tested. Three lines of evidence support this notion. Blastocysts of different mammalian species including human IVF blastcysts have shown the ability to biotransform several steroids and this includes the aromatization of testosterone to oestrogen *in vitro*[72–74]. It has also been reported that human endometrial stromal cells exhibit high degree of aromatase activity under progesterone dominance in culture[75]. We have observed that endometrial tissue obtained from ovariectomized monkeys receiving only progesterone and subjected to traumatization had detectable amount of immunoreactive oestradiol[53]. Future studies using techniques of cell biology and molecular biology[76, 77] will shed further light into the endocrine-paracrine type of regulatory mechanisms involved in embryo and endometrial development as well as blastocyst–endometrial interaction during the peri-implantation stage in the primate.

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Secretion of endocrine signals by the primate embryo during the peri-implantation period

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Development of preimplantation embryos and blastocyst implantation are critical early events in the establishment of pregnancy. In primates, embryonic signals, secreted during the peri-implantation period, are believed to play a major role in the regulation of embryonic differentiation and implantation. However, only limited progress has been made in the molecular and functional characterization of embryonic signals, partly due to severe paucity of primate embryos and the lack of optimal culture conditions to obtain viable embryo development. Two embryonic (endocrine) secretions, i.e. chorionic gonadotrophin (CG) and gonadotrophin releasing hormone (GnRH) are being studied. This article reviews the current status of knowledge on the recovery and culture of embryos, their secretion of CG, GnRH and other potential endocrine signals and their regulation and physiological role(s) during the peri-implantation period in primates, including humans.

BLASTOCYST implantation and early establishment of pregnancy in primates relies on endocrine mechanisms that are distinct from those in non-primate species. Embryo-specific endocrine signals are believed to control embryonic differentiation and implantation in primates. But, very little is known of their activation and sequential expression. In contrast, sufficient knowledge is available on placental endocrine secretions and their