International Journal of Veterinary Science and Medicine 6 (2018) S6–S14

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Contents lists available at ScienceDirect

International Journal of Veterinary Science and Medicine

journal homepage: www.elsevier.com/locate/ijvsm
www.vet.cu.edu.eg

Review Article

Cytokines, growth factors and macromolecules as mediators of implantation in mammalian species

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ARTICLE INFO

Article history:
Received 2 October 2017
Revised 8 December 2017
Accepted 9 December 2017
Available online 19 December 2017

Keywords:
Implantation
Integrin
Osteopontin
Hyaluronan
Mucin

ABSTRACT

Implantation is one of the most critical steps in mammalian reproduction and implantation failure constitutes a major cause of infertility in both animals and humans. The mechanism of implantation is exclusively under the control of ovarian steroids progesterone and oestrogen whose actions are mediated in a complex phenomenon that involves a number of cytokines and growth factors. According to a plethora of literature on implantation in mammalian species, prominent of these cytokines and growth factor playing crucial roles in implantation include integrin, osteopontin, integrin, insulin-like growth factor and leukaemia inhibitory factor. Others are cluster domain 44, hyaluronan system and many non-adhesive molecules such as glycoprotein mucin 1. In this review, the specific roles played by these molecules are expatiated. Generally, they function as adhesive molecules that facilitate attachment of ligands/proteins on the trophectoderm to their respective receptors on endometrial luminal epithelia or vice versa. Sometimes, they also function as signalling molecules that enhance communication between implanting blastocyst and receptive endometrium. This is of particular importance in embryo culture and embryo transfer where *in vitro* derived blastocyst unlike the *in vivo* condition, is not exposed to these substances and hence, their absence may be partly responsible for the low implantation rate observed in the surrogate. Appreciation of the roles played by these cytokines, growth factors and molecules as revealed in this review will spur further research on these topics, facilitate their inclusion in embryo culture media (if positively required) and are considered as vital aspect while developing strategies to improve fertility.

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Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.
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https://doi.org/10.1016/j.ijvsm.2017.12.001
2314-4599/

1. Introduction

Sub-fertility is a pervasive problem affecting both human and animal species. In humans, available evidence suggests that pregnancy loss predominantly during pre-implantation and the first few weeks of pregnancy is one of the major causes of subfertility. According to the global statistics presented by Boivin and Bunting [1], an estimate of over 72 million women between the ages of 20–40 years are infertile. In domestic animals, subfertility is a limitation to animal production and is one of the main issues for dairy cows selected for milk production [2]. An estimate of 60% pregnancy loss occurs in dairy cattle, with a significant number observed during early stages of embryo development [3]. Fertilization rates in cattle are around 90%, however, one-third of embryos fail to survive the first 18 days of pregnancy [4]. This implies that reproductive losses in dairy cows due to early embryo death are 3–4 times greater than losses due to fertilization failure. In spite of almost 100% fertilization rate in sheep [2], only 60–80% of the fertilized eggs proceed to live birth, while higher percentage of these losses occur before day 18 of pregnancy [5]. Collectively, this suggests that implantation failure constitutes a major source of pregnancy loss and infertility in both human and animal subjects.

The early stage of pregnancy is thus termed 'a critical period' because of the high risk of embryo loss. One event known to occur during this critical period is blastocyst implantation to the maternal endometrium. Implantation is a complex process and has been generally acknowledged as the most critical step in mammalian reproduction. In primate and human, embryo stays momentarily in the oviduct before being transported to the uterus between 72 and 96 h post fertilization, readily prepared for implantation. In domestic ruminants, definitive implantation is achieved by adhesion of the mononuclear trophoblast cells to the endometrial luminal epithelium and formation of syncytia by the fusion of trophoblast binucleate cells with the luminal epithelium. The protracted period of peri-implantation embryo has made the ruminant especially sheep a unique model for classical study on molecular mechanism of implantation in mammalian species generally [6].

The contact between the embryo and the maternal tissue soon after fertilization is crucial for subsequent development and survival of the embryo *in utero* because it creates the medium of interaction between the two entities and also generates the platform that eventually leads to the formation and development of placenta [7] which is necessary to facilitate the exchange especially of micronutrients and gases from the mother to the conceptus.

Endometrial cells undergo cyclic renewal, differentiation and eventually apoptosis and shedding (in primate) as well as secretory with the primary purpose of allowing implantation of a viable embryo in a conceptive cycle. Many of these physiological processes depend on the timely expression of cell adhesion, bridging and signalling molecules as well as disappearance of others (such as non-adhesive molecules) which maintain tissue microarchitecture by mediating cell-to-cell and cell-to-substratum attachments that constitutes endometrial remodelling. In other words, endometrial remodelling is a prerequisite for the uterus to attain structural and functional capacity during implantation. This remodelling occurs only during the receptive phase of reproductive cycle [8] and this period is termed 'window of receptivity' when attachment of blastocyst to the maternal endometrium is physiologically possible [9].

Window of receptivity in mammalian species is exclusively under the influence of ovarian steroids, progesterone and oestrogen [10–12]. High level of oestrogen at ovulation causes uterine cell proliferation while subsequent increase progesterone during dioestrus/pregnancy suppresses proliferation and causes cell differentiation in preparing the uterus for receptivity [13]. In addition to the steroids, a plethora of other molecules, including macromolecules, growth factors and pro-inflammatory cytokines mediate and modulate the actions of these steroid hormones to bring about the required changes in the uterine extracellular matrix [14]. Among these cytokines/growth factors playing crucial roles in implantation are integrins (ITG), osteopontin (OPN), insulin-like growth factor (IGF) and leukaemia inhibitory factor (LIF). Others are hyaluronan (HA) system, cluster domain 44 (CD44), and many other non-adhesive molecules such as glycoprotein mucin 1 (MUC1). To date, the list is inexhaustible and continues to grow, however, the molecular mechanism underlying the phenomenon of implantation still remains, in the word of Aplin [9], elusive. For the purpose of simplicity in this review, they are better classified as (i) adhesive and bridging molecules for those that initiate the visible actual attachment observed, (ii) signalling molecules that induce the transcription and translation of other genes and proteins that initiate communication/interaction between the receptive maternal endometrium and implantation-competent blastocyst and then (iii) the protective non-adhesive molecules on the endometrium that have to be removed before implantation could occur.

As reproductive biologists persist in their continued effort to understand the mechanisms underlying implantation process for a better development of strategies towards improving its success rate, our present understanding on the mechanism of implantation is still far from being complete. The objective of this review is to highlight the roles of ITG, OPN, IGF, LIF, HA system, CD44 and the non-adhesive MUC1 as mediators of implantation in mammalian species.

2. Methodology

The preliminary search strategy involved using the Unites States National Library of Medicine (https://www.ncbi.nlm.nih.gov/pubmed/) while matching the word implantation with cytokines, growth factors and adhesive molecules. The plenty of papers generated each time were selected based on their relevance to the subject matter of this review by going through the titles and abstract. These were read one by one and key references from them were also reviewed to generate a broad knowledge on the roles of ITG, OPN, IGF, LIF, HA, CD44 and MUC1 as mediators of implantation in mammalian species. Other relevant textbooks on the subject were also consulted to come up with a broad knowledge

Table 1
Sources and roles of cytokines, growth factors and macromolecules mediating and modulating implantation process in mammalian species.

Cytokine/growth factor	Sources/location	Proposed roles in implantation	References
ITG	Endometrium and blastocyst	Adhesive molecules	[146]
OPN	Placental and endometrial immune cells	Adhesive molecule	[35]
IGF	Oviduct/Endometrium	Metabolic indicator/ signalling	[63,147]
LIF	Endometrium	Signaling	[85,148]
HA	Endometrium and blastocyst	Adhesive molecule	[95,99]
CD44	Endometrium and blastocyst	Adhesive and signalling	[119]
MUC1	LE of endometrium	Anti-adhesive	[149]

CD44; cluster domain 44, HA; hyaluronan, IGF; insulin-like growth factor, ITG; integrin, LIF; leukaemia inhibitory factor, LE; luminal epithelium, MUC1; mucin, OPN; osteopontin.

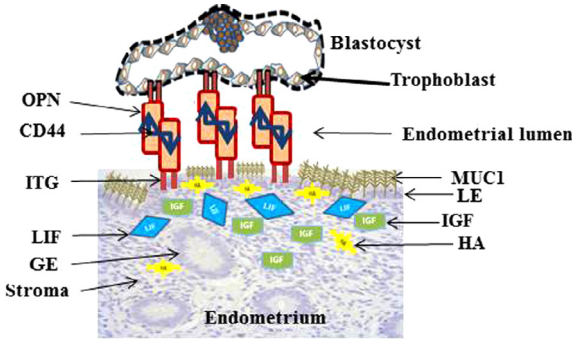

Fig. 1. Proposed Interactions between trophectoderm of implantation-competent blastocyst and luminal epithelial cells of receptive endometrium mediated by implantation-related cytokines and growth factors in mouse model. During implantation, there is increase in expression of cytokines and growth factor such ITG, OPN, LIF, IGF, HA and CD44 at endometrium, while MUC1 is proposed to reduce at implantation site of endometrial LE in response to embryo signal. ITG is also produced by the blastocyst and OPN being a receptor for ITG binds to OPN on the endometrium to establish attachment with OPN on the trophoblast leading to adhesion of the luminal epithelia and trophectoderm. Legends: CD44; cluster domain 44, GE; glandular epithelia, HA; hyaluronan, IGF; insulin-like growth factor, ITG; integrin, LIF; leukaemia inhibitory factor, LE; luminal epithelium, MUC1; mucin, OPN; osteopontin.

which has been summarised in the discussion that follows. It is noteworthy to state that the aforementioned mediators are not the only cytokines, growth factors or macromolecules involved in implantation, they are selectively chosen based on compelling evidence that suggests their synergistic and interlinked interactions during implantation from bodies of previous studies on this subject as summarized in Table 1 and subsequently illustrated in Fig. 1.

3. Integrins

ITGs are members of a larger family of cell adhesion protein believed to have major roles in cellular processes such as differentiation, motility and attachment, apoptosis and cell survival [15]. Roles of ITG in implantation are hypothesized to be either through ITG attachment of cells to the extracellular matrix [16] or ITG initiating a signalling transduction from the embryo to the extracellular matrix (ECM) leading to the transcription and translation of genes critical for implantation [17]. The two major players of implantation, maternal endometrium and blastocyst reportedly expressed ITG [18,19]. Therefore, conceptualising a role for ITG during implantation is reasonable.

ITG undergoes dynamic temporal and spatial patterns of expression on endometrial cells during the menstrual cycle and in the early stages of human pregnancy [20]. There are many isoforms of ITG in mammals, however, only three isoforms α1β1, α4β1 and αVβ3 are found to be particularly involved in implantation, with αVβ3 seemingly playing more conspicuous roles than others [21]. In human, these isoforms were shown to be expressed in the endometrium during the window of implantation (day 20–24) when the endometrium is structurally and physiologically conducive to implanting blastocysts. On day 16 of bovine oestrous cycle, ITG αvβ3 and the oestrogen receptor are detected in uterine environment as molecular markers for the adhesion and signalling [22]. ITG subunits are detected at sites of attachment between uterine epithelial cells and trophectoderm on Days 12–15 of swine pregnancy [23]. Expression of ITG αVβ3 at the foeto-maternal interface in many species including sheep, pigs, baboon and human during blastocyst attachment and implantation further substantiates the concept that ITG is an adhesion as well as bridging molecule during the process of implantation [24–27].

On the contrary, several conditions that interfere with expression of ITG in the endometrium culminate into implantation failure. The blockade of the ITG αVβ3 inhibits implantation in mouse [28]. ITG expression was also significantly reduced in human glandular epithelial cells and endometrial lumen as well as stromal cells of the hydrosalpinx group when compared with those of the control group [29,30], sequel to which is infertility in the hydrosalpinx group. In addition, some treatments in IVF protocol such as ovarian stimulation may adversely affect the expression of ITG which may partly responsible for low success outcome. For instance, all the three variants of ITG (α1β1, α4β1 and αVβ3) were reduced in glandular endometrium coupled with a reduced expression of the αVβ3 in the luminal epithelium after ovulation induction with gonadotropin [31]. This was partially restored with administration of GnRH agonist and not GnRH antagonist in mice [32].

Synthetic ITG ligands are currently been explored in other fields of research. Novel synthetic cyclic ITG αVβ3 binding peptide ALOS4 was reported to initiate antitumor activity in mouse melanoma models thorough inhibition of cell migration [33]. Considering the importance of ITG in implantation, reduced or absence of ITG in surrogate endometrium may be detrimental to the success outcome of assisted conception. The question is, 'Is it possible to compensate for the reduced ITG in the endometrium through exogenous addition of synthetic ITG in the embryo transfer media or intrauterine infusion?' Further studies are warranted to determine the possible roles of ITG as a media component for embryo culture and embryo transfer since it is not included presently.

4. Osteopontin

Osteopontin (OPN) otherwise called secreted phosphoprotein 1 is an ECM proteins/cytokine capable of undergoing extensive phosphorylation, glycosylation and cleavage to yield molecular mass variants ranging from 25 to 75 kDa [34]. It has multiple functions by binding cell surface receptors to mediate cell-cell adhesion and cell-ECM communication as well as cell migration.

OPN is hypothesised to play significant roles in mammalian implantation in a number of ways. Firstly, OPN is a component of histotroph required for adhesion and signal transduction at the uterine-placental interface resulting into conceptus attachment [35]. Secondly, it is a gene product expressed by uterine stroma as it decidualizes in response to conceptus invasion, and thirdly, as a constituent of resident placental and uterine immune cells that regulates immune cells behaviour and cytokine production [36,37]. All mammalian uteri including human contain endometrial glands that secrete substances comprising enzymes, growth factors, cytokines, lymphokines, hormones, transport proteins and other substances altogether refer to as histotroph [38]. Histotroph plays a role in conceptus nourishment, production of maternal pregnancy recognition signals, immuno-tolerance of semi-allograft embryo, blastocyst attachment and implantation as well as placentation [38,39].

Uterine gland secretion is active and support pregnancy in human during the first trimester (10 weeks) of gestation [40]. Previous studies with ewes in which uterine glands were epigenetically ablated (known as uterine gland knockout, UGKO ewes) by neonatal progestin exposure confirmed that histotroph is required to maintain pregnancy during peri-implantation period [41,42]. OPN, a key component of histotroph is distinctly absent in uterine secretion of UGKO ewe such that UGKO ewes are infertile due to failure to support pregnancy at the very early stage [43]. In sow, OPN was detectable at the maternal-placenta interface from day 25 and remained elevated till day 85 [44]. Localisation of OPN at the point of maternal-placenta interface is suggestive of ITG

interaction with its receptors on the conceptus and uterus to promote conceptus development and signalling. *In vitro* modelling of early implantation with human endometrial cells (Ishikawa) and mouse or human embryos or ligand-coated beads showed that OPN of epithelial origin binds the receptor ITG αvβ3 at the maternal surface to support adhesion during the early stages of implantation [45].

Non-interaction of ITG αvβ3 and OPN at the foetal-maternal interface in ruminants especially bovine was reported by Kimmins et al. [46]. On the contrary, other workers reported co-localisation of both OPN and ITG αvβ3 in sheep uterus and trophectoderm to induce adhesion between endometrial luminal epithelium and trophectoderm during period of implantation [24,47,48] while a summary of these works has been reviewed elsewhere [49]. There are limited studies of these cytokines in cattle possibly due to size of the animal. Certainly, further studies are required to clarify this ambiguity as regards simultaneous expression of OPN and ITG αvβ3 at the foetal-maternal interface during implantation in ruminants.

5. Insulin-like growth factor

According to Clemons [50], the insulin-like growth factor (IGF) family includes the two ligands-IGF1 and IGF11, their receptors-IGF1R and IGFIIR and six binding proteins (insulin-like growth factor binding protein, IGFBP1-6). IGFs have structural (50%) homology with pro-insulin and hence the name insulin-like growth. IGF system is extremely complex and functions in a wide variety of physiological and pathological conditions in tissues of various types. It may promote differentiation and migration in some cells while inhibiting apoptosis in some other cell type. Besides, the binding of IGFs to their respective receptors may elicit intracellular signalling cascade using Gi-coupled receptor or Mitogen Activated Protein Kinase (MAPK) [51] and may involve α5β1 integrin [52].

IGFs form a part of the body immune response mechanism and may be produced in response to endotoxins [53], however, they are reported to be involved in foetal and placental development [54]. During oestrous cycle, IGFs and their binding proteins are expressed in the cow oviduct [55]. IGF1, IGF11 and IGF1-R are also expressed in sheep endometrium during oestrous cycle as well as in early pregnancy [56]. There is an increase in foetal hepatic IGFI, IGFBP-2, -3 and -4 concentrations during gestation in sheep [57] while high concentrations of IGF1 and 11 are detectable in human maternal circulation during early pregnancy [58]. These data suggest endocrine roles of IGFs in regulation of placental and foetal growth.

Reproduction is a secondary characteristic and occurs essentially when the primary metabolic requirement has been fulfilled. IGF system also seems to be a link between nutrition and reproduction. The study of Fenwick et al. [59] demonstrated the effect of negative energy balance on level of circulating IGF1 after calving that may impede embryo development and causing embryo mortality in dairy cow. In related study, low concentration of IGF1 of live heifer calves indicated the survivability of calf post partum in dairy [60]. Collectively, it could be inferred that IGFs are key metabolic signalling molecules that may be used as indicator/marker of metabolic stress in domestic farm animals [61]. Stress is a limitation to attainment of full reproductive potential in mammalian species.

The use of IGF in assisted reproductive technology is well documented. A reasonable number of studies have shown that IGF1 especially with oestrus cow serum can facilitate embryo development and increase blastocyst rate [62], possibly by increasing embryo signalling via MAPK [63]. *In vitro* culture of oocyte, luteal and follicular cells and embryo are important aspect of assisted reproductive technology. The morphological and functional characteristics of cultured luteal cells are retained in media containing insulin and luteal angiogenesis is also facilitated with IGF1 [64,65]. IGF1 produces anti-apoptotic effect in regressing porcine corpus luteum [66] possibly through stimulation of progesterone secretion as was observed in cultured luteal cells obtained from early pregnant subject [67].

Inclusion of IGF11 to embryo culture media improves blastocyst rate and blastocyst hatching *in vitro* in mouse [68]. Addition of 100 ng IGF-I per mL of embryo culture media shortens the transition from the morula to the blastocyst stage and increases the proportion of blastocysts and hatched blastocysts on day 13 [62]. One of the major limitations of assisted conception is failure of the blastocyst to implant. A recent study has shown that IGF1 improves attachment of mouse blastocysts to Ishikawa cells *in vitro* [69], thus indicates the potential of IGF1 to enhance adherence of implantation-competent blastocysts in the surrogate. Summarily, in spite of several reports of beneficial inclusion of IGF in experimental studies, the clinical application of these findings to improve results of assisted conception in animal and human has not been optimised.

6. Leukaemia inhibitory factor

Leukaemia inhibitory factor (LIF) belongs to a group of cytokines known as interleukin-6 (IL-6) family. Other members in the group are IL-6 and IL-11. Receptors for LIF are LIF receptor alpha (LIFRα), LIF receptor beta (LIFRβ) and glycoprotein gp130 [70].

The LIF receptor is expressed during secretory and post ovulatory phases of the oestrous cycle and is restricted to the luminal epithelium [71]. The associated signal-transducing component of the LIF receptor, gp130 is also expressed in both the luminal and glandular epithelium throughout the mestrual cycle; however, maximal expression of LIF was reportedly expressed during the secretory phase at which time implantation occurs [72]. The coexistence of a high level of LIF protein, LIF receptor and gp130 on day 4 of gestation in gravid uterus further emphasizes the importance of LIF in blastocyst implantation in mouse and LIF possible involvement in signalling between the foetus and the endometrium [73]. The role of LIF in implantation was clearly demonstrated in studies using LIF knockout model in which a LIF null female mice exhibited failure of implantation that was restored on LIF administration [74]. Such implantation failure was partly attributed to profound disturbance of normal luminal epithelial and stromal differentiation during early pregnancy in LIF-null mice which was characterised by non-development of apical pinopods, increased glycocalyx and failure of endometrial cell decidualization during the peri-implantation period [75]. In addition, absence of LIFR to activate Janus kinase-signal transducer and activation of transcription 3 (Jak-Stat3) signalling pathway in the LE that could have induced receptivity in the LE [76].

At early stage of pregnancy, LIF has also been detected in uterus of mouse [77], rabbit [78], sheep [79], western spotted skunk [80] and uterine flushing in human with good prognosis for implantation success [81]. Uterine expression of LIF in human is proposed to play significant role in embryo implantation, possibly through an autocrine/paracrine interaction between LIF and its receptor at the luminal epithelium [72]. Similar effect is proposed in rabbit [82]. To buttress this line of thought, low expression of endometrial LIF at the proliferative phase of the cycle has been associated with infertility primarily due to implantation failures [83]. This makes LIF one of the candidate genes/molecules to be considered when investigating infertility in human especially with those implantation failures after embryo transfer [84]. Progesterone and IFNt were shown to regulate expression of LIF and its receptor

LIFR in sheep GE and trophectoderm during the period of implantation. This was also associated with increased phosphorylation of signalling molecules STAT3 and MAPK3/1 protein [79] as well association of LIFR and gp130 forming a functional heterodimer in the uterus during the attachment reaction to direct LIF signalling [85].

These results indicate that LIF is involved in blastocyst implantation to the endometrium. This is possible through generation of paracrine signalling that complements the endocrine signal between the mother and the conceptus [86] or through the indirect local effect of LIF on other implantation-related cytokines produced by the endometrium or conceptus.

7. Hyaluronan system

Hyaluronan, otherwise known as hyaluronic acid or hyaluronate (HA) is a unique high molecular weight anionic members of a group of macromolecules called glycosaminoglycans (GAGs) that constitute major components of the ECM in all animal tissues [87]. At low concentrations, HA is ubiquitous in the body tissue and fluid. It is detectable in tendon, muscle, joint, uterus and cartilage with more than 50% of total HA body content existing in the skin where it keeps the dermis moisturized [88]. It is also present in various fluids and tissues of the reproductive tract, in amounts that vary from one mammalian species to another. In human follicular fluid, HA concentrations range between 48 and 72.8 ng/mL with significant variation in fluid with fertilized and unfertilized oocytes [89]. In the serum, HA level gradually increases as the pregnancy proceeds with the highest concentration (about 100.4 ng/mL) occurring during labour [90]. There is a high concentration of HA in the foetal circulation and amniotic fluid. In mice, the HA content of the uterus is 4053.0 ± 651.4 ng/g during dioestrus [91].

HA is produced by three trans-membrane enzymes hyaluronan synthases (HAS1, HAS2 and HAS3) and systematically degraded by hyaluronidase (HYALs). It is also capable of altering cell structure and function by binding to its receptor, mainly cluster domain 44 (CD44) and receptor for HA mediated motility (RHAMM) [92,93]. Many roles of HA in mammalian reproduction that include cumulus expansion and oocyte maturation, sperm-oocyte interaction, cervical ripening and dilation as well as development of embryo have been reviewed elsewhere [94]. HA increases about five to six folds in mouse uteri on the day of implantation with potential to support attachment from observation of an embryo cultured on HA-coated tissue culture plates [95].

During the early stage of its discovery, HA was originally thought of fulfilling the functions of space filling and tissue hydration alone. Evidence in the last two decades implies that HA is involved in diverse physiological processes in the body. The issue of finding a suitable embryo transfer media partly brought HA into the scope of reproductive biotechnology research. According to available data, HA seems to be beneficial in assisted reproductive technologies involving *in vitro* fertilization (IVF) and embryo transfer (ET). Its use becomes attractive since HA is a naturally occurring substance. HA is the only non-sulphated GAG that has been detected in various segments of the mammalian reproductive tract including oviduct and uterus [96,97] and expressed at different stages of embryo development [98]. If HA is produced by the two principal partakers of implantation (endometrium and embryo), then, conception of HA roles in implantation is rational. Besides, HA is up-regulated in endometrial stroma at the time of implantation in human [99] and enhancement of implantation in many clinical trials in human using HA-supplemented media for embryo transfer (ET) supports this line of thought [100–102].

As much as there is a plethora of data suggesting beneficial roles for HA in human embryo implantation, the mechanism through which HA promotes implantation still remains ambiguous. It is generally proposed to be attributed to early stages of implantation facilitating apposition and attachment of the trophectoderm to the maternal endometrium [103]. A possible mechanism of HA involvement in implantation may also be through the ability of HA to promote angiogenesis [104], a process which is fundamental to embryonic development [105]. Other known roles of HA including facilitation of cell adhesion, cell to cell matrix and HA mediated signalling [106,107], induction of heat shock protein and the suppression of apoptosis by low molecular weight HA are all processes essential for embryonic development and implantation [108]. Besides, HA or its related protein has been closely linked with low molecular weight cytokines or growth factors such as prostaglandins, IGF, epidermal growth factor and LIF [109], which have been individually implied to be involved in embryo implantation. Moreover, sheep endometrial cell culture treated *in vitro* with low molecular weight HA upregulated transcript expression of LIF, CD44, IGF but reduced MUC1 expression into the culture media [110].

Despite these studies indicating beneficial roles of HA in embryo development, many other studies have failed to find a positive influence of HA inclusion in embryo transfer media on pregnancy rate [102,111]. The result of a recent study clearly demonstrated the need for HA clearance at the foetal-maternal interface in sheep for successful implantation to occur [112]. More studies are required to clarify this ambiguity on the role of HA inclusion in embryo transfer media and implantation.

8. Cluster domain 44 (CD44)

CD44 is a single-pass trans-membrane glycoprotein located on the surface of most vertebrate cells [113]. It has been detected in various segments of the reproductive tract in bovine, ovine, mouse, mare and human under normal physiological [96,97,114,115] or pathological conditions such as neoplastic endometrium in human [116]. CD44 is also detected during early stages of embryo development in mouse, bovine and human [117,118]. The specific roles of CD44 at the blastocyst-endometrial interface during implantation were demonstrated by the study of Illera et al. [119] where intra-uterine administration of anti-CD44 impeded implantation in the rabbit, while no effect was seen in control rabbits with intra-peritoneal administration of the same antibodies.

The signalling properties of CD44 embrace physiological processes like oocyte maturation and implantation [120]. It is a major receptor for HA [121] and also a receptor for OPN [122]. OPN, as a primary ligand of ITG binds to cell surface integrins primarily αVβ3 heterodimer expressed by trophectoderm and uterus to promote cell-cell attachment and cell spreading [47]. ITG has been acknowledged to bridge the gaps between the ITG family in the maternal endometrium and trophoblast, an event that is critical for initiation of initial attachment during implantation [123]. In the scheme of attachment cascade, CD44 is proposed to play a crucial role of activating the OPN which unites ITG in the trophectoderm and the endometrial luminal epithelia via the ITG αvβ3 (Fig. 1).

In vitro maturation of oocytes is widely used for *in vitro* fertilization. The localisation of CD44 in the cumulus cells which produces HA matrix of the cumulus oophorus [124] is suggestive of HA-CD44 induced signalling during oocyte maturation. The mechanism is that HA-CD44 interaction regulates the tyrosine phosphorylation of Connexin 43 (the major gap protein found in the cumulus oophorus) leading to closure of the gap junctions and subsequent upregulation of maturation promotion factor activity [120]. The latter brings about resumption of meiotic division in the oocytes. This line of thought is corroborated by the earlier work of Schoenfelder and Einspanier [125] in which HA and its receptor CD44 were reportedly involved in maturation of bovine oocyte.

It should be reiterated that many studies on HA-CD44 signalling have focussed on cancer. HA-CD44 signalling in cancer [106,126], though well-documented in the literature is beyond the scope of this review. However, HA-CD44 signalling is also observed under physiological conditions. HYAL2 reportedly caused increased phosphorylation of MAPK1/3 signalling in bovine embryos [127]. A recent study also showed induction of signalling by HA in human placenta through MAPK1/3 and Phospho inositol 3 kinase signalling pathways; an event that enhanced trophoblast growth and invasion possibly through placenta angiogenesis [128]. Even though the latter study did not show clearly that the signalling was through HA binding to CD44, it is likely to be through HA-CD44 because CD44 is the major receptor for HA and earlier studies have shown expression of CD44 in the human endometrium [96] and trophoblast [117] where it was proposed to play a significant role in placenta angiogenesis [129]. Collectively, it may be concluded that CD44 is germane to implantation process specifically and/or as receptors for other key cytokines OPN and HA already shown to be involved in implantation cascades.

9. Glycoprotein mucin 1 (MUC1)

Mucins encompass a family of highly glycosylated and high molecular weight (>250 kDa) glycoproteins expressed on the epithelia surface subsequent to its production by epithelial tissue [130]. There are about 15 variants of mucin in mammals, however, MUC1 is the most widely distributed in reproductive tract [130,131]. MUC1 expression has been reported in endometrial luminal epithelia in most mammals that including human, sheep, horse, pig and rabbit [132]. MUC1 is a non-adhesive molecule whose regulation, expression and functions with regards to implantation vary according to species. In sheep, endometrial MUC1 expression is cyclically regulated by both oestrogen and progesterone *in vivo* and *in vitro*, and directly down regulated by interferon tau treatment *in vitro* [133]. Interferon tau is an agent of maternal recognition of pregnancy produced by the blastocyst to abrogate luteolysis in ruminants [134].

In some animals like mice, rats, pigs, rabbits and ruminants, MUC1 expression at the uterine luminal epithelium is attributed to non-receptivity of the uterus and continued expression during the period of implantation will hysterically hinder the access of receptors to their ligands [6]. Therefore, in these species, it is regarded as an anti-adhesive molecule that inhibits successful interaction between the maternal endometrium and the implanting embryo. In sheep, MUC1 is up-regulated in the LE of a progesterone-dominant endometrium observed during the luteal phase. This property is in consonance with its protective function against pathogens because there is likelihood of reduced maternal immune response during this period. Henceforth, for a successful implantation to occur, MUC1 expression in the endometrial epithelia has to be cleared. This down-regulation also coincides with loss of progesterone receptor in the endometrial epithelia [135]. Removal of MUC1 from the epithelial surface at implantation sites is accomplished locally through signals apparently produced by the blastocyst [136]. In addition it is successively followed with up-regulation in the expression of other adhesive molecule like OPN and ITG in the LE [24]. In this context, MUC1 down-regulation in the endometrial epithelia can be used as a marker of endometrial receptivity in these species.

Human and mouse have the same haemochorial mode of placentation. For obvious ethical reason, our current understanding on human embryo implantation is based mostly on experiments derived from animal models especially mice, even though there is inverse expression of MUC1 in the endometrium during implantation in the two species. In a clear contrast to mice, MUC1 expression is highest at the time when the human endometrium is receptive, however, it is systematically removed from the apical endometrial epithelia in a paracrine pattern by the embryo just at the time of implantation [137]. MUC1 is regarded as inherent constituent of secretory endometrium and its down-regulation during this period is associated with recurrent miscarriage in human [138].

In mare, MUC1 does not inhibit implantation. MUC1 protein is expressed at foeto-maternal interface throughout the period of gestation. Therefore, the protracted period of implantation in this species is not attributable to adhesive property of MUC1 observed in the LE and the trophoblast before implantation [139]. In the sow, MUC1 expression is also unique. MUC1 protein was detected in the attachment and inter-attachment region of the endometrium between day 13 and 24 of gestation [140] and was found to be down-regulated during the time of implantation in this species [23,141].

It is concluded that MUC1 plays crucial roles in successful implantation and embryo survival, possibly through establishment of stromal decidualization and its down-regulation either locally at the region of implantation sites or generally along the entire endometrial luminal epithelia that is essential to allow ligands on the trophoblast gain access to their respective receptors on the endometrial epithelia and vice versa. Altogether, this implies that MUC1 expression and regulation in endometrial epithelia during implantation are species-specific.

10. Conclusions

Finally, cytokines, growth factors and macromolecules are all chemical messengers that mediate intercellular communication whose biological actions are mediated locally by specific receptors. They have been associated with many functions in the body including injury, inflammation, immune response and implantation. Pro-inflammatory mediators are produced in response to inflammation very similar to what are observed during implantation [142]. Apart from the aforementioned cytokines and growth factors discussed in this review, there are many more that partake in implantation in mammalian species. Indeed, there are repertoires of genes 'working behind the veil' in a molecular template to bring about the actual observable event of cellular adhesion of the trophoblast to the maternal endometrium during mammalian implantation [143–145] and the aforementioned have been shown to play significant roles.

For implantation to be fruitful, the blastocyst must be implantation competent, while the endometrium has to be receptive. Certainly, the different modes of implantation across many mammalian species dictate different molecular mechanisms to be involved. Suitable universal markers and mediators of implantation have proved difficult to be identified partly because very few morphological or molecular correlates of the receptive/implantation states are common to all species. No single reliable universal marker that is strictly restricted only to the receptive phase is yet to be identified across board. Therefore, a combination of markers/mediators as done in this study seems logical.

The Assisted reproductive technology (ART) has contributed significantly towards improving animal and human fertility. Embryo culture is an emerging technical component of ART. However, *the in vitro* environment, no matter the level of simulation, the immediate macro-environment remains sub-optimal when compared to the *in vivo* condition. In the latter, the embryo is innately exposed to arrays of growth factors, cytokines and macromolecules some of which are the subject matter of this review. The high rate of implantation failure associated with embryo transfer in assisted conception may possibly be due to absence of some of

these cytokines/growth factors. It is hoped that a clear understanding of the roles played by these cytokines and growth factors in mammalian implantation as revealed in this review will motivate further research on these topics to unravel some unresolved ambiguity on their roles in implantation. Such endeavours will facilitate their inclusion in embryo culture media (if found positive) and elevate them as a vital aspect to be considered along with steroid functions while developing strategies to improve fertility or investigating infertility in mammals.

Competing interests

There are no competing interests.

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