Basic and practical aspects of pregnancy establishment in cattle

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Abstract

Bovine embryos are increasingly produced using reproductive technologies, e.g. ovum pick-up (OPU), in vitro embryo production (IVP) and embryo transfer (ET). Such in vitro manipulated embryos are known to deviate in several aspects compared to in vivo derived embryos. Pregnancy establishment in cattle involves timed biological events including fine-tuned communication, initiated and carried out by both the embryo and the endometrium. This stimulates research to increase the understanding of events and interactions taking place in the uterus after embryo transfer, both from a biological and systems biology point of view. This review will focus on the biological events taking place during early embryonic development, implantation and beginning of placentation, with focus on transfer of in vitro produced embryos, including a systems biology approach for selection of superior embryo recipients.

Keywords: embryo recipient quality, in vitro embryo production, pregnancy establishment, reproductive technologies, systems biology.

Introduction

For food producing animals such as cattle, reproductive health plays an important role in relation to farm economy and is essential for improving breeding progress. Several biological and management-related factors have an impact on successful fertilization, establishment and maintenance of pregnancy, such as oocyte competence, semen quality, hormone levels, nutrition, milk production and parity. In cattle, the fertilization rate after insemination or natural mating has been estimated to 90%, with an average calving rate of 55% (Sartori et al., 2002; Diskin et al., 2006; Wiltbank et al., 2013). Most pregnancy losses occur during the early embryonic period, and the biological reasons include both the embryo and the mother in terms of oocyte and embryo quality, impaired function of the endometrium and sub-optimal embryo-maternal communication.

The last decades have shown an increase in both development and use of new technologies for improved reproductive efficiency and for improving the genetic merit of a herd. Among these are ovum pick-up (OPU) followed by oocyte in vitro maturation (IVM), in vitro fertilization (IVF) also using sex-sorted semen, in vitro embryo culture (IVC) and embryo transfer (ET). In 2015, more than 520,000 in vivo derived and 427,000 in vitro produced bovine embryos were transferred worldwide (Perry, 2016). In vitro produced embryos are still showing impaired results compared to in vivo derived, both in tolerance to cryopreservation, pregnancy rates and early embryo loss (Alberto et al., 2013). To improve the output of OPU-IVP-ET, the identification and selection of high-quality oocyte donors and embryo recipients as well as improved culture systems, resulting in improved embryo development and pregnancy rates, would make these technologies even more attractive.

One important aspect of the increasing use of artificially produced and in vitro manipulated embryos is a growing knowledge about the delicate interactions existing between the embryo and the endometrium. These interactions are dependent on the quality of both parts, the embryo and the endometrium.

This review will focus on the biological events taking place during early embryonic development, implantation and beginning of placentation, with focus on transfer of in vitro produced embryos, including a systems biology approach for selection of superior embryo recipients.

Establishment of the pregnancy

Early embryonic development

In the few hours after ovulation of the mature oocyte, gamete interaction occurs in the ampulla of the oviduct. At that time greater portions of the oocyte’s zona pellucida is devoid of cumulus cells and the fertilizing spermatozoon has easy access to the zona surface (Hyttel et al., 1988). At 2-3 h after ovulation, the spermatozoon has undergone the acrosome reaction, penetrated zona pellucida, and both the sperm head and tail are found in the ooplasm. Consequently, the oocyte is activated resulting in resumption of meiosis and release of the cortical granules that elicits zona hardening and the resulting block to polyspermic penetration. Over the coming hours, the second polar body is abstracted, and smooth endoplasmic reticulum is attracted to the sperm head as well as to the retained maternal chromatin in order to build nuclear envelopes of the two pronuclei. Around 5-7 h after ovulation, the pronuclei have developed to spherical structures that
migrate to close apposition around 15-19 h after ovulation. During this developmental period the S-phase of the first mitotic cell cycle takes place (Laurincik et al., 1994). At around 20 h, the nuclear envelopes are dissolved into the smooth endoplasmic reticulum, and the maternal and paternal chromosomes align during formation of the prophase and metaphase of the first mitotic division. Around 24 h after ovulation, the cleavage to the 2-cell stage occurs followed by two rather short cell cycles bringing the embryo to the 8-cell stage. This fourth cell cycle is considerably longer and includes major activation of the embryonic genome during which the blastomere’s nucleoli are activated to initiate transcription and ribosome production (King et al., 1988; Laurincik et al., 2000). Around the morula stage (16-32 cells), the embryo passes from the oviduct to the uterus. With the activation of the embryonic genome, the embryo achieves the ability to form different cell lineages, and at the 16-32-cell stage compaction of the morula occurs. The pluripotent inner cell mass (ICM) is formed, and the outer trophoblast (TE) is sealed by tight junctions and desmosomes and develops water transporting capacity leading to the formation of the fluid filled blastocyst. Around day 8 after ovulation, the blastocyst hatches from the zona pellucida. Around the time of hatching, the ICM develops into an upper pluripotent epiblast and a lower epithelium, the hypoblast (Maddox-Hytel et al., 2003). The latter epithelium develops on the inside of the blastocyst to form an inner lining of both the epiblast and the TE. Around day 12, the TE covering of the epiblast (Rauber’s layer) becomes extremely thin, and finally the epiblast penetrates the TE and becomes incorporated into the outer layer of the conceptus, which at this time of development is two-layered. The epiblast establishes the embryonic disc of pluripotent cells that will give rise to the embryo proper. Around day 14 after ovulation, the embryonic disc initiates gastrulation by formation of the primitive streak through which cells start to ingress to form endoderm and mesoderm. The endoderm becomes integrated in the hypoblast whereas the mesoderm forms a loose mesenchyme between the epiblast and the hypoblast as well as the longitudinal rod, the notochord (Maddox-Hytel et al., 2003). The epiblast located longitudinally over the notochord will develop into the neural ectoderm, whereas the more peripheral parts of the overlying epiblast will develop into the surface ectoderm. Along with the development of the embryonic disc and gastrulation, the conceptus elongates to a length of several centimeters on day 15 after ovulation at the time of embryonic-maternal signaling. The implantation process begins day 16-18, i.e. after embryo elongation, with placentation starting around day 22. Implantation and placentation occur at the caruncular areas of the endometrium. Reduction or loss of an anti-adhesive molecule from the uterine endometrium is necessary to prepare for implantation. Also, an appearance of cell-adhesion molecules (e.g. Integrins) on the surface of the endometrium is important to attachment and invasion (Mansouri-Attia et al., 2009). Fusions of placental cotyledons with the caruncles form placentomes that are involved in fetal-maternal gas exchange and provision of nutrients.

Maternal-embryonic communication

From the mid-1970ies, the view on maternal-embryonic communication during early pregnancy has changed. At that time, the function of the oviduct and the uterine horn was considered to simply keep and transport the gametes under proper conditions, but with no specialized signaling and interaction. In some ways, the success with in vitro embryo production (IVP) during the same period only supported such a view, also because these processes could be performed in a well-defined medium at the right temperature and atmosphere. However, the reports on Large Offspring Syndrome (LOS) from the early 1990ies challenged this view (e.g. Lazardi et al., 2002), even though the solution seemed to be a simple reduction in serum concentration in IVP media.

Parallel to the technological omics-revolution, a quite new insight into the mechanisms has gradually appeared, illustrating that the events are complex, interactive and fine-tuned involving both the embryo and the endometrium. The complex biological events have been expressed as “Thus, a receptive endometrium, an implantation-competent blastocyst and a synchronized dialogue between maternal and embryonic tissues is a pre-requisite for successful implantation” (Salilew-Wondim et al., 2012).

Today, it is well described that there is “cross-talking” going on at many points of the reproductive process in different species (e.g. Oestrup et al., 2011; Alminana et al., 2012; Forde et al., 2012a; Salilew-Wondim et al., 2012; Ulbrich et al., 2013; Fazelli and Holt, 2016; Klein, 2016). This cross-talking reflects quality at several points with some species differences, but the overall pattern is the same. In the oviduct, it involves timing of ovulation, tube collection of the ovulated cumulus-oocyte-complex, the oocyte-sperm interactions and oviductal cilia movements to transport the zygote and early embryo (Avilés et al., 2015; Maillo et al., 2016a). In vivo, it has been demonstrated in mice that unfertilized vs fertilized oocytes trigger a different gene response in the oviduct (Lee et al., 2002), and in the horse are unfertilized oocytes not allowed to pass the utero-tubal junction, probably influenced by missing embryo secretion of prostaglandin E2 (Klein, 2016). Today, the active role of the oviduct is becoming more and more clear with a growing list of activities both as preparation for an embryo to arrive as well as reactions to its actual presence (Artemenok et al., 2015; Gonella-Diaza et al., 2015, 2017; Maillo et al., 2016b). One challenge for this kind of work seems to be able to detect the changes when they are only caused by a single embryo (Maillo et al., 2015).

Some of the cross-talking involves presence of semen in the reproductive tract. The first reports were in vitro studies with oviduct epithelial cells responding to...
the presence of spermatozoa in cattle (Ellington et al., 1993) and in horse (Thomas et al., 1995). Since then, a number of studies have demonstrated various reactions in the oviduct from the arrival of sperm (Maillo et al., 2016b), also showing that the oviduct seems to be able to differentiate between X- and Y-bearing spermatozoa (Alminana et al., 2014). Seminal plasma is described to have a positive influence on embryo development, implantation and pregnancy in different species, although there are mixed results with respect to bovine (Maillo et al., 2016b). In several species an ovulation-inducing-factor been demonstrated in seminal fluid that induces ovulation and possibly influences the properties of the progesterone-producing capacity of the corpus luteum (Ratto et al., 2012).

In the uterus, the interaction and communication continues through hatching and implantation. The hatching is a mutual process, where the embryo is active with collapses and re-expansions, the zona is thinned by secretion of trypsin from the endometrium, and small projections of specialized cellular TE through the zona induce the focal opening(s) through one of which the embryo escapes, aided by TE proteinase secretion (Kirkegaard et al., 2013). In human, an active embryo secretion seems to be essential for establishment of the pregnancy (Brosens et al., 2014).

Mechanisms of communication between embryo and recipient are also under investigation. It has been known for a longer time that an immunological reaction is initiated by the alien sperm/embryo that alert the maternal immune system, however without rejection of the gametes (Fazelli and Holt, 2016). Therefore, it must be well under control, and interferon (IFN-γ) secreted by the elongating conceptus (around day 15 in cattle to block luteal regression) is considered to be one of the most important candidates in regulating the immune response (Oestrup et al., 2011). During the last ten years a messenger system including small vesicles has appeared (miRNA, exosomes; Saadeldin et al., 2015) that is so far speculated to be one way of communication between the maternal tract and the gametes (Maillo et al., 2016b).

Taken together, it is getting more and more clear that there is a testing of process and product quality going on at several points during the early phase of the reproductive process – and that this has regulatory consequences, sometimes resulting in embryonic/fetal loss, sometimes leading to long-term effects observed in the offspring (e.g. Fleming et al., 2015).

Preparations in the embryo recipient

During the estrous cycle, the cow prepares herself for a potential pregnancy by establishing an environment supporting embryonic development (Pohler et al., 2012; Atkins et al., 2013). High levels of estradiol produced by ovarian follicles during proestrus and estrous result in increased uterine blood flow, promote uterine contractions assisting sperm transport, and affect the uterine environment increasing the chance of embryo survival, possibly by sustaining embryonic growth and the development of placenta (Madsen et al., 2015) The ovulatory follicle’s production of estradiol and the subsequent corpus luteum’s progesterone production are now also shown to be related to changes in tissue, cells and secretions in both the oviduct (Gonella-Diaz et al., 2015, 2017) and the uterus (Binelli et al., 2015). These changes are also stimulated by the conceptus (INF-γ, prostaglandins, cortisol), and the endometrium (prostaglandins, cortisol), and altogether are affecting uterine physiology and receptivity (Forde et al., 2009, 2011). The uterine preparation for pregnancy includes thickening of the endometrium and development of uterine muscles and glands, including production of uterine histotroph required for embryo survival and growth. The histotroph consists of different substances (e.g. amino acids, carbohydrates, proteins, lipids) transported into the uterine lumen by endometrial epithelial cells from the blood and as specific secretory products encoded by genes expressed in the endometrial epithelium (Bazer, 1975; Gray et al., 2001; Forde et al., 2014). Embryo development in uterine-gland knock-out sheep has been shown to be retarded from day 9-14, indicating the importance of the endometrial epithelial secretions (Gray et al., 2002).

On the molecular level, the progesterone-induced changes in gene expression in the endometrium result in up- or down-regulation of genes involved in processes such as cellular transports, cell cycle, cell growth and differentiation, lipogenesis, metabolism, cell adhesion, signal transduction, biosynthesis and immune response (Bauersachs et al., 2006; Forde et al., 2009, 2011, 2012b; Simmons et al., 2009; Binelli et al., 2015). The progesterone-induced changes in the endometrial transcriptome seem to be independent of pregnancy status up to the time of conceptus elongation and maternal recognition of pregnancy on day 15 (Forde et al., 2011), but an embryo-dependent programming of endometrial function has recently been demonstrated already from day 7 in the estrous cycle (Sponchiado et al., 2017). Also on day 7, Binelli et al. (2015) showed endometrial gene expressions in the uterine horn contralateral to an AI that illustrated the readiness and preparedness of the endometrium to receive an incoming embryo. In addition, pretransfer endometrial biopsies from heifers on day 7 of the estrous cycle revealed differences in gene expression according to pregnancy diagnosis in the following cycle after transfer of in vivo derived embryos (Salilew-Wondim et al., 2010) and in vitro produced embryos (Ponsuksili et al., 2012). Differences in endometrial gene expression have shown to be related to the chance of pregnancy in fertility-classified heifers on day 14 (Minten et al., 2013). For pregnant animals, a difference in endometrial gene expression was demonstrated on day 17 between fertile and subfertile dairy cow strains (Walker et al., 2012). This information on endometrial transcriptomic profiles express the status of uterine receptivity at least at a given time, but it is still not known how well it can characterize that animal (or strain).

As stated above, progesterone stimulates and
progesterone have shown to advance conceptus elongation (Carter et al., 2008). Several studies have investigated the effect of exogenous post-insemination progesterone treatment in heifers and cows, but results differ with regard to a potential beneficial effect on embryo development and pregnancy outcome (Sreenan and Diskin, 1983; Mann and Lamming, 1999; Yan et al., 2016).

In vitro produced embryos

In vitro produced embryos are in general less robust in establishing pregnancies compared to their in vivo counterparts. In terms of cryopreservation, in vitro produced embryos have decreased survival rates post-thawing after conventional freezing and post-warming after vitrification (Papadopoulou et al., 2002). Furthermore, there are morphological differences such as an overall lower cell number of both TE and ICM compared to in vivo derived embryos (Farin et al., 1995). The increasing use of in vitro produced embryos adds a challenge to the successful outcome of ET, and prediction of embryo quality prior to transfer could result in a major improvement of pregnancy rate.

The impact of culture conditions during IVP on bovine embryos is still not sufficiently investigated, and therefore the knowledge of how this parameter is reflected in the pregnancy establishment can be improved. It is, however, well known that culture conditions and IVP media have a high impact on embryonic gene expression and hence on embryo quality. Addition of serum to the embryo culture medium was earlier considered to cause abnormalities in vitro produced embryos (Farin et al., 1995). The increasing use of in vitro produced embryos adds a challenge to the successful outcome of ET, and prediction of embryo quality prior to transfer could result in a major improvement of pregnancy rate.

Application of quantitative genetics for selection of embryo recipients

While the heritability of fertility traits is low (0.05), OPU-IVP related traits (number of cumulus-oocyte complexes, quality of cumulus-oocyte complexes, number and proportion of cleaved embryos at day 4, and number and proportion of total and transferable embryos at day 7 of culture) have shown a heritability from 0.10 to 0.25 (Merton et al., 2009). Thus, genetic improvement could be faster for ART traits such as OPU-IVP-ET than for conventional fertility traits in dairy cattle (Kadarmideen et al., 2000). Alternative approaches have to select successfully for this type of traits, and a possibility is to use molecular genomic information in animal breeding including genomic selection (GS; Kadarmideen, 2014).

Genomic selection is based on computing genomic estimated breeding values (GEBVs) by estimating SNP effects from prediction equations (Meuwissen et al., 2001). Two major advantages of genomic selection compared with traditional selection based on pedigree and phenotype alone are: (i) it can select animals accurately early in life using their GEBVs from genomic prediction and (ii) increased accuracy of GEBVs for phenotypes that are very difficult or expensive to measure including fertility
(Hayes et al., 2013). Genomic selection has made a substantial economic impact (Kadarmideen, 2014; Suravajhala et al., 2016) increasing the genetic gain or income with 60-120% compared to traditional methods of progeny testing and performance tests in livestock (Schaeffer, 2006; Pryce and Daetwyler, 2012). Numerous genomic prediction models have been developed, which vary according to several assumptions regarding the variance of traits of interest and the distribution of the SNP effect.

The principles behind genomic selection of recipient cows is the same as for any traditional phenotype in cattle breeding. Genomic prediction accuracy gets better with increasing trait heritability and reference population used for calculating GEBVs. Before applying any quantitative genetics or breeding method, the high quality reproductive data recording traits will be an essential step. Thus, it is important to set up a reference population where a large number of recipient cattle are recorded for pregnancy rates after OPU-IVP-ET. Once a good reference population is collected, the Best Linear Unbiased Prediction (BLUP) methods (e.g. GBLUP and single-step BLUP) (Henderson, 1975; Meuwissen et al., 2001; Aguilar et al., 2010; Goddard et al., 2011) can produce GEBVs for all these animals. Based on the ranking of GEBVs, breeding animals can then be selected and used in OPU-IVP-ET, increasing the recipient cattle reproductive performance, i.e. pregnancy success.

In this context, integrative systems biology could provide useful information for GS. IVP and ET performances are complex traits, so more holistic approaches are needed to identify biological mechanisms and biomarkers associated with these traits. Systems biology approaches identify the emerging properties of a biological system (Kitano, 2002; Breitling, 2010). Therefore, systems biology represents a promising tool for OPU-IVP-ET related traits. The function of the endometrium is important to the chance of embryo implantation in recipient cows. Therefore, transcriptomic of endometrial tissue can be used to perform systems biology analysis of recipient cow performances (Orozco-Lucero and Sirard, 2014). The biological mechanisms and the molecular markers identified through the systems biology analysis of transcriptomic data could be integrated in multi-omics analysis, for example eQTL studies. The eQTL studies integrate transcriptomic with genomic data to identify genomic regions controlling the expression of a certain gene (Westra and Franke, 2014). If the expression of the genes is associated to the trait of interest, the eQTL identified can be indirectly associated with the traits (Ponsuksil et al., 2010). The information provided by integrative systems biology studies, for example eQTLs, could be included in GS methods utilizing functional information.

The understanding of the biological basis of the molecular regulation of the complex reproductive events is improving significantly these years. One main reason is the fruitful interaction between the biological and molecular sciences that form a very strong platform, and the combined action “can provide a strong continuation to the understanding of traits related to ARTS” (Mazzoni et al., 2017).

Conclusions and perspectives

The establishment of a pregnancy in cattle includes interactions between the embryo and the mother at all stages of the pregnancy. Timed biological events and communication take place to maintain and accomplish the pregnancy and to reach the final goal, i.e. the birth of a healthy live offspring. The embryo and the endometrium handle and adapt to different challenges and conditions, based on signaling from both sides and influenced by e.g. the use of reproductive technologies and the origin of the embryo. Also, many factors affect uterine receptivity and finally, the synchrony between the embryo and recipient is important. Therefore, to improve in vitro embryo production conditions and to increase the output from OPU-IVP-ET, it is highly relevant to continue the research into the complex biological mechanisms, but also to further investigate and develop methods based on a systems biology approach. One ultimate goal for this combined action will be to obtain a tool to improve selection of recipients for transfer of in vitro produced embryos.

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