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Review Article

Maternal Recognition of Pregnancy in the Context of Equine Embryo Transfer

Claudia Klein*

Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada

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ABSTRACT

Maternal recognition of pregnancy refers to the sequence of events by which embryo-derived signals prolong luteal function, ultimately assuring ongoing progesterone secretion by the corpus luteum beyond its normal lifespan of the estrous cycle. In this overview, key aspects of maternal recognition of pregnancy in the mare are reviewed and discussed in the context of equine embryo transfer. In mares, the uterine tube has a species-specific, well-developed tunica muscularis which necessitates production of prostaglandin E2 by the equine embryo to facilitate transport to and passage through the uterotubal junction. After entry into the uterus, an acellular glycoprotein capsule forms beneath the zona pellucida, eventually replacing the zona pellucida and surrounding the embryo until the third week of pregnancy. Embryonic mobility is a key aspect of early pregnancy in the mare and is driven by embryo-derived prostaglandins that stimulate uterine contractility. Endometrial prostaglandin production is temporarily attenuated during early pregnancy. After formation of the chorionic girdle and invasion of chorionic girdle cells into the endometrium, endometrial cups are formed resulting in the production of equine chorionic gonadotropin. Equine chorionic gonadotropin is instrumental in the formation of accessory corpora lutea, whose progesterone production is essential in maintaining pregnancy until the placenta produces sufficient amounts of progestogens.

1. Introduction

Maternal recognition of pregnancy (MRP) is a phrase coined by Short [1] almost five decades ago. It refers to the sequence of events by which embryo-derived signals prolong luteal function, ultimately assuring ongoing progesterone secretion by the corpus luteum (CL) beyond its normal lifespan of the estrous cycle. In the presence of an embryo, lifespan of the primary CL is extended in the mare, making it the sole source of progesterone for the first 40 days of pregnancy. At around 40 days of pregnancy, newly formed accessory corpora lutea contribute to progesterone secretion, sustaining high levels thereof until approximately day 100 of gestation, at which time the developing placenta takes over and secretes progestogens [2]. The continuum of events leading to maintenance of gestation after successful fertilization and descent of the developing conceptus into the uterus are species specific; it appears each species has adopted a different pathway to prevent demise of the primary CL. For example, in ruminants, conceptus-derived interferon tau [3], and in pigs, estrogens are critical for establishing pregnancy [4]. Maternal recognition of pregnancy, and embryo–maternal communication in a wider sense, is a reciprocal process involving both the embryo and the uterine environment. Among other things, this is reflected in low pregnancy rates when transferring embryos from aged donor mares to young recipient mares [5]. In the following, key aspects of MRP in the mare will be reviewed and, when possible, discussed in the context of equine embryo transfer.

* Corresponding author at: Claudia Klein, Faculty of Veterinary Medicine, University of Calgary, 3280 Hospital Dr NW, Calgary, AB T2N 4Z6, Canada.
E-mail address: cklei@ucalgary.ca.

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2. Embryo–Maternal Communication During the Oviductal Period

Considering the high success rate of equine embryo transfer, during which the embryo is transferred into the uterus of a recipient mare, the oviductal period of embryo development appears not to be essential to establishment and maintenance of pregnancy. Nevertheless, embryo–maternal communication takes place during this early phase of pregnancy and has implications for equine embryo transfer.

2.1. Early Pregnancy Factor

It was first noted in the mid 1970s that lymphocytes retrieved from mice shortly after mating displayed altered reactivity during rosette inhibition testing [6]. During rosette inhibition testing, lymphocytes are incubated with heterologous erythrocytes and the formation of rosettes, that is, the arrangement of erythrocytes around a central lymphocyte, is observed. The inhibition of rosette formation observed in females during early pregnancy is attributed to the presence of an immunosuppressive protein, the "early pregnancy factor" (EPF). Early pregnancy factor is undoubtedly the earliest sign of the developing embryo impacting the maternal environment, and its presence has been detected in a wide range of species including the horse [6,7]. It was not until 20 years after EPFs initial discovery that its identity was confirmed as chaperonin 10, also known as heat-shock protein 10 [8]. Heat-shock protein 10 forms a heterodimer with heat-shock protein 60, and in concert, this heterodimer facilitates protein folding, a classic heat-shock protein attribute [9]. Both the endometrium and embryo express heat-shock protein 10 and heat-shock protein 60 [10–12]. The rosette inhibition test as a means to detect the EPF has been proven useful to detect pregnancy in both Pony and Thoroughbred mares [13,14]. Early pregnancy factor activity in blood can be detected as early as 24 hours after mating and remains present until the second trimester of gestation [14]. Takagi et al [15] assessed EPF activity in blood within the context of equine embryo transfer. In donor mares, EPF activity rose to pregnant levels within 2 days after ovulation and declined to nonpregnant levels within 3 days after the flush of the embryo. In recipient mares that were detected pregnant on ultrasonography 12 to 14 days after embryo transfer, EPF activity rose to pregnant levels within 3 days after embryo transfer. In 3 of 4 mares that were diagnosed nonpregnant on ultrasonography, a temporary rise in EPF activity above the nonpregnant threshold was observed, whereas in the fourth mare, levels remained low. These data, albeit a low number of mares were enrolled in the study, indicate monitoring EPF activity could be a useful tool within a commercial embryo transfer program. Major drawback, however, is the time consuming and labor intensive nature of the rosette inhibition test which has thus far prevented the routine use of measuring EPF activity levels when managing embryo donor and recipient mares. In light of this, a commercially available "stall-side" test designed to detect EPF in the serum of mares has been evaluated (early conception factor lateral flow test; Concepto Diagnostics, Knoxville, TN). The general consensus is, however, that this test is not a reliable tool to confirm pregnancy or nonpregnancy [16–18]. To the knowledge of the author, this test is no longer commercially available. In pigs, an enzyme-linked immunosorbent assay (ELISA) has been tested for early pregnancy diagnosis; sensitivity and specificity, however, were depended on the dilution ratios of the serum samples, which puts the validity and reliability of the test in question [19]. In summary, while at a first glance, EPF activity appears to be ideally suited to manage donor mares in an embryo transfer program, difficulty in assessing EPF in a time efficient, and high throughput manner has thus far prevented clinical use of this factor.

2.2. Preimplantation Factor

The preimplantation factor (PIF) is an embryo-specific 15 amino acid peptide (MVRIKPGSANKPSDD) secreted by viable embryos in a number of mammalian species, including human [20], bovine [21], and murine [21]. Exogenous PIF promotes embryo development in vitro [21,22]. The preimplantation factor targets and interacts with proteins known to play a role in protecting cells against protein misfolding and oxidative stress [23], revealing the basis for the autotrophic and protective role of PIF during embryo development. The PIF is intriguing, as to date, the corresponding gene resulting in its transcription and translation has not been identified. Although its expression by equine embryos has not been examined, PIF has been shown to bind to, and presumable be up-taken, by equine blastocysts [23]. Unlike EPF, PIF can be detected in the systemic circulation of cattle during early pregnancy using an ELISA, with high specificity and sensitivity by day 20 after ovulation [22].

2.3. Selective Transport of Embryos Through the Uterine Tube

The selective transport of embryos through the uterotubal junction, with unfertilized oocytes remaining within the uterine tube, is a remarkable reflection of embryo–maternal communication in the mare [24–28]. Coinciding with this selective transport of embryos, the embryo secretes increasing amounts of prostaglandin E2 (PGE2) [29]. Specific binding of PGE2 to the uterine tube between 2 and 5 days after ovulation has been confirmed, indicating that the uterine tube has the capacity to respond to embryo-derived PGE2 during the tubal stage of embryonic development [30]. Indeed, continuous infusion of PGE2 into the uterine tube significantly hastens the transport of embryos, so that embryos can be recovered from the uterus as early as 4 days after ovulation; in addition, unfertilized oocytes and oviductal masses gain access to the uterus [31]. The specific time the embryo enters the uterus dictates the earliest time point a uterine flush can be carried out; 6, 6.5, and 7 days after ovulation, 0/20 (0%), 9/17 (53%), and 12/23 (52%) embryos, respectively, were retrieved [32], indicating the earliest time a uterine flush should be attempted is 6.5 days after ovulation.

In recent years, the uterine tube has gained increasing attention as a cause for infertility. Laparoscopic application of PGE2 onto the serosal surface of the uterine tube surface