Getting Cows Pregnant and Keeping Them Pregnant Requires Progesterone

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Abstract

Early embryonic mortality in sheep and cattle averages 38%, with the majority of losses occurring prior to 30 days post-conception. This loss is primarily due to two factors: 1) genetic abnormalities in the embryo (7 to 10%), and 2) inadequate secretion of progesterone at critical times (approximately 30%). Supplementation of progesterone in cattle increased pregnancy rates by 13 to 30%. Thus, the requirement for continued secretion of progesterone has been clearly documented. Therefore, it is important to understand the complex hormonal interactions between the developing embryo, uterus, pituitary gland and ovary that regulate the secretion of progesterone from the corpus luteum. The mechanisms whereby the pregnant uterus prevents regression of the corpus luteum, which occurs at the end of a normal reproductive cycle, are complex and sensitive to a number of environmental and management factors. These factors include heat stress, level of nutrition, genetics and general herd health. Any biological process which retards the growth of the embryo may result in early embryonic mortality due to a lack of synchrony between the embryo and the uterus. The basic physiological mechanisms involved in establishing and maintaining pregnancy are presented and discussed.

Introduction

Successful reproduction in domestic ruminants begins with development of a follicle, which ovulates and becomes a corpus luteum. The corpus luteum secretes the sex steroid hormone, progesterone, which regulates the uterine processes necessary for establishment of pregnancy. If the female does not become pregnant, the corpus luteum stops secreting progesterone and undergoes luteolysis. This allows a new follicle to develop and ovulate and another chance for pregnancy to occur. The complicated physiological processes whereby the uterus signals the corpus luteum that pregnancy has occurred are collectively termed maternal recognition of pregnancy. When the pregnant uterus fails to provide the proper signal(s), the corpus luteum regresses and the embryo is lost.

Early embryonic mortality in sheep and cattle averages 38%[25,36,40] with the majority of losses occurring prior to 30 days post-conception[8,20,30]. A simple calculation puts this problem in perspective for the beef industry. There are approximately 30 million beef cows in the United States x an average of 35% embryonic wastage (first cycle) x a loss of 1.5 lb (0.68 kg) day weaning weight x a 21-day estrous cycle x $1.00/per pound (mean for last year) = 330 million lb (150 million kg) of calf and $330 million lost in the first cycle. Of the cows that rebreed, 35% will again suffer embryonic loss in the second cycle, which equals an additional loss of 115 million pounds and $115 million. This phenomenon continues to repeat itself during subsequent non-fertile cycles, with over $450 million in potential revenue lost annually. In dairy cattle, loss of milk production due to failure to maintain pregnancy makes the problem even more dramatic on a per-head basis. It is not unusual to have pregnancy rates at first service of 30 to 40% in dairy cattle[23]. The National Health Monitoring System reported that infertility in cattle cost producers $382 million in 1996[5]. The problem has a relative economic impact in the sheep industry with losses totaling nearly $7 million annually. The mechanisms involved in regulating luteolysis and maternal recognition of pregnancy in sheep and cattle appear to be virtually identical.

Embryonic Wastage

Early embryonic loss is due primarily to two factors: 1) genetic abnormalities in the embryo, and 2) inadequate secretion of progesterone at critical times. The relative importance of these factors appears to vary depending upon a number of management factors including breed, climate and nutrition[25,36,40]. Based on several studies, embryonic wastage in cattle is estimated to be 38% (range 6 to 64%), while in nine studies it was concluded that there were 7.2 to 10.4% genetically abnormal embryos[40]. Roche[31] reported that up to 40% of bovine embryos are lost by day 25 of pregnancy and that "It is reasonable to conclude that most females become pregnant but may lose the embryo early in pregnancy" and proposed that spontaneous regression of the corpus luteum was involved. Thus, it seems logical to conclude

that at least 25% of bovine embryos are lost due to inadequate secretion of progesterone. In sheep, embryonic wastage ranges from 6 to 48%, with an average estimated at 30%.

As reviewed in Zavy[40], progesterone supplementation has been used to decrease embryonic mortality due to progesterone insufficiency. The increased rates of pregnancy in cattle ranged from 13 to 30%. In ewes, progesterone supplementation increased pregnancy rates from 0[7] to 28%.[28] In experiments where management is optimal and pregnancy rates in control animals are already high (approximately 80%), additional progesterone has no beneficial effect.[7] However, optimal management is not economically feasible or even possible under applied conditions such as those which exist on most ranches, and progesterone insufficiency remains a major cause of embryonic wastage.

Inadequate secretion of progesterone from the corpus luteum during early pregnancy may be due to a number of factors, including failure of the embryo to adequately inhibit uterine secretion of $PGF_2\alpha$[12] or to prevent its luteolytic actions.[26] Other factors such as stress[11] and nutrition,[28] can also influence synthesis and secretion of progesterone. More detailed information is needed regarding the molecular mechanisms involved in progesterone biosynthesis to understand how these factors exert their effects.

Biosynthesis of Progesterone

The general pathway for biosynthesis of progesterone in a luteal cell is depicted in Figure 1. Under normal conditions, cholesterol present in lipoproteins in blood is the precursor for all steroid biosynthesis. Once inside the cell, the rate-limiting step in progesterone biosynthesis is the transport of cholesterol to the inner mitochondrial membrane,[3,6,16,38] which also appears to be the primary site of acute hormonal regulation[3,13,38] of this process. To date, a minimum of three essential proteins have been identified that are involved in the transport of cholesterol from the outer to the inner mitochondrial membrane. These include steroidogenic acute regulatory (StAR) protein, peripheral type benzodiazepine receptors (PBR) and endosepine, the natural ligand for PBR.

Control of Progesterone Biosynthesis in Domestic Ruminants

Unfortunately control of progesterone synthesis in the corpus luteum is not as straightforward as suggested in Figure 1. There are two morphologically and biochemically distinct steroidogenic cell types in the corpus luteum of cattle and sheep, as well as in most other mammalian species.[27] In the ewe, small luteal cells are

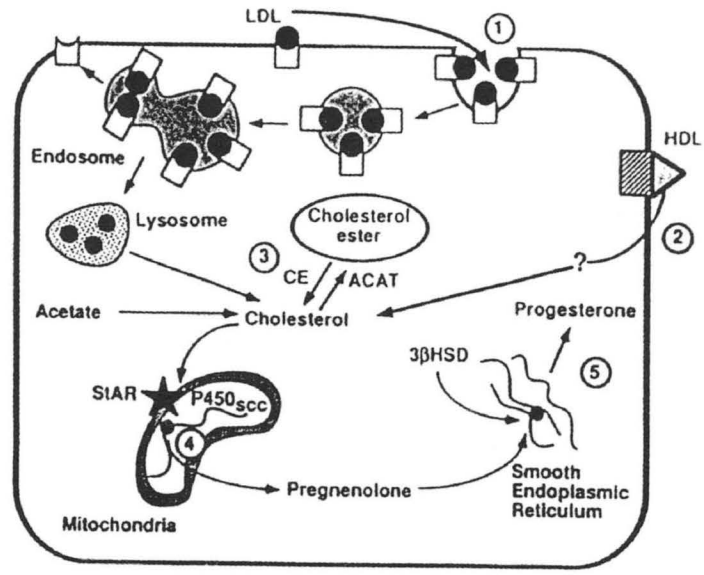

Figure 1. Pathway for progesterone biosynthesis in a generic luteal cell. Three sources of cholesterol can be utilized for substrate: 1) low density lipoprotein (LDL), 2) high density lipoprotein (HDL), or 3) hydrolysis of stored cholesterol esters by cholesterol esterase (CE). The free cholesterol is transported to the mitochondria with involvement of cytoskeletal elements[17] and StAR. Cholesterol is then transported from the outer to inner mitochondrial membrane and this process appears to involve StAR, peripheral type benzodiazepine receptors and endosepine. This transport process is the rate-limiting step, as well as the site of acute positive and negative regulation of steroidogenesis by hormones[16,38]. Cholesterol is converted to pregnenolone by cytochrome P450scc[3], transported out of the mitochondria and converted to progesterone by 3β-hydroxysteroid dehydrogenase, Δ^5, Δ^4 isomerase (3β-HSD)[21] which is present in the smooth endoplasmic reticulum. Progesterone appears to diffuse from the luteal cell.

12 to 20 μm in diameter, thought to be of follicular thecal cell origin, contain receptors for luteinizing hormone (LH), respond to LH or cyclic adenosine monophosphate (cAMP) with a 5- to 15-fold increase in secretion of progesterone, and often contain numerous lipid droplets.[36] Large luteal cells (>20 μm) are primarily of granulosal cell origin, secrete high basal levels of progesterone, and although LH receptors are present, do not respond to LH or cAMP with increased secretion of progesterone.[27] Large cells contain receptors for prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and respond to this hormone with activation of the protein kinase C second messenger pathway which decreases the secretion of progesterone. Binding of $PGF_2\alpha$ to its receptor also increases intracellular levels of free calcium,[26] which appear to induce apoptosis and luteal cell death (Figure 2).

The step in progesterone secretion that is acutely stimulated by LH in luteal cells appears to be transport of cholesterol to the inner mitochondrial membrane[3,38]. This effect of LH is mediated by the protein kinase A (PKA) second messenger pathway, and appears to be due to enhanced cholesterol transport following PKA phosphorylation of StAR. In humans, mutations in the gene encoding StAR are responsible for reduced steroid production (congenital lipid adrenal hypoplasia) and, if left untreated, death[25]. There are also long-term effects of LH on luteal steroidogenesis. Stimulation by LH is critical for the long-term steroidogenic capability of luteal cells, including maintenance of the steroidogenic pathway, including 3β-hydroxysteroid dehydrogenase/ Δ^5, Δ^4 isomerase (3β-HSD), cytochrome P450 side-chain cleavage enzyme (P450scc) and StAR.[18,19] Growth hormone (GH) also increases concentrations of progesterone in sera[33] and is necessary for normal luteal development.[19]

Prostaglandin $F_2\alpha$ causes a dramatic decrease in secretion of progesterone from the ovine corpus luteum *in vivo* and from large luteal cells *in vitro*. The antisteroidogenic effects of $PGF_2\alpha$ are mediated via activation of the PKC second messenger system.[26] The primary mechanism involved in acutely decreased progesterone synthesis following $PGF_2\alpha$ treatment appears to be decreased transport of cholesterol to the inner mitochondrial membrane.[3,13,38] Treatment with $PGF_2\alpha$ also has long-term molecular effects including decreased luteal concentrations of mRNA encoding LH receptor,[14] low density lipoprotein (LDL) receptor,[35] StAR,[10,18] and 3b-HSD,[22] while mRNA encoding high density lipoprotein (HDL) receptor[35] and P450scc[22] are not dramatically altered. Thus, both luteotropic and luteolytic hormones appear to regulate secretion of progesterone acutely by affecting transfer of cholesterol to the inner mitochondrial membrane and more long-term by regulating levels of mRNA species which encode for important receptors, transport proteins and steroidogenic enzymes required for regulation of luteal cell function. The effects of luteotropic and luteolytic stimuli are clearly different in the two steroidogenic cell types.

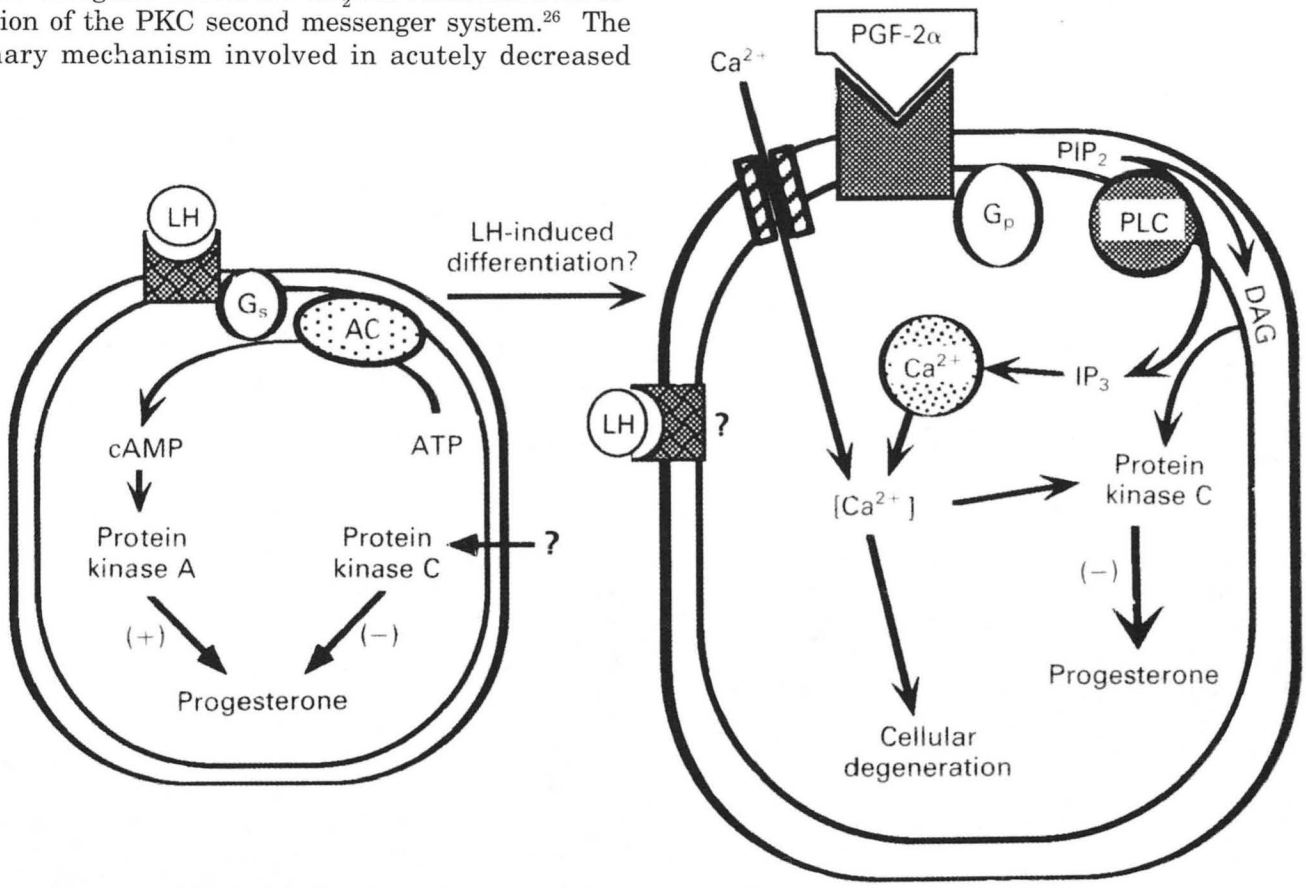

Figure 2. Current working model for the second messenger pathways involved in regulating small (left side) and large (right) luteal cells. See summary in text for details. Abbreviations are: luteinizing hormone (LH), G-protein causing stimulation of adenylate cyclase (Gs), adenylate cyclase (AC), adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP), prostaglandin $F_2\alpha$ ($PGF_2\alpha$), free calcium concentration ($[CA^{2+}]$), phosphatidylinositol 4,5-bisphosphate (PIP_2), inositol 1,4,5-trisphosphate (IP_3), diacylglycerol (DAG).

Luteolysis

The intracellular effects of $PGF_2\alpha$ that cause luteal cell death are mediated by increased intracellular levels of calcium ion. The increased calcium induces specific enzymes, which cause apoptotic cell death[39]. Thus, the negative effects of $PGF_2\alpha$ on luteal function are due to activation of two separate, second-messenger pathways. Activation of the PKC pathway reduces the secretion of progesterone and increases intracellular levels of calcium, which results in cell death.

The $PGF_2\alpha$ which causes luteolysis has been thought to be of uterine origin. This is due to the facts that: 1) normal luteolysis does not occur in hysterectomized ewes or heifers; 2) endometrial tissue from ewes or heifers during the late luteal phase of the estrous cycle synthesizes and secretes $PGF_2\alpha$, and 3) oxytocin stimulates the release of $PGF_2\alpha$ from the endometrium under appropriate conditions. However, $PGF_2\alpha$ levels do not reach maximal levels until circulating concentrations of progesterone are already decreased by at least 50%. Therefore, we hypothesized that $PGF_2\alpha$ from the uterus causes the decreased serum concentrations of progesterone through activation of PKC which also activates COX-2, which is a key enzyme in prostaglandin biosynthesis in luteal cells. This results in very high levels of $PGF_2\alpha$ being produced by the corpus luteum itself, which ultimately causes cell death. To test this concept, a biodegradable implant which contained an inhibitor of $PGF_2\alpha$ synthesis was placed directly into the corpus luteum. Local inhibition of $PGF_2\alpha$ synthesis prevented normal luteolysis in ewes and provides evidence supporting our hypothesis. Thus, it appears that uterine secretion of $PGF_2\alpha$ starts the processes involved in luteolysis but intraluteal, synthesis of $PGF_2\alpha$ is required for cell death.

Maternal Recognition of Pregnancy

If the cow or ewe becomes pregnant, the trophectoderm of the conceptus secretes a protein, interferon-tau, which prevents uterine secretion of $PGF_2\alpha$ and luteolysis. The cellular mechanisms involved are complicated, but it appears that interferon-tau prevents synthesis of receptors for estradiol in the endometrium, which prevents estradiol from stimulating an increase in receptors for oxytocin. Therefore, during early gestation, oxytocin is unable to stimulate uterine secretion of $PGF_2\alpha$, the corpus luteum is maintained and secretion of progesterone and pregnancy continues.[1]

Once the embryo has survived the critical period of maternal recognition of pregnancy, continued secretion of progesterone from the corpus luteum is required for varying times, depending upon the species. In sheep, the corpus luteum can be removed on day 50 of pregnancy and pregnancy will continue.[4] In cattle, luteal progesterone is required for 200 days of pregnancy.[9] In both ewes and cows that have been ovariectomized, pregnancy can be maintained with progesterone supplementation.[2,24,34]

Progesterone Therapy

Since progesterone has a relatively short half-life in blood (approximately two hours)[15], it is difficult and quite expensive to provide long-term supplementation of this hormone. Although LH stimulates the secretion of progesterone, its half-life in blood is approximately 0.5 hours. Therefore, numerous studies have been performed using human chorionic gonadotropin (hCG), which mimics the actions of LH but has a half-life in cows of approximately 2 days. Injections of hCG have been given to cows 12 to 14 days post ovulation to increase serum concentrations of progesterone, particularly through the time of maternal recognition of pregnancy. The results of these studies have been quite variable, and conclusions controversial[23,29,32]. About half of the studies report increased pregnancy rates after hCG treatment and half found no effect. These results are similar to those obtained when progesterone therapy was used to increase pregnancy rates in ewes and cows. It appears that treatment with hCG had little effect in situations where pregnancy rates in controls were already high[32], but was beneficial if pregnancy rates in untreated animals were relatively low. Part of this variability in response is due to the various conditions under which the experiments were performed. For example, apparently small management differences, such as the acute feeding regime, appears to have an effect on circulating levels of progesterone in lactating dairy cows.[37]

Conclusions

Normal pregnancy during early gestation in all domestic ruminants requires continued secretion of progesterone from the corpus luteum. If the female does not become pregnant, $PGF_2\alpha$ causes decreased secretion of progesterone and luteal cell death. If the animal becomes pregnant, the developing conceptus secretes interferon-tau which blocks uterine secretion of $PGF_2\alpha$ and allows continued secretion of progesterone and pregnancy to continue. Management factors such as heat, cold, under-nutrition, over-nutrition and infectious diseases can influence the rate of embryo development, and/or directly influence secretion of progesterone. In these cases, uterine function and embryo development become asynchronous, and early embryonic wastage occurs. Thus, good management practices are important to ensure maintenance of pregnancy and prevent early embryonic loss.

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