Anim. Reprod., v.7, n.2, p.75-79, Apr./Jun. 2010

Effects of flunixin meglumine on reproductive parameters in beef cattle

E. Lucacin[1], A. Pinto-Neto[1,5], M.F. Mota[2], A. Acco[3], M.I.L. Souza[4], J. Alberton[1], A.V. Silva[1]

[1]Animal Science, Paranaense University (UNIPAR), Umuarama, PR, Brazil.
[2]Veterinary Medicine, Paranaense University (UNIPAR), Umuarama, PR, Brazil.
[3]Pharmacology, Federal University of Paraná (UFPR), Curitiba, PR, Brazil.
[4]Physiology, Federal University of Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil.

Abstract

Flunixin meglumine (FM) has been used as an attempt to inhibit luteolysis in ruminants. The effects of FM on seric concentration of progesterone, pregnancy rate and ovarian status of cows were assessed in this study. Fifty-seven cows were divided into Control Group (CG; n = 30) and Treated Group (TG; n = 27) on an estrus synchronization program involving intramuscular (IM) estradiol benzoate (EB) and intravaginal progesterone-releasing insert. After seven days the intravaginal insert was removed, prostaglandin-F2alpha (PGF2α) IM was given, and then EB was administered 24 h later. Fixed-time artificial insemination (FTAI) was conducted 30 h after EB administration. Animals from the TG received 1.1 mg/kg of FM IM daily between the days 11 and 16 of the estrous cycle (day 0 = FTAI), whereas the animals from the CG received physiological solution. Blood from all animals was collected on days 0, 6, 9, 11 through 18, and 21. Thirty animals from the Nellore breed were selected for the measurement of serum concentrations of progesterone by radioimmunoassay. Animals which returned to estrus were inseminated again (artificial insemination; AI) and allocated back to their groups. Pregnancy diagnosis was conducted by transrectal ultrasound in either FTAI or AI cows after 30 days. The ovarian condition of the non-pregnant animals was reevaluated 4 days later. Progesterone concentration among the animals from the experimental groups was similar until the day 18 of the estrous cycle ($P > 0.05$). On day 21, pregnant animals presented higher progesterone concentrations ($P < 0.05$) than non-pregnant animals from the TG and TN. Pregnancy rate was similar among the groups ($P > 0.05$). However, non-pregnant animals from the TG presented higher ($P > 0.05$) follicular persistence than that observed for the animals in the CG, 78.6% (11/14) vs. 33.3% (5/15), respectively ($P = 0.025$). Results indicate that FM administered during the luteolysis period of beef cattle does not influence progesterone concentration and pregnancy rate, although it influences the occurrence of follicular persistence.

Keywords: bovine, luteolysis, NSAIDs, pregnancy, progesterone.

Introduction

In sexually mature cows, two events may be physiologically expected after the ovulation of the dominant follicle: new reproductive cycle and new ovulation between 17 and 23 days later when there is neither copulation nor pregnancy beginning after conception. In the latter, pregnancy success depends on a number of factors related to genetics, sanity, physiology, and reproductive management of cattle.

Maternal recognition of pregnancy (MRP) occurs especially between days 15 and 19 after ovulation. This is defined as a combination of events performed by mother and conceptus aiming the normal establishing of pregnancy mainly by keeping the corpus luteum producing high progesterone concentrations (Thatcher *et al*., 2001).

The failure of MRP induced luteolysis, promoted by prostaglandin F2α (PGF2α), with consequent progesterone concentration decrease and beginning of a new reproductive cycle (Thatcher *et al*., 1997; Niswender, 2000; Thatcher *et al*., 2001).

In order to increase bovine reproductive efficiency, several anti-luteolytic strategies involving the specific inhibition of the enzymes participating in the PGF2α synthesis such as phospholipase C, protein kinase C, phospholipase A2, and COX (constitutive COX-1 and induced COX-2) have been researched (Binelli *et al*., 2005). The COX, which converts arachidonic acid into prostaglandin-H2, further converted into PGF2α through prostaglandin-F-synthase (Burns *et al*., 1997), are the primary targets of nonsteroid anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, and flunixin meglumine (FM).

The administration of ibuprofen lysinate, a COX-1 and COX-2 inhibitor by day 17 of the estrous cycle (estrus = day 0) in cattle resulted in higher pregnancy rates for treated animals (Elli *et al*., 2001). In another study, the use of aspirin (acetylsalicylic acid: COX-2 inhibitor) on day 7 of the estrous cycle induced a significant pregnancy rate increase (Pugh *et al*., 2004). These authors used FM in therapeutic doses and found pregnancy rate to be lower than while using aspirin, but higher than that for non-treated animals.

The administration of FM as an antiluteolytic agent during the critical period is well established for

[5]Corresponding author: adalgiza@unipar.br
Phone: +55(44)3055-2247; Fax: +55(44)3621-2550
Received: April 8, 2009
Accepted: August 20, 2010

goats as an attempt to prevent early regression of the corpus luteum, mainly associated with multiple ovulation and embryo transfer programs (Soares *et al*., 1998). Salles *et al.* (1998) suggest a 1.1 mg/kg dose of FM, daily, as an antiluteolytic agent for goats.

Based on these reports, this study aimed at assessing the effects of FM on the following reproductive parameters: progesterone concentration, pregnancy rate, and follicular persistence of cows submitted to FTAI.

Materials and Methods

This study was performed at Fazenda e Haras Simone, located in the city of Moreira Sales, Northeastern Paraná, Brazil, from December 2006 to March 2007.

Cows (n = 57) from different breeds (2 Blond D'Aquitaine, 4 Gir, 1 Guzerá, 8 Holstein, 6 Jersey, 6 Limousin, and 30 Nellore) were kept in *Brachiaria brizantha* pasture, with mineral supplement and access to water *ad libitum*, and homogeneously assigned into two groups: control group (CG) and treated group (TG), containing 30 and 27 animals, respectively.

Apart from the treatment, all animals were submitted to the estrus synchronization protocol which started with the intramuscular (IM) administration of 1 mg of estradiol benzoate (BE: Estrogin®, FarmaVet, São Paulo) and the intravaginal implant of a device containing 1.9 g of progesterone (CIDR®, Pfizer, São Paulo).

Seven days after the beginning of the synchronization, 300 μg of $PGF_2\alpha$ was administered and the intravaginal device was removed. Twenty-four hours after implant removal, 1 mg of EB was intramuscularly administered, and the FTAI was carried out 30 h later.

The animals from the TG were submitted to daily administration of 1.1 mg/kg of flunixin meglumine (FM; Banamine, Schering-Plough Laboratories, São Paulo) between days 11 and 16 of the estrous cycle (day 0 = FTAI day), whereas the animals from the CG received the equivalent volume of physiological saline solution.

Blood samples were collected from all animals by puncture of the caudal artery blood on days 0, 6, 11-18, and 21 at 7:00 am. The blood collected was chilled, centrifuged, aliquoted, and frozen for further seric progesterone analysis through solid phase radioimmunoassay (CAC solid phase – DPC-MedLab) in the Laboratório de Endocrinologia da FMVZ-UNESP in Botucatu, SP. Nellore animals (n = 32) were homogeneously selected from the samples (considering pregnancy or non-pregnancy and treatment or non-treatment).

The animals which returned to estrus (n = 14) after FTAI were re-inseminated (AI) and reassigned into their original experimental groups. The pregnancy diagnosis was performed by transrectal ultrasonography 30 days after FTAI and AI and was confirmed by the visualization of fetal heart beat. On the day of pregnancy diagnosis, the ovarian status of non-pregnant animals was assessed through ultrasonography for the presence of follicle > 12 mm, which was repeated after 4 days, when the follicle was considered persistent once detected in the first assessment presenting neither atresia nor ovulation.

The results were analyzed by using the InStat program in which the progesterone concentration was submitted to ANOVA for comparison of the groups (control and treated, pregnant and non-pregnant), by Tukey´s Multiple Range Test, the pregnancy rate by Mantel-Haenszel chi-square test, and the follicular size and persistence by Fischer´s Exact Test.

Results

In order to assess the seric progesterone concentrations, two non-pregnant animals were discarded as they did not present active corpus luteum (one from the treated group and another from the control group), and one non-pregnant animal from the control group and three pregnant (one from the control group and two from the treated group) as it overcomes the parameter for correction used (means ± 2 x SD).

Table 1 presents the mean (±SD) of the progesterone concentration among the animals from the control group (pregnant-CG and non-pregnant-CN), and treated group (pregnant-TG and non-pregnant-TN) with FM. Fig. 1 illustrates the progesterone profile presented by these animals.

The analysis of the progesterone concentration among the animals from the control (C) and the treated (T) with FM, pregnant (P), and non-pregnant (N) was similar ($P > 0.05$) until day 18 of the estrous cycle. On day 21, the pregnant animals presented progesterone concentrations higher ($P < 0.05$) than those observed for non-pregnant animals from the control and treated groups (CN and TN). However, neither did the pregnant animals from the control group (CG) differ from the pregnant animals treated (TG) nor from the non-pregnant animals from both groups (CN and TN; Table 1).

While assessing the ovarian status of the non-pregnant animals, it was observed that the animals from the treated group had higher ($P < 0.05$) follicular persistence than that observed for the control animals, 78.6% (11/14) and 33.3% (5/15), respectively.

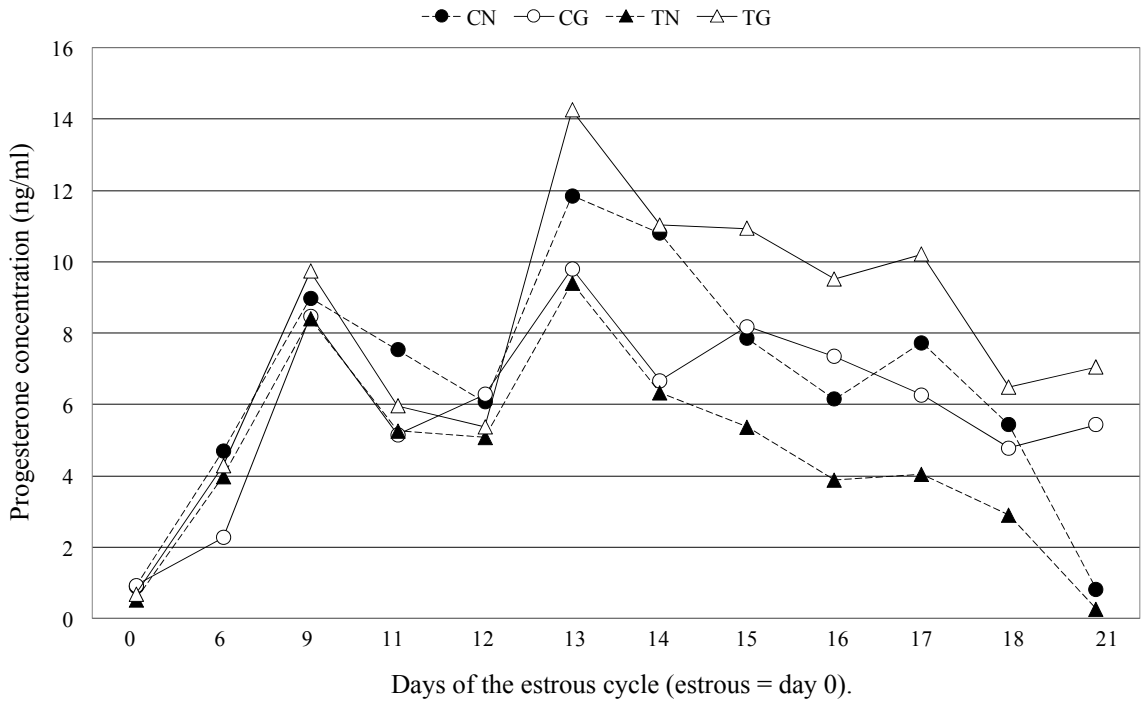

Figure 1. Mean progesterone concentration profiles (ng/ml) for pregnant (CG) and non-pregnant (CN) control cows and pregnant (TG) and non-pregnant (TN) treated cows with flunixin meglumine during days 11 and 16 of the estrous cycle.

Table 1. Mean (±SD) progesterone concentration (ng/ml) for cows in the control group [pregnant (CG) and non-pregnant (CN)] and treated group [pregnant (TG) and non-pregnant (TN)] with flunixin meglumine during days 11 and 16 of the estrous cycle.

Days of the estrous cycle	Experimental groups			
	CG (n = 6)	CN (n = 7)	TG (n= 7)	TN (n = 4)
0	0.9 ± 0.9	0.9 ± 0.8	0.7 ± 0.4	0.5 ± 0.2
6	2.3 ± 1.5	4.7 ± 3.1	4.3 ± 2.2	4.0 ± 0.7
9	8.5 ± 2.0	9.0 ± 2.4	9.7 ± 2.8	8.4 ± 3.7
11	5.2 ± 3.6	7.5 ± 3.7	6.0 ± 3.9	5.3 ± 0.9
12	6.3 ± 4.4	6.1 ± 3.2	5.4 ± 3.1	5.1 ± 1.5
13	9.8 ± 6.0	11.9 ± 6.4	14.3 ± 4.7	9.4 ± 3.1
14	6.7 ± 2.7	10.8 ± 6.5	11.0 ± 5.4	6.3 ± 1.5
15	8.2 ± 5.0	7.8 ± 3.7	10.9 ± 6.5	5.4 ± 2.8
16	7.4 ± 5.0	6.2 ± 2.6	9.5 ± 6.6	3.9 ± 4.1
17	6.3 ± 3.6	7.7 ± 3.5	10.2 ± 7.0	4.0 ± 5.0
18	4.8 ± 3.9	5.4 ± 1.8	6.5 ± 3.6	2.9 ± 4.6
21	5.4 ± 3.9[ab]	0.8 ± 0.6[a]	7.0 ± 4.6[b]	0.3 ± 0.3[a]

[a,b]Means followed by different letters in the same line indicate significant differences ($P < 0.05$).

Discussion

Thatcher *et al*. (1997) reported that the presence of the conceptus in the uterus of pregnant animals induces a number of complex metabolic reactions which signal the mother the need to sustain pregnancy by keeping proper levels of progesterone. Such interaction is not noticed in non-pregnant animals presenting a decrease in progesterone concentration induced by the synthesis and release of prostaglandins,

which results in luteolysis (Wathes and Lamming, 1995).

Pinto-Neto *et al*. (2008) found no difference in progesterone concentration while using FM during a critical period in cows and heifers. However, they reported differences related to the decrease of progesterone concentration in relation to luteolysis; pregnant animals from the control group presented sudden decrease when compared to the progressive decrease noticed for the pregnant animals in the treated group.

New theories regarding the control of luteolysis and the maintenance of pregnancy were described, indicating mechanisms of action based on endometrial blood flow and synthesis-release of different prostaglandins (Krzymowski *et al*., 2002) as well as the action of the luteal oxytocin as a mediator of luteolysis (Shirasuna *et al*., 2007). Therefore, new experiments should clarify the complex mechanism of maternal recognition of pregnancy.

In the present study, both experimental groups showed similar pregnancy rates, 37.8% (14/37) and 38.2% (13/34) for the animals from the CGs and TGs receiving FM treatment, respectively. Pugh *et al*. (2004) reported pregnancy rates higher than that observed for the animals receiving FM at the moment of the embryo transfer, possibly as a result of the inhibition of the prostaglandin release from the manipulations associated with the procedure. Elli *et al*. (2001), using ibuprofen on day 7 of the estrous cycle, which presents an action over the COX-2 similar to FM, showed higher pregnancy rates for the treated animals when compared to the non-treated.

The administration of prostaglandin system inhibitors is considered by some authors as a pharmacological tool to prevent the endogenous release of these eicosanoids at the moment of embryo transfer and during the maternal recognition of pregnancy (Odensvik *et al*., 1998; Scenna *et al*., 2005). However, under the conditions of this study, progesterone concentration and pregnancy rate were similar for animals treated and non-treated with FM. Moreover, the FM dose used, the amount administered and the duration of the treatment could also have contributed to the results in this study as the half-life of the drug used in bovines increases substantially according to the amount of administrations (from 4 h in a single administration to 26 h after four daily administrations), as described by Odensvik and Johansson (1995).

While assessing the ovarian condition of the non-pregnant animals, it was noticed that non-pregnant animals from the TG group had a higher follicular persistence than the control animals, 78.6% (11/14) and 33.3% (5/15), respectively.

Peters *et al*. (2004) reported that while administering the COX-2 inhibitor NS-398 in cows, there was decrease of PGF2α, PGE2 synthesis and a blockage of ovulation in the treated animals. These authors also mentioned that NS-398 did not interfere with follicular luteinization and consequent progesterone production. Pall *et al*. (2001) clearly demonstrated the blockage of ovulation in women treated with the selective inhibitor of COX-2. Therefore, it is consistent to support that, under the conditions of this study, FM could have interfered in the synthesis of intrafollicular prostaglandins, mainly PGF2α, thus preventing ovulation, what could justify the follicular persistence observed in the treated animals.

As a result of follicular persistence for the animals treated with FM, higher levels of serum estradiol could have been expected for these animals. The role of estradiol, originating from the dominant follicle, is also essential for luteolysis through the regulation of cytokine receptors in the uterus (Silvia, 1999), which are associated with the release of endometrial PGF2α and consequent luteolysis (Wathes and Lamming, 1995, Liu *et al*., 1997). Such interaction, which culminates with the decrease of progesterone concentration, could explain the lack of pregnancy for the animals presenting follicular persistence treated with FM.

In vitro experiments demonstrate that FM is efficient for reducing the production of PGE2 in an isolated bovine uterus model, but it does not induce down-regulation of mRNA for COX-2 (Braun and Kietzmann, 2004). Parent and Fortier (2005) mentioned that the PGE2 carries on action against the PGF2α, by promoting the establishment of pregnancy through luteotrophic action and immune suppression as a means of prevention against the rejection of the conceptus. The PGE2 action, whether inhibited by the FM action as it was administered to the treated animals, could subsidize the results for progesterone concentration and the pregnancy rate found in this study.

In addition, the roles played by prostaglandins on reproduction, mainly on ovulation and luteolysis, as well as other parts of the organism, relate to the process of cellular death by apoptosis and immunological events (Yadav *et al*., 2005; Kisliouk *et al*., 2007). These aspects suggest that other endocrine and/or paracrine mechanisms may be involved in the regulation of cyclooxigenases and prostaglandins in reproductive events, setting up perspectives for further experiments.

In conclusion, FM, as used under the conditions of this study, did not influence the pregnancy rate and the concentration of serum progesterone in cows submitted to FTAI; however, it significantly influenced the occurrence of follicular persistence.

Acknowledgments

The authors acknowledge the financial support by the UNIPAR Research and Post-Graduation Department (Project 612-05); Fundação Araucária and CNPq – for the scientific research scholarship granted (PEBIC-UNIPAR); and Professor Eunice Oba, Animal Reproduction Laboratory, UNESP - Botucatu, for allowing us to assay progesterone in her laboratory.

References

Binelli M, Machado R, Bergamaschi M, Bertan CM, Baruselli S. 2005. Estratégias para inibir a luteólise e aumentar a fertilidade em bovinos. *Rev Bras Reprod Anim*, 16:1-6.

Braun M, Kietzmann M. 2004. Ischemia reperfusion injury in the isolated hemoperfused bovine uterus – a model for the investigation of anti-inflamatory substances? *ALTEX*, 21(suppl 3):49-56.

Burns D, Graf GA, Haynes SH, Silvia WJ. 1997. Cellular mechanisms by which oxytocin stimulates uterine PGF2alpha synthesis in bovine endometrium: roles of phospholipases C and A2. *Domest Anim Endocrinol*, 14:181-191.

Elli M, Gaffuri B, Frigerio A, Zanardelli M, Covini D, Candiani M, Vaginali M. 2001. Effect of a single dose of ibuprofen lysinate before embryo transfer on pregnancy rates in cows. *Reproduction*, 121:151-154.

Kisliouk T, Friedman A, Klipper E, Zhou QY, Schams D, Alfaidy N, Meidan R. 2007. Expression pattern of prokineticin 1 and its receptors in bovine ovaries during the estrous cycle: involvement in corpus luteum regression and follicular atresia. *Biol Reprod*, 76:749-758.

Krzymowski T, Stefańczyk-Krzymowska S. 2002. Uterine blood supply as a main factor involved in the regulation of the estrous cycle - a new theory. *Reprod Biol*, 2:93-114.

Liu J, Carriere D, Dore M, Sirois J. 1997. Prostaglandin G/H synthase-2 is expressed in bovine preovulatory follicles after the endogenous surge of luteinizing hormone. *Biol Reprod*, 57:1524-1531.

Niswender GD, Juengel JL, Silva J, Rollyson KM, McIntush EW. 2000. Mechanisms controlling the function and life span of the corpus luteum. *Physiol Rev*, 80:1-29.

Odensvik K, Johansson IM. 1995. High-performance liquid chromatography method for determination of flunixin in bovine plasma and pharmacokinetics after single and repeated doses of the drug. *Am J Vet Res*, 56:489-495.

Odensvik K, Gustafsson H, Kindahl H. 1998. The effect on luteolysis by intensive oral administration of flunixin granules in heifers. *Anim Reprod Sci*, 50:35-44.

Pall M, Friden BE, Brannstrom M. 2001. Induction of delayed follicular rupture in the human by the selective COX-2 inhibitor rofecoxib: a randomized double-blind study. *Hum Reprod*, 16:1323-1328.

Parent J, Fortier MA. 2005. Expression and contribution of three different isoforms of prostaglandin E synthase in the bovine endometrium. *Biol Reprod*, 73:36-44.

Peters MW, Purley JR, Smith GW. 2004. Inhibition of intrafollicular PGE_2 synthesis and ovulation following ultrasound-mediated intrafollicular injection of the selective cyclooxygenase-2 inhibitor NS0398 in cattle. *J Anim Sci*, 82:1656-1662.

Pinto-Neto A, Lucca FM, Alberton J, Mota MF, Lucacin E, Borges AB, Acco A, Fonseca JF, Silva-Filho JM, Silva AV, Brandão FZ. 2008. Avaliação dos efeitos do flunixin meglumine sobre a concentração sérica de progesterona e ciclo estral em novilhas e vacas mestiças. *Rev Bras Ciênc* Vet, 15:10-14.

Pugh ML, Moreira MB, Gilbert GR, Youngs CR. 2004. Influence of prostaglandin F2α synthesis inhibitors on pregnancy rate of embryo transfer recipient heifers. *In*: Abstracts of the 15th International Congress on Animal Reproduction, 2004, Porto Seguro, Brazil. Belo Horizonte: CBRA. pp. 399.

Salles HO, Soares AT, Andrioli A, Moura Sobrinho PA, Azevedo HC. 1998. Diferentes posologias de flunixin meglumine na prevenção da regressão prematura de corpos lúteos em cabras superovuladas. *Ciênc Anim*, 8:69-74.

Scenna FN, Hockett ME, Towns TM, Saxton AM, Rohrbach NR, Wehrman ME, Schrick FN. 2005. Influence of a prostaglandin synthesis inhibitor administered at embryo transfer on pregnancy rates of recipient cows. *Prostaglandins Other Lip Mediat*, 78:38-45.

Shirasuna K, Shimizu T, Hayashi K-G, Nagai K, Matsui M, Miyamoto A. 2007. Positive association, in local release, of luteal oxytocin with endothelin 1 and prostaglandin F2alpha during spontaneous Luteolysis in cow: a possible intermediatory role for luteolytic cascade within the corpus luteum. *Biol Reprod*, 76:965-970.

Silvia WJ. 1999. The role of uterine and ovarian hormones in luteolysis: a comparison among species. *Reprod Domest Anim*, 34:317-328.

Soares AT, Simplício AA, Andrioli-Pinheiro A, Salles HO, Moura-Sobrinho A, Azevedo HC. 1998. Eficiência do flunixin meglumine no controle da regressão lútea prematura em cabras superovuladas. *Arq Bras Med Vet Zootec*, 50:35-39.

Thatcher WW, Binelli M, Burke J, Staples CR, Ambrose JD, Coelho S. 1997. Antiluteolytic signals between the conceptus and endometrium. *Theriogenology*, 47:131-140.

Thatcher WW, Guzeloglu A, Mattos R, Binelli M, Hansen TR, Pru JK. 2001. Uterine-conceptus interactions and reproductive failure in cattle. *Theriogenology*, 56:1435-1450.

Wathes DC, Lamming GE. 1995. The oxytocin receptor, luteolysis and the maintenance of pregnancy. *J Reprod Fertil Suppl*, 49:53-67.

Yadav K, Lakshmi G, Medhamurthy R. 2005. Prostaglandin F2alpha-mediated activation of apoptotic signaling cascades in the corpus luteum during apoptosis. *J Biol Chem*, 280:10357-10367.