Developments in Primatology: Progress and Prospects
Series Editor: Louise Barrett

Kathryn B.H. Clancy
Katie Hinde
Julienne N. Rutherford *Editors*

Building Babies

Primate Development in Proximate and Ultimate Perspective

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We dedicate this book to babies, and to those who build them.

Preface

The building of primate babies is a confluence of genes, ecology, nutrition, activity, health, and social dynamics experienced by both the parent and the offspring in time and space. To maximize lifetime reproductive success, parents must allocate finite resources to maintenance and reproduction, precipitating trade-offs among parental condition, current and future reproduction, and quality and quantity of offspring. Moreover, reproductive function and parental style themselves are shaped by early life and intergenerational factors. As a result, each individual's developmental trajectory varies as a function of parental investment and behavioral care. The developing primate, however, is not a passive recipient of parental investment, but can exploit physiological and behavioral mechanisms to extract parental resources to a greater extent than is in the parent's interest to provide. Consequently, development is perhaps an overly simplistic term for a process that is a dynamic relationship between progeny and parent.

Adult primates encounter complex social dynamics and diverse foraging tasks that directly influence survival and reproduction, the currency of natural selection. Yet the physical, physiological, cognitive, neurobiological, and behavioral capacity to confront these challenges reflects, in part, the unique ontogeny of each individual. For these reasons, investigations of developmental processes inform our understanding of what it means to be a primate. Primates are generally characterized by slow life histories, complex neurobiology and social dynamics, relatively large brains, and high parental investment in dependent offspring. Development is directly relevant to all these phenomena, as the chapters in this book demonstrate. Here we address ontogeny in a comparative framework, one that explicitly includes humans as primates, not just nonhuman primates, as model systems for human biology and evolution. In this way, we gain a deeper insight into the evolution, function, and causation of developmental trajectories in the order *Primates.*

Building Babies explores the dynamic multigenerational processes of development from many perspectives. The book is organized thematically along the developmental trajectory: conception, pregnancy, lactation, the mother–infant dyad, broader social relationships, and transitions to independence and adulthood. In this volume, we showcase the myriad approaches to understanding primate developmental trajectories

from both proximate and ultimate perspectives. These collected chapters provide insights from experimental manipulations in captive settings to long-term observations of wild-living populations, and consider levels of analysis from molecule to organism to social group to taxon. In addition, strepsirrhines, New World monkeys, Old World monkeys, apes, and humans are all well represented in this volume. Contributions by anthropologists, microbiologists, psychologists, population geneticists, and other experts passionate about primates provide *Building Babies* a uniquely diverse voice. We thank the authors for the care with which they put together their chapters from the outset and through the course of this book's development.

Each chapter in this book was reviewed by two or more of the editors in addition to being anonymously reviewed by two to four external referees. We are very appreciative of the numerous colleagues who peer reviewed chapter drafts and in so doing greatly enhanced the quality of this volume. We thank David Abbott, Michael Bailey, Jacinta Beehner, Alison Bell, Michelle Bezanson, Kristin Bonnie, Graham Burton, Katharine Campi, Isabella Capellini, David Coall, Herbert Covert, Jeremy De Silva, Amanda Dettmer, Leslie Digby, Christine Edwards, Peter Ellison, Melissa Emery Thompson, Paul Garber, Peter Gray, Robin Hudson, Laura Klein, Richard Lawler, Steve Leigh, Rebecca Lewis, Zarin Machanda, Dario Maestripieri, William Mason, Thom McDade, Talia Melber, Krista Milich, Carson Murray, Michael Nelson, Teague O'Mara, Ivy Pike, Meredith Reiches, Jeff Rogers, Michael Rudolph, Wendy Saltzman, Mar Sanchez, Karen Strier, Erin Sullivan, Elizabeth Sweet, Lin Tao, Zaneta Thayer, Jan Thornton, Wenda Trevathan, Claudia Valleggia, Eric Vallender, Derek Wildman, Jesse Young, and several anonymous others for their thoughtful comments.

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JNR—I would like to thank George Goehl for his perennial support and inspiration to do more always. My mother, Victoria Rutherford, has always championed my work, my writing, and me. I am grateful to my late father, John Rutherford, for his excellent paternal care. I built a baby during the building of this book, and have learned a great deal about the depths of maternal investment from my daughter Adelaide Caledonia Goehl. I was generously supported by funding from the National

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Lastly, we Lady Editors thank each other, having proved that the best starting point for an edited volume is finding co-editors with whom you agree unanimously on almost all editorial decisions and with whom you can really have an awesome time!

Cambridge, MA — Katie Hinde
Urbana, IL — Kate B.H. Clancy
Chicago, IL — Julienne N. Rutherford

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Conception and Pregnancy

Chapter 1
Inflammation, Reproduction, and the Goldilocks Principle

Kathryn B.H. Clancy

Keywords Inflammation • C-reactive protein • Ovarian function • Endometrial function • Pregnancy • Reproductive ecology • Human

1.1 Introduction

Great apes are a very low fertility clade among the already relatively low fertility primates. Giving birth largely to singletons, apes have interbirth intervals that range from 2 years to over 8 years (Furuichi et al. 1998; Galdikas and Wood 1990; Knott 2001; van Noordwijk and van Schaik 2005; Watts 1991; Wood 1994); confining human analyses to foragers ups the low end of the range to almost 4 years (Galdikas and Wood 1990; Lancaster et al. 2000). Apes undergo repeated, often very frequent copulations in the periovulatory period, and it still takes up to a year after lactational amenorrhea or anestrus has ceased for many to conceive (Watts 1991; Wood 1994).

Ultimate determinants of great ape low fertility include high parental investment and slow life histories (Kaplan 1996; Kaplan and Lancaster 2003; Walker et al. 2008). Because large-bodied hominoids, especially humans, rely on their parents and community for so long, a longer interbirth interval maximizes quality of each offspring, particularly as dependence of each offspring can overlap significantly. This longer interval also helps prevent maternal depletion, given the very high energetic costs of gestation and lactation, which then permits investment in future offspring.

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The study of proximate determinants of reproduction has largely fixated on energetics and ovarian suppression and to a lesser degree advancing age as it impacts chromosomal abnormalities and irregular cycles. Examining energetics and age reflects an attempt to understand the competing interests of current and future reproduction, growth and maintenance, and size, number, and sex of offspring because of the balance of weighing one's somatic investment with reproductive and sexual maturation when investing in offspring. This research has made it possible to understand how energy availability and energy balance impact reproductive cycles across multiple reproductive states and ages and is of enormous value to reproductive ecology and life history theory.

While energetic constraint is crucial to variation in ovarian hormones and reproductive cycles, other ecological stressors can also pull resource away from reproduction. Systemic inflammation offers a window into the ways in which ecological stressors increase somatic maintenance. Allocating energy towards maintenance demonstrates life history trade-offs in favor of survival over growth or reproduction, which may be necessary in marginal or otherwise constrained environments. Inflammatory processes are additionally important to attempts to implant an embryo and support a pregnancy. This means that determining whether inflammation is a cause or consequence of reproductive variation will be a continual challenge.

Inflammation is a biological response to trauma, from physical injuries to pathogens. Inflammation is a way the body tries to remove harmful stimuli and begin the healing process. And while acute stimulation of inflammatory processes is beneficial to the immune system, constant stimulation such that inflammation becomes chronic is correlated with several negative health outcomes. Prolonged inflammatory processes can lead to a constant cycle of cell destruction and healing. This may strain resources, shift the sensitivity of the immune system, or lead to amyloidosis, the depositing of excessive amyloid proteins in tissues of the body that is a precursor to diseases ranging from arthritis to Alzheimer's disease (Cunnane 2001; Merlini and Bellotti 2003).

Women's reproductive functioning involves significant tissue remodeling, the cycle of growth and repair that leads to the selection of a dominant follicle, ovulation, implantation, placentation, pregnancy, parturition, and lactation. Tissue remodeling is not only a kind of maintenance effort but is itself an inflammatory process. So inflammation can be caused by ecological stressors or simply the benign, normal functioning of the body. It is possible that activation of inflammatory processes from ecological stressors could disrupt normal tissue remodeling in female reproductive physiology, as well as shunt resources towards immune rather than reproductive function. Thus, there are two avenues that could connect the relationship from ecological stress to inflammation to reproductive function, disruption of function or diverting of resources, and an avenue from reproduction back to inflammation via normal tissue remodeling.

The reproductive system's sensitivity to ecological stressors, not just the stressors themselves, may be important to understanding variability in reproductive outcomes. An array of molecularly focused, clinical data demonstrate how the inflammatory

environment impacts reproductive success: too high an inflammatory response, or too low, and fetal loss follows. The Goldilocks principle seems useful here: the mother wants the environment to be "just right" in order to be willing to sustain a fetus. The way in which one's inflammatory milieu must be "just right" for a pregnancy to take hold reflects multiple maternal trade-offs: between immunocompetence and reproductive success, maternal and paternal strategies, and maternal and fetal strategies.

This means an inflammatory milieu supportive of implantation, but how do we know "just right" when we see it? Further, is "just right" population dependent, and what are the evolutionary implications for this? The study of the inflammatory milieu is an ideal place to expand our understanding of proximate and ultimate determinants of reproductive success, and the purpose of this chapter is to expose the intersection between the two.

Therefore, I will review the factors that produce variation in inflammatory processes, the evidence for sensitivity to attenuations in inflammatory processes for reproduction, and the broader implications of this work. These data show that building babies requires not only adequate energy but an adequate prediction of immunological and psychosocial investment to be successful.

1.2 What Modulates Inflammation?

The maternal environment is context dependent, and due to high physiological investment during pregnancy and lactation, fairly choosy around the circumstances under which conception, implantation, and gestation occur. This complicates achievement of a "just right" maternal inflammatory environment. Factors related to energetics, diet composition, immunological health, and psychosocial stress all impact systemic inflammation: the maternal environment is shaped by past and current variation in these factors, and they in part dictate the relative ability of a mother to support the fetus.

C-reactive protein (CRP), an acute phase protein largely produced in the liver, is the most frequently used biomarker for inflammation. CRP is easy to measure (McDade et al. 2007), covaries with IL-6, and is used clinically to predict cardiovascular disease (CVD) risk (Honda et al. 2006; Pradhan et al. 2001; Rutter et al. 2004; Williams et al. 2004). CRP is particularly responsive to acute phase stimuli, which allows for sensitive measurement of the severity of inflammation and a more nuanced understanding of different disease states, physical trauma, and autoimmune disease (Steel and Whitehead 1994). Therefore, while a better understanding of the maternal inflammatory environment should eventually include examination of several inflammatory factors, CRP will be the primary biomarker discussed in this chapter due to its much higher prevalence in the literature and responsiveness to current stimuli. This section directs the reader towards specific points of inquiry and unanswered questions about how or whether these factors specifically influence the maternal inflammatory environment.

1.2.1 Energetics

The best-studied factors that influence inflammatory processes are anthropometric determinants of fatness and weight, and several measures of adiposity correspond to elevated CRP concentrations. Increases in waist circumference and weight with age are positively correlated with CRP in samples of adults in the Philippines (McDade et al. 2009, 2010; Rutherford et al. 2009). CRP and BMI correlate in a number of studies (i.e., Guzelmeric et al. 2007; Oh et al. 2009), which often leads study authors to control for BMI when trying to estimate the effects or relationship of CRP to other variables. This has also led to the idea that excess weight produces inflammation, as a stressor on the body (Baynard et al. 2008; Guzelmeric et al. 2007; Williams et al. 2004).

Broadly speaking, increasing weight and sedentism are correlated with increased CRP concentrations. Physical activity itself produces a short-term acute-phase response, particularly after strenuous activity. However over time physical activity has an anti-inflammatory effect (Kasapis and Thompson 2005). While specific kinds of physical activity exert different degrees of inflammation suppression over time, these effects are inconsistent, while the inverse relationship between the amount of time spent on physical activity and CRP concentrations is consistent across many studies (Kasapis and Thompson 2005).

Recent evidence indicates that the relationship between physical activity and CRP in women may be cycle phase dependent. In a study of rural Polish women, strenuous activity was associated with CRP concentrations, but only in the luteal phase, or latter half of the cycle (Clancy et al. 2012). Further, when women were grouped by CRP concentrations, those with low CRP performed significantly more strenuous physical activity. CRP was also inversely correlated with luteal progesterone in this population (Clancy et al. 2012). CRP may help highlight the conflicting relationship between progesterone and physical activity: physical activity tends to suppress ovarian function and thus progesterone concentrations (Jasienska and Ellison 1998), yet progesterone has been found to increase activity in mouse models (Lightfoot 2008) and is itself anti-inflammatory (Finn 1998).

Reductions in calorie intake reduce CRP concentrations (Belalcazar et al. 2010; Heilbronn et al. 2001; Kasim-Karakas et al. 2006; Nicklas et al. 2004, 2005; Noakes et al. 2005). This may be because adipose tissue is an active endocrine organ in its own right and secretes CRP as well as tumor necrosis factor-alpha (TNF-α), leptin, and interleukin (IL)-6 (Forsythe et al. 2008; Yudkin et al. 1999). Independent of intake, diet composition has been found to impact CRP. Micronutrients can prevent lipid oxidation, and fiber, as a prebiotic, can support healthy gut flora; both reduce CRP (King et al. 2003; Koyanagi et al. 2004). The consumption of refined carbohydrates, sodium, and certain fats upregulate the innate immune system and increase CRP (Liu et al. 2002; Lopez-Garcia et al. 2004). Further, inflammation is higher in populations that eat a western-style diet high in simple carbohydrates and saturated fats, as opposed to a Mediterranean diet higher in fruits, vegetables, and unsaturated fats (Chrysohoou et al. 2004; Lopez-Garcia et al. 2004). In a sample of rural Polish women, there were

trends to support negative relationships with fiber and monounsaturated fat intake ($p = 0.08$ and 0.06, respectively) (Clancy et al. 2011).

CRP is positively correlated with energy status, energy availability, and energy balance, and these relationships are population specific. Further, CRP correlates with markers of a poor quality diet, including sugars, fats, and refined carbohydrates. Therefore, CRP is not necessarily independent of energetic factors, and any desire to understand the impact of other ecological stressors on CRP needs to control for this. This provides support that CRP indicates resource allocation towards somatic maintenance.

1.2.2 Psychosocial Stress

Psychosocial stress, as in clinical depression, is associated with both CRP and IL-6 (Miller et al. 2002). In this study, neither smoking nor infection was found to explain the relationship, and adiposity only partly explained it. Clinical depression also attenuates the relationship between acute stressors and inflammation: in a study of 36 women with depression and an equal number of age- and ethnicity-matched controls, participants were subjected to a mock job interview acute stressor. While CRP increased after the stressor in both groups, clinically depressed participants were more sensitive to stress at the beginning of the study, but that sensitivity declined after the stressor; the inverse was true for the nondepressive participants (Miller et al. 2005). That is, when inflammatory processes initiate, depressed participants had an impaired capacity to halt these processes once the stressor was over (Miller et al. 2005). The study of inflammation and psychosocial stress, then, needs to take into account variable sensitivity to stress as well as the stress response and activation of inflammatory cytokines.

Adversity in early life, or stressors that impact the maternal environment, may profoundly and differentially impact adult stress sensitivity. In a community sample of older adults, childhood adversity was associated with serum IL-6 concentrations, even after controlling for factors such as age, BMI, and gender (Kiecolt-Glaser et al. 2011). Danese et al. (2009) performed a longitudinal study that assessed adversity at 3 years of age and then again at 32 years of age. Individuals with adverse psychological experiences at 3 years were more likely to have higher CRP at 3 and 32 (Danese et al. 2009). Finally, in an effort to parse relationships between early adversity, inflammation, and other familial factors, Rooks et al. (2012) examined adult male twins from the Vietnam Era Twin Registry. Early life trauma was positively associated with adult CRP concentrations, and the between-pair, not within-pair, effects were positively associated with early trauma. This suggests that familial factors linked to early life trauma help explain adult CRP concentrations.

CRP varies by sex and race within the US, where women and people of color have on average higher CRP (McDade et al. 2006; Nazmi and Victora 2007). These group differences have been further supported in other studies (Chenillot et al. 2000; Hutchinson et al. 2000) and in a meta-analysis that supported both nonwhite race

and poverty as risk factors for elevated CRP (Nazmi and Victora 2007). But more importantly, self-reported discrimination (Lewis et al. 2010), perceived discrimination (Flores et al. 2008; Guthrie et al. 2002; Pascoe and Smart Richman 2009), and other aspects of racial discrimination and identity (Mays et al. 2007; Paul et al. 2008; Slopen et al. 2010; Thurston and Kubzansky 2007) positively associate with CRP concentrations. Therefore, constitutional, systematic, and institutional stressors must be considered as factors that influence systemic inflammation and perhaps reproductive functioning.

1.2.3 Immune Stress, Maintenance and Development

Like intrapopulation variation, interpopulation variation in systemic inflammation may be due to reaction norms from developmental exposure to immune or other stress. Microbial exposure in infancy, measured by diarrheal episodes and animal fecal exposure, corresponds to lower CRP in a sample of Philippine adults (McDade et al. 2009a, b). In this same sample, CRP was elevated in adults with a lower birth weight (McDade et al. 2009a, b). It is interesting to note that these two states can often co-occur in individuals from certain populations, which indicates trade-offs between maintenance and growth. Priming via exposure may impact the sensitivity of the immune system and offset the elevated CRP from a lower birth weight. These results also lend support to the idea that microbial exposure in childhood was an important aspect of human evolution; this does not require symptomatic disease but simply exposure to the benign microbes that characterized our ancestral environment (Barnes et al. 1999; McDade et al. 2009a, b; Rook 2008, 2009). This means careful attention must be paid to the many factors that push and pull on CRP concentrations when making comparisons or links to reproductive functioning.

Slightly different results have been found in microbial exposure after infancy, where higher exposure to infectious agents predicted elevated CRP in a sample of Philippine women (McDade et al. 2008). Increasing measures of pathogen exposure is also associated with CRP concentrations in a sample of patients with coronary heart disease (Zhu et al. 2000). This relationship has also been found in Tsimané children between the ages of 2 and 15 (McDade et al. 2005). One meta-analysis of pathogen exposure, periodontal disease, and cardiovascular disease even considered elevated CRP a proxy for adult pathogen exposure (Mustapha et al. 2007).

CRP concentrations mean something very different in developed versus developing populations. Where CRP can predict CVD risk in developed countries like the USA (Aiello et al. 2009; McDade et al. 2006), it is not associated with CVD in a sample of lean horticulturalists (Gurven et al. 2009). Further, the relationship between adiposity and CRP, which has been confirmed in several studies of developed populations, is different in countries with different subsistence patterns and lifestyles. McDade et al. (2009, 2010) found that CRP concentrations were lower in a sample of Philippine subjects compared to US subjects with the same waist circumference. This is not unlike the finding that the relationship between weight and

energetic biomarkers varies by population: forager Ache subjects had similar leptin concentrations to US anorectics despite a much higher BMI (Bribiescas 2005), and HDL is lower in a sample of Cebu compared to US participants at a similar BMI (Rutherford et al. 2010).

Overall CRP decreases with energetic stress, increases with psychosocial stress, increases with adult immune stress and decreases with childhood immune stress. CRP reflects ecological stressors and maintenance effort. This means the body has to navigate many environmental factors in order to achieve a population-specific "just right" inflammatory milieu. Under ancestral conditions, where individuals were not presumably traveling great distances to environments with different pathogen exposures or greatly different energy availability, reaction norms that set adult systemic inflammation may be an adaptive trait that helps the body determine the appropriate allocation of effort towards maintenance, growth, and reproduction.

1.3 How Much Inflammation Is "Just Right?"

Maternal and fetal strategies are not always in alignment. The fetus should always want to secure more resources, while the mother wants to reserve enough for her own survival and future reproduction (Haig 1993; Sterner et al. 2012). Maternal–fetal conflict should produce variation in trophoblast invasion, as the degree of invasiveness should correspond to the degree of maternal and fetal control of maternal resources (Crespi and Semeniuk 2004). Developmental trajectories during the mother's life may set the amount of resources available from the mother, which helps explain why the appropriate degree of inflammation for reproduction is context dependent. Inflammatory milieus are dependent on sex, race, and population, but the level of inflammation considered normal is dependent not only on those factors but on the desired reproductive state. For instance early pregnancy requires a slight inflammatory response; no response, or too much of a response, and fetal loss can result (Sacks et al. 2004). Thus, this section will identify the ways in which inflammation impacts certain reproductive pathways and states.

Previous research both on psychosocial stressors and inflammatory processes have found tenuous relationships with reproductive variables (Chisholm et al. 2005; Coussons-Read 2007; Dole et al. 2003; Ellis and Garber 2000; Ellison et al. 2007; Flinn and England 2003; Hogue et al. 2001; Nepomnaschy et al. 2006; Sanders and Bruce 1999; Wadhwa et al. 2001). In one study of normal women, there was a trend for psychosocial stress to associate positively with menstrual cycle length (Sanders and Bruce 1999); in another on newly incarcerated women, the authors found a significant positive relationship between stress and cycle length irregularity (Allsworth et al. 2007). The study of incarcerated women found not only a higher rate of oligomenorrhea and amenorrhea in this population than the general population, but having a parent with drug or alcohol problems, and having been a victim of childhood physical or sexual abuse, were all significant predictors of menstrual disturbances (Allsworth et al. 2007). Nepomnaschy et al. (2004) examined the effects

of daily stress on a sample of rural Mayan women. In the follicular phase, LH and FSH were positively correlated with cortisol, and cortisol was negatively correlated with progesterone when controlled for age. In the luteal phase cortisol was positively associated with FSH and positively associated with LH, estradiol, and progesterone at midcycle and near the menstrual phase (Nepomnaschy et al. 2004). This demonstrates a subtle, time-dependent interaction between stress and reproductive function (Nepomnaschy et al. 2004). Other studies have not shown a relationship between ovarian function and stress; for example, psychosocial stress did not impact ovarian hormones in a sample of college-aged American women studying for the MCAT (Ellison et al. 2007).

External factors that increase systemic inflammation, as well as immunological factors that produce an over- or under-expression of immune function, are some of the major culprits for reproductive pathologies. However, little is known about how to connect the mechanisms studied at the molecular level with lifestyle or population variation. As nice as it is to know what interleukins are in higher or lower concentrations for a particular reproductive pathology, what is stimulating that interleukin concentration variation in the first place? The following subsections of this chapter describe current knowledge about the relationship between inflammation and ovarian function, endometrial function and pregnancy. Pathological conditions help inform our understanding of what constitutes normal function. These subsections will be instrumental in building testable hypotheses to inform future areas for research in the final section of this chapter.

1.3.1 Ovarian Function

Ovulation occurs when the extracellular matrix (ECM) degrades at the follicular wall and the dominant follicle ruptures its surface. The corpus luteum, the site of follicular eruption on the ovary, is the most quickly vascularizing tissue in the body, and increases in size 20-fold over only a few days. Thus, the process of ovulation and ovarian maintenance of the endometrium and other reproductive processes rely on cyclical tissue remodeling, which itself relies on inflammatory processes (Smith et al. 2002).

While a vast literature exists to test relationships between multiple cytokines and inflammatory factors with reproduction, few are ever linked to reproductive outcomes or environmental determinants of stress. Ovarian remodeling is characterized by degradation and replacement of the ECM. The main players are cytokines—particularly IL-1, IL-6 and TNF-α—and matrix metalloproteinases (MMPs). However, CRP is also correlated to many of the above factors.

It was once thought that follicle recruitment occurs continuously (Baird 1987) or at only a single time during the menstrual cycle (Bakos et al. 1994; Gougeon 1979; Lenz 1985; O'Herlihy 1980; Pache et al. 1990; Queenan et al. 1980; Renaud et al. 1980). In fact, follicular recruitment occurs in waves as a normal feature of the menstrual cycle and healthy ovarian functioning. These follicular waves have been

documented during the human menstrual cycle, using histologic, endocrinologic and/or ultrasonographic techniques (Baerwald et al. 2003a, b; Block 1951; Dervain 1980; Hackeloer et al. 1979). This is important for understanding normal variation in human ovarian physiology and fertility (Fehring et al. 2006; Lipson and Ellison 1996; Noyes et al. 2001; Wilcox et al. 2000). While endocrine hormones regulate follicle wave dynamics (Baerwald et al. 2003a, b), the factors which determine the type and number of follicle waves in women are not fully known.

Variation in inflammation may help to explain why women experience different follicle wave dynamics. Clancy et al. (in revision) have found that women with three follicular waves had greater serum CRP concentrations than women with two waves. Furthermore, when more follicular waves emerged in the follicular phase and were spaced more closely together, greater CRP concentrations were detected (Clancy et al. in revision). And though CRP correlates with age and BMI in other samples (Guzelmeric et al. 2007; Hutchinson et al. 2000; McDade et al. 2008, 2009a, b), such relationships were weak or nonexistent in this dataset. These data suggest considerable variation in follicle wave dynamics even in normo-ovulatory women and a link to inflammation that has considerable clinical implications (Clancy et al. in revision).

Existing studies reveal significant differences in the relationships found between CRP and ovarian hormones. Jilma et al. (1997) found that CRP was significantly higher in single blood samples of women at midcycle and the midluteal phase compared to the follicular phase and that these increases were correlated with progesterone concentrations. Wander et al. (2008) found that CRP is positively associated with progesterone, but negatively with estradiol, in a sample of spontaneously cycling women. Conversely, Wunder et al. (2006) found no associations between CRP and menstrual cycle phase. However, methodological differences hamper adequate comparisons between these studies of women in industrialized populations, and none performed daily measures of reproductive hormones.

Recent work by Clancy et al. (in preparation) has found significant negative relationships between urinary CRP and both estradiol and progesterone in a sample of rural Polish women; further, women with high CRP had significantly lower progesterone through the luteal phase than those with low CRP (Fig. 1.1). The negative relationship between CRP and progesterone is counter to that found in spontaneous cycles in other samples. However, all other studies that have examined CRP through the menstrual cycle have been in industrialized populations, and the measure of CRP via urine may produce a value more averaged over time. This study is both the first to examine nonindustrialized menstrual cycle variation with CRP and to compare baseline CRP (not including CRP during ovulation or menstruation to avoid internal influences) to daily salivary hormones throughout the entire cycle.

The physiology of individuals at the edges of normal, those labeled pathological, can be instructive to our understanding of how and when the inflammatory milieu is disrupted. For instance, polycystic ovarian syndrome (PCOS) is a syndrome whose diagnostic criteria include elevated androgens, male pattern hair growth, cycle irregularities, a greater than normal number of immature follicles, and/or anovulation. Sonographic evidence supports the idea that women with PCOS have more than the

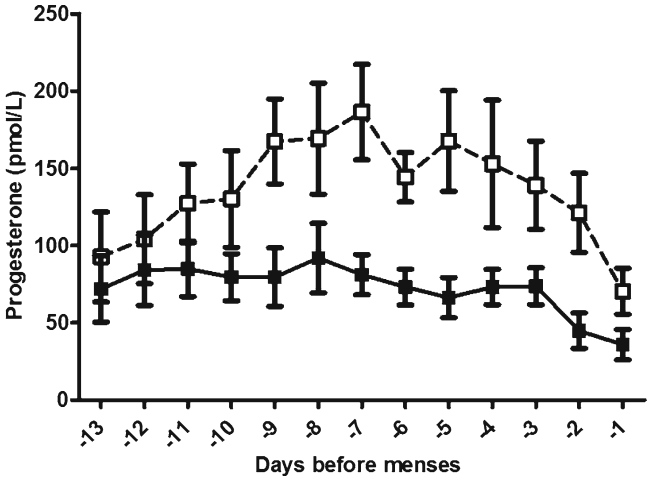

Fig. 1.1 Progesterone concentrations of individuals in high and low CRP tertiles (mean ± SEM). High CRP is represented by *black* points and a *solid line*, where low CRP is represented by *white* points and a *dotted line* (Clancy et al. in prep)

normal number of follicular waves: one subject was measured to have six follicular waves before being diagnosed with PCOS and excluded from the normo-ovulatory sample (Baerwald, personal communication). The literature broadly supports a relationship between CRP and PCOS (Guzelmeric et al. 2007; Morin-Papunen et al. 2003; Oh et al. 2009).

Endometriosis produces local inflammatory processes where the endometrial tissue external to the uterus is activated. When this tissue is on the ovary, it appears to increase risk of ovarian tumors (Brinton et al. 2004; Hoshiai 2000; Ness and Cottreau 1999; Nishida et al. 2000; Sainz de la Cuesta et al. 1996; Yoshikawa et al. 2000). Pelvic inflammatory disease, an infection caused by sexual transmitted diseases like chlamydia and gonorrhea, is better known as a cause for cervical cancer but may increase risk of ovarian cancer as well (Risch and Howe 1995). Therefore even those pathologies that we presume are genetic or energetic such as ovarian cancer may be additionally explained through inflammation.

1.3.2 Endometrial Function

The endometrium is composed of the functionalis and basalis layers; the functionalis comprises two thirds of the endometrium and is the part that proliferates and sheds each reproductive cycle. The basalis is adjacent to the myometrium and is the place from which the endometrium regenerates after menses. The proliferative (also known as follicular) phase is when estradiol promotes proliferation of endometrial tissue, whereas the secretory (also known as luteal) phase is characterized

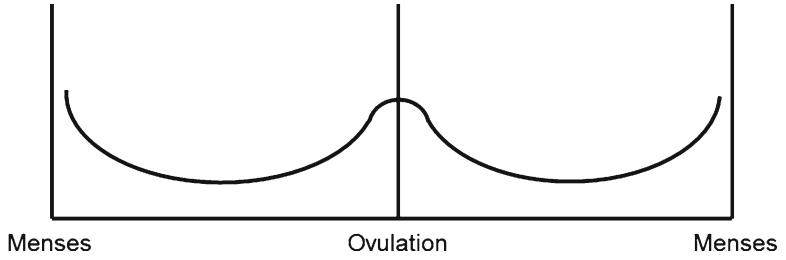

Fig. 1.2 Conceptual model for the increase in inflammatory processes at ovulation and menses during the ovulatory human menstrual cycle

by progesterone control of decidualization (the sum of the proliferation and secretion, as well as increase in vascularization of the endometrium) and menstruation. When endometrium proliferates, it often does so with narrow, straight glands and a thin surface epithelium, and angiogenesis continues as ovulation nears (King and Critchley 2010). After ovulation and during the secretory phase, the endometrium differentiates: endometrial glands become increasingly secretory, and by the late secretory phase spiral arterioles form. If implantation does not occur, the corpus luteum degrades, progesterone declines, and this triggers a cascade of events to produce menstruation: the state of the corpus luteum determines whether or not menstruation will occur.

Menstruation is a key inflammatory process of the endometrium when the functionalis is shed at the end of the human reproductive cycle. The basalis regenerates over the course of the next cycle. The demise of the corpus luteum and the associated withdrawal of progesterone precipitate inflammatory mediators that cause tissue degradation (Maybin et al. 2011). The withdrawal of progesterone is also associated with an increase in endometrial leukocytes and IL-8, which regulate the repair process (Maybin et al. 2011). At this time, other inflammatory factors promote MMP production to break down endometrial tissue (Maybin et al. 2011). Further, it is thought that progesterone withdrawal, not an increase in estradiol concentrations, leads to the repair of the endometrium so that it can resume activity for the next cycle (Maybin et al. 2011). Thus, variation in progesterone concentrations may lead to variation in inflammatory activity, degradation, repair, and cycling in the endometrium. Figure 1.2 demonstrates the likely timepoints along the reproductive cycle where inflammatory processes increase.

Another important component of menstruation is the control over vessel radius and blood flow. Prostaglandin $F_{2\alpha}$ and endothelin-1 cause vasoconstriction of the spiral arterioles to decrease blood flow in normal menstruation. Heavy menstrual bleeding, or menorrhagia, can be caused by unrestrained inflammation or impaired repair processes. Menorrhagia is alternately defined as menstrual bleeding in excess of 80 or 120 mL, where normal blood loss is around 30 mL. However, excessive prostaglandin concentrations and concurrently exaggerated inflammation, as well as immature spiral arterioles and lower concentrations of vasoconstrictor endothelin-1 are associated with heavy menstrual bleeding (Maybin et al. 2011). Women with

menstrual bleeding over 80 mL have reduced vascular endothelial growth factor (VEGF) mRNA expression (Maybin et al. 2011) and reduced VEGF-A, MMP-2, and MMP-9 concentrations in menstrual effluent (Malik et al. 2006).

Other endometrial pathologies appear to have inflammatory mechanisms or origins, particularly endometriosis, fibroids, endometritis, and endometrial cancer; understanding these pathologies may help us understand the extreme end of the range of endometrial functioning. Endometriosis affects about 10% of women in the USA and is caused by hormonally sensitive endometrial tissue that migrates out of the uterus found mostly on the ovaries, pelvic cavity, and peritoneum. Endometriosis is caused, in a sense, by retrograde menstruation, menses that flows back into the vagina and enters the abdominal and pelvic cavities. Epithelial to mesenchymal transitions (EMT), which is the loss of adhesion and increased cellular mobility in cellular remodeling, appear to play a role in the production of endometriotic lesions (Demir et al. 2005).

Like endometriosis, uterine fibroids can cause dysmenorrhea and infertility, depending on their placement. Fibroids, or leiomyomas, are benign tumors of myometrial origin that affect up to 70% of reproductively aged women. This pathology is controlled by ovarian steroids, but the mechanism of their production is mediated by inflammatory factors. And like menorrhagia, the cause of fibroids is suspected to relate to tissue repair dysfunction. Women with fibroids appear to have excessive inflammation of the endometrium, which may explain their increased risk for infertility. Women with fibroids have both higher TNF-α and endometrial macrophage infiltration (Agic et al. 2006; Miura et al. 2006).

Endometritis is an endometrial infection that tends to occur when debris is not fully cleared from the uterus after parturition but can also occur with bacterial and viral infections (Donofrio et al. 2010; LeBlanc 2010). IL-8, a cytokine scarcely present in healthy individuals, ratchets up in individuals with endometritis in a bovine model (Donofrio et al. 2010). IL-8 attracts granulocytes like macrophages and neutrophils to sites of infection.

Weight gain and obesity increase endometrial cancer risk in postmenopausal women, and it is generally assumed that estriol from fat cells is the cause (Barrett et al. 1995; Gull et al. 2001; Kaaks et al. 2002; Trentham-Dietz et al. 2006). Weight gain, overweight, and obesity are associated with CRP and thus systemic inflammation (Guzelmeric et al. 2007; McDade et al. 2006, 2008, 2009a, b; Rutherford et al. 2009; Williams et al. 2004); therefore, an inflammatory origin may augment the hormonal explanation for endometrial cancer, especially since inflammation can increase estrogen concentrations (Modugno et al. 2005). Chronic inflammation, such as that associated with the systemic inflammation caused by overweight, can induce rapid cell division, which increases the risk of mutations that can lead to cancer (Modugno et al. 2005).

From these pathologies, we can learn about the ways in which understanding hormones may be insufficient to understand endometrial functioning. While many of the mechanisms described above are hormonally mediated, it is unclear whether hormones always drive the variation that leads to pathology. Paracrine and perhaps even autocrine processes drive the basic endometrial cycle of proliferation, decidualization,

and degradation (Beier and Beier-Hellwig 1998; Bischof et al. 1998; Lessey 2003; Wu et al. 2009). Thus, measurements of local inflammatory factors are important to future work on variation in endometrial functioning.

1.3.3 Pregnancy

Like menstruation, implantation is an inflammatory process of the endometrium. In the proliferative phase, estrogen primes the endometrium and its receptors for progesterone secreted by the corpus luteum. The endometrium decidualizes, but chemokine and cytokine expression, and leukocytes also increase. Uterine natural killer cells (uNKs, a kind of leukocyte) are present in the implantation window and may aid in trophoblast invasion (King and Critchley 2010).

Implantation is a breach of the mucosal barrier of the endometrium, the first line of immune defense. Therefore, innate immune cells need to be activated in order to keep the site free of infection while not overexpressing to the point of attacking the trophoblast (King and Critchley 2010). The fact that the fetus is semiallogenic or partially unrelated to the mother means that the required immunological changes also need to be timed appropriately. The fetus is protected from maternal attack via embryologically derived trophoblast cells that then become the placenta (Rusterholz 2007). Further, the placenta itself secretes immunological and inflammatory factors that interplay with maternal factors: these factors may actually contribute to the regulation of local and systemic immunological changes needed for a successful pregnancy (Hauguel-de Mouzon and Guerre-Millo 2006; Rusterholz 2007). The placenta also expresses molecules that prevent destruction by NK cells and cytotoxic T lymphocytes (Rusterholz 2007). Further, the maternal immunological milieu is biased towards T-helper cell 2 (Th2) immunity during pregnancy, both at the intrauterine and systemic levels (Rusterholz 2007). This means that the humoral response is favored over the cell-mediated response, which is more likely to be destructive to a semiforeign body like a fetus (Rusterholz 2007).

Pregnancy itself is a state of mild inflammation, where the expression of several cytokines is increased compared to the nonpregnancy state (Rusterholz 2007). Maternal CRP concentrations are elevated as early as 4 weeks gestation, and remain elevated through pregnancy (Sacks et al. 2004). Related to this, supraphysiologic estradiol concentrations in women undergoing assisted reproductive technologies (ART) positively correlated with CRP (Almagor et al. 2004; Orvieto et al. 2004); however, normal concentrations were negatively correlated with CRP (Wander et al. 2008). A shift in the relationship between estradiol and CRP may occur at high concentrations of estradiol, from a negative to a positive correlation. The increment of increase in CRP has been further related to pregnancy outcome (Almagor et al. 2004; Orvieto et al. 2004); women with a serum CRP increase between oocyte retrieval day and 5–7 days posttransfer were more likely to have conceived. Other research has reported higher CRP in women with successful versus unsuccessful IVF outcomes (Sacks et al. 2004). Increased maternal CRP may

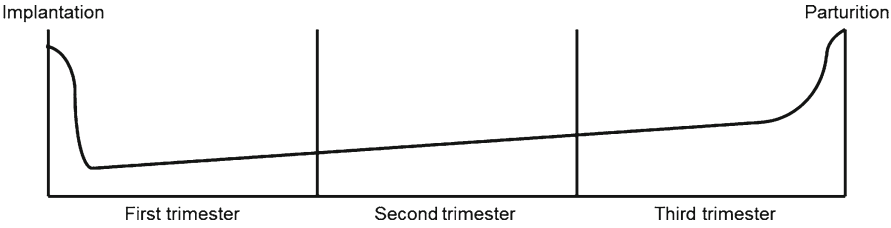

Fig. 1.3 Conceptual model for the changes in CRP from implantation to parturition in an uneventful pregnancy

be associated with protection from pregnancy loss, particularly in losses greater than 12 weeks gestation (Boggess et al. 2005), and that intentional local inflammation during blastocyst transfer improves IVF outcome (Gnainsky et al. 2010). Thus, an inflammatory response is thought to be a positive signal for implantation, but too much (or too little) inflammation could indicate pathology (Sacks et al. 2004). Figure 1.3 demonstrates a conceptual model of the behavior of CRP through pregnancy based on current evidence.

IL-6, strongly correlated with CRP, has also been studied in infertility patients. Demir et al. (2009) measured serum concentrations on day 3 of the menstrual cycle in infertility and fertility groups and found that IL-6 levels are higher in women diagnosed with unexplained infertility compared to controls. Bedaiwy et al. (2007) demonstrated that follicular fluid concentrations of IL-6 were higher in pregnant cycles compared to nonpregnant IVF cycles. However, Hammadeh et al. (2002) results in an IVF sample using the intracytoplasmic sperm injection (ICSI) method were not correlated with follicular fluid IL-6, nor was IL-6 correlated with peripheral blood measures of ovarian hormones. Other researchers have reported variation in follicular fluid IL-6 among different ovarian stimulation protocols (Ficicioglu et al. 2010).

The timing of parturition is one of the places that inflammatory factors can produce pathology. Normal parturition is associated with an increase in inflammatory factors, and these are necessary to activate the uterus and ripen the cervix. IL-1 and IL-8 can be measured in the cervicovaginal fluid and increases in these cytokines are strongly associated with the commencement of labor and the rupture of fetal membranes (Tanaka et al. 1998). Choriodecidual inflammation is a leading cause of late miscarriage and preterm birth and may be precipitated by NF-κB cells (De Silva et al. 2010). NF-κB, mentioned above, is inhibited by progesterone concentrations, but in its absence can stimulate IL-1 and IL-6 production (De Silva et al. 2010). Other inflammatory factors considered include MMPs, as tissue inhibitor of metalloproteinases (TIMPs), or molecules which inhibit MMPs, are reduced in women with a history of pregnancy loss (Anumba et al. 2010; Shibahara et al. 2005). Insulin resistance is also a concern, as insulin resistance beyond the normal range of maternal insulin resistance found in pregnancy may impact endometrial receptivity and activity (Levens and Skarulis 2008). Obese pregnant women who