

PLASMA PROTEIN PROFILE AS AN INDEX OF PREGNANCY IN THE BLACK BEAR

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Abstract: We analyzed plasma protein profiles in an attempt to identify physiological parameters associated with pregnancy in black bears (*Ursus americanus*) that might be used as early confirmation of this reproductive state. We collected serial blood samples from 2 animals over 3 consecutive fall periods. Both females were pregnant during the 1st and 3rd years; cubs from the 1st litters were removed early so females were in a non-pregnant, anestrus state during the 2nd year. We qualitatively analyzed the plasma proteins using polyacrylamide gel electrophoresis. Several distinct differences were apparent between samples from pregnant and non-pregnant animals and the protein profile changed during pregnancy as the time of implantation approached. Results suggest that 1 or more plasma proteins are associated with early stages of pregnancy in the black bear.

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Many physiological changes occur in association with pregnancy that provide support and regulation of embryonic development. One such well-documented change is the appearance of human chorionic gonadotropin (hCG), a hormone of embryonic origin, in the blood of pregnant women (Saxena et al. 1974). This glycoprotein has been used as an antigen to which specific antibodies have been produced in the development of pregnancy testing kits. Pregnancy-specific antigens that are blood-borne have also been described in mink (Larsen et al. 1971), rat (Lin et al. 1974), mouse (Lin et al. 1974; Morton et al. 1974, 1976), and sheep (Cerini et al. 1976a, b; Morton et al. 1979). Many of these antigens appear late in pregnancy and are associated with placental function; however a few, such as hCG in humans and those in sheep, appear very early. With the exception of mink, where an antigen was identified during the latter half of pregnancy, up to now no report of such blood components has been made for a species that exhibits an obligate delay in embryonic development. The black bear is 1 such species that exhibits this reproductive phenomenon. Pregnancy in black bears is characterized by 5 months of embryonic quiescence after conception in June, with implantation in late November, and a 2-month postimplantation development period before parturition in late January (Fig. 1, Wimsatt 1963, Foresman and Daniel 1983). Concomitant with studies designed to identify plasma levels of progesterone during pregnancy (Foresman and Daniel 1983) studies were performed to address the question of the existence of pregnancy-specific plasma proteins in this species.

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METHODS

During 3 consecutive fall periods (1977-79) 2 female bears were anesthetized (2 mg ketamine hydrochloride/2 mg xylazine, intramuscularly) either twice a month (1977-78) or once a month (1979). During the 1st and last years both females were pregnant; an anestrus condition existed during the middle year (1978) because cubs from 1977 were removed from their mothers in the fall (bear 1, 13 November; bear 2, 14 September). Sampling was discontinued in December in response to the zoo's desire to produce successful litters, and the females were allowed to retreat into denning facilities. Samples were also obtained from 1 male during fall, 1978 as an additional control.

Blood samples obtained from either the jugular vein or the femoral artery were drawn into 20 ml heparinized syringes and immediately transferred into heparinized vials. Whole blood was centrifuged at 600 x g and 4 C for 10 min and the resultant plasma was stored at -20 C until assay.

Plasma protein determinations were made by the Lowry method (Lowry et al. 1951). Protein profiles were analyzed employing slab polyacrylamide gel electrophoretic procedures. Native slab gels (1.5 mm thick, Bio-Rad Model 220 vertical slab cell) consisted of an 8% stacking layer and 12% running gel. Ninety µg of protein/sample were layered on these gels and

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electrophoresed in a Tris/Borate buffer [0.065M] at 325 V (constant) until the dye front migrated to within 5 mm of the lower gel surface (approximately 2 h). The sodium dodecyl sulfate (SDS)- polyacrylamide procedure (Weber and Osborn 1969) was used for molecular weight determinations. Running gels consisted of 10% acrylamide to which samples containing 60 μ g of total protein were applied. Electrophoresis was performed at 80 V (constant) in a sodium phosphate-SDS [0.05M, pH 7.0] buffer system, until the dye front migrated to within 5 mm of the gel surface (4–5 h). A molecular weight standard preparation consisting of phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme (Bio-Rad) was used for comparative purposes. Following electrophoresis, the protein bands were identified by the standard staining/destaining procedure of Weber and Osborn (1969) using Coomassie Brilliant Blue R-250.

RESULTS

The plasma protein profile during the late preimplantation period of a normal pregnancy was significantly altered from the pattern observed during non-pregnancy. Though there was a degree of variability in the protein profiles between animals, and within an animal between pregnancies, a representative pattern can be presented (Fig. 2). Five major proteins or classes of protein consistently appeared during both pregnancy and non-pregnancy, and in male samples. Molecular weight determinations of these bands gave values of approximately 30,000, 38,000, 60,000–62,000, 66,000 (albumin), and 78,000–80,000. One to 3 additional bands appeared only during pregnancy and in apparent association with the period encompassing implantation (Fig. 2A). These bands had approximate molecular weights in the 42,000–50,000 range. When electrophoresis was performed on native samples (without SDS treatment), where protein separation is primarily on the basis of charge distribution, the bands specifically associated with pregnancy were slowly migrating, running far behind albumin (Fig. 3).

DISCUSSION

Though only 4 pregnancies and 2 non-pregnancies were followed in the present study, preliminary evidence suggests that 1 or more plasma proteins is associated with early stages pregnancy. The appear-

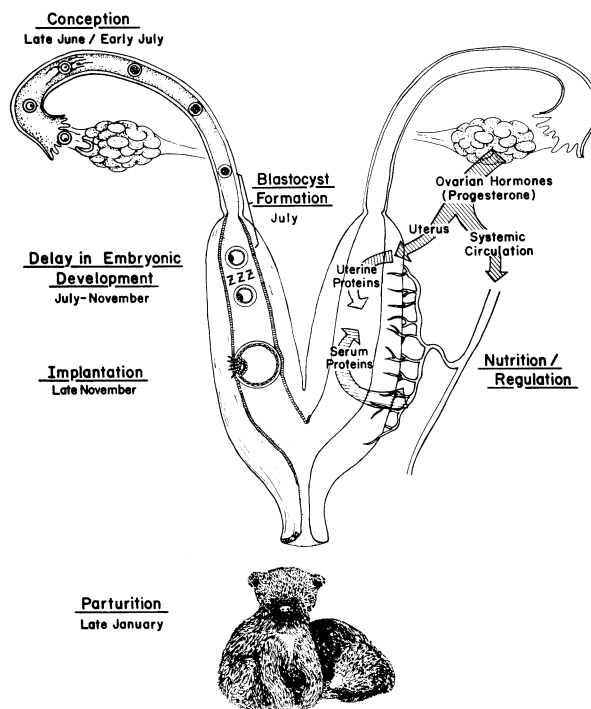


Fig. 1. Reproductive events in the black bear. The gestation period is approximately 225 days of which 145–165 days are spent in arrested development before embryonic attachment to the uterus. Ovarian hormones, in particular progesterone, are thought to regulate these events through the synthesis of uterine- and plasma-specific proteins that serve both nutritive and regulatory functions.

ance of these proteins coincided with the late preimplantation period suggesting their association with events leading up to and culminating in implantation of the embryo.

Pregnancy-specific antigens have been identified in several other species though, with very few exceptions, these have been viewed as being associated with progressive placental and fetal development and appear toward the later stages of pregnancy. In the rat and mouse 4 serum antigens have been detected within the last 3 days of pregnancy that were not observed in newborns or in the maternal system following parturition (Lin et al. 1974). Several proteins, in particular α -fetoprotein (Gitlin and Gitlin 1975), human pregnancy zone protein (PZP, von Schoultz 1974), and human pregnancy associated macroglobulin (PAM, Stimson 1975) have been identified during mid-to-late pregnancy in humans and are commonly used as indicators of fetal development. Relatively few proteins have been identified in the general circulation during very early stages of preg-

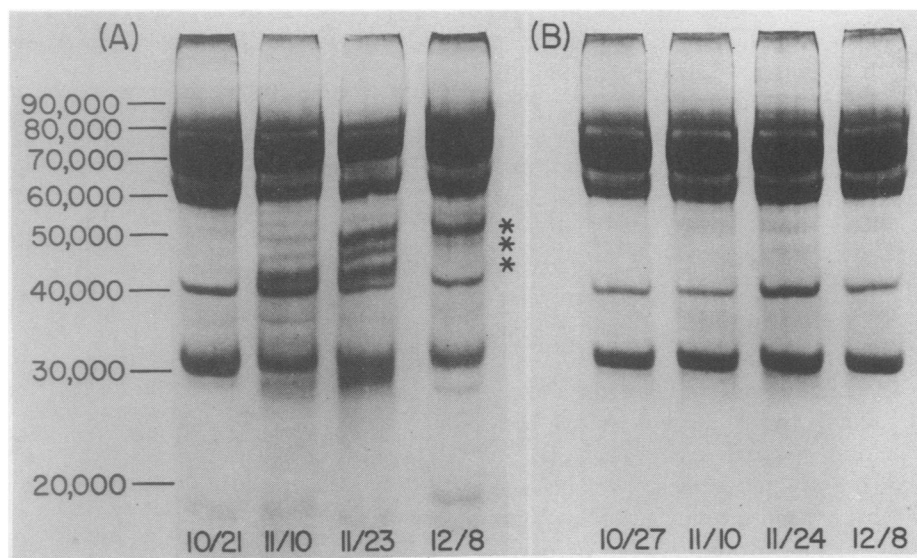


Fig. 2. Representative electrophoretic pattern of plasma proteins during (A) pregnancy (1977) and (B) non-pregnancy (1978) in female number 2. Gels were purposely overloaded with protein (60 μ g) to highlight bands in lower con-

centrations. The molecular weight scale is identified on the left and the sampling date is displayed below each column. (*) denotes suspected pregnancy-specific proteins.

nancy at or just following implantation. Human chorionic gonadotropin, surely the most thoroughly studied of these, is produced by syncytiotrophoblastic tissue beginning at, or slightly before implantation (Saxena et al. 1974). This glycoprotein stimulates luteal secretion of progesterone and thus functions to maintain the pregnancy. Its early appearance serves as the basis for the immunological test for pregnancy in women (Hobson 1974). Recent research in sheep has also demonstrated the existence of pregnancy-specific antigens, most likely of embryonic origin, which are detectable within 6 days of fertilization (Cerini et al. 1976b). Initial investigations in sheep employed rabbit antisera to Day 14 whole ovine conceptuses subsequently treated to remove non-pregnancy specific antigens by absorption with liver and kidney homogenates from non-pregnant sheep. Immunofluorescent techniques provided evidence that a pregnancy-specific antigen was present not only in trophoblastic and uterine tissues but erythrocytes as well. More recent studies employing calf antisera to Day 16 whole ovine conceptuses similarly purified confirmed the existence of ovine pregnancy-associated antigen (oPPA) but have been unable to detect this in erythrocytes (Cerini et al. 1977, Staples et al. 1978, Staples 1980). Purification of this antigen has led to molecular weight determinations which indicate that it exists in dimeric form with a native weight of 42,600.

It is interesting to note that the appearance of new plasma proteins in the black bear occurs as circulating progesterone levels are on the rise coincident with impending implantation (Foresman and Daniel 1983). Progesterone has been shown to stimulate the

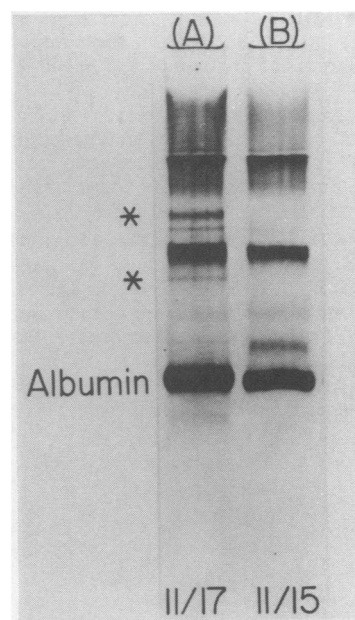


Fig. 3. Representative electrophoretic pattern of plasma proteins run on native samples during (A) pregnancy (1977) and (B) non-pregnancy (1978) in female number 1. Note slowly migrating pregnancy-specific proteins (*).

synthesis of proteins, particularly of uterine origin, in association with embryonic development and implantation in many species (e.g., rabbit (Daniel 1976), spotted skunk (Mead et al. 1979), pig (Basha et al. 1980)) as well as to induce the influx of serum proteins into the uterine lumen at this time (Mead et al. 1979).

This report should be viewed as a preliminary attempt to identify plasma components which are characteristically associated with a pregnancy. The evidence suggests that such blood components exist in the black bear though much more research is required in more animals before more definitive conclusions can be drawn. Studies are currently in progress to further characterize these proteins and this research is being expanded to include polar bears (*Ursus maritimus*) so that procedures can be developed for the early detection of pregnancy in these and other ursids.

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