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„Placental scar counts and the assessment of reproductive performance in female brown bears“

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ABSTRACT

Analysis of the uteri (n=181 complete and 8 incomplete) of female Scandinavian brown bears (*Ursus arctos*) in the 3 core areas for female reproduction in Sweden, in the harvest years 1992 and 1997-2005, provided a unique opportunity to reveal "12" years of reproductive performance and thereby contribute to a better understanding of a brown bear's life-history. I tested the accuracy of placental scar counts in the stained uteri for the estimation of female reproductive traits (such as age at primiparity, litter size, litter production and cub mortality) through the comparison between estimated and observed litter sizes in a number of females with known reproductive history. Staining the uterine tissue proved indispensable for the visibility and classification of 49.0% (n=98) or at least 20.4% (n=98; knowing how to classify the orange-coloured scars) of the new and 80.3% (n=66) of the old placental scars. Moreover, I tried to assess the reliability of the method in due consideration of: corroborative evidence from confirmed lactation, the status of the uterus (endometrial development), the age- and handling-dependent appearance of placental scars, as well as the results of estimated reproductive performance in comparison to the observed performance in the field reported in literature. In the females examined, placental scars usually were found to persist for 21 month postpartum. Stained new placental scars had a vibrant, commonly dark appearance and a complete scar-pattern. Stained old placental scars appeared faded and their scar-pattern was no longer complete. The method to count placental scars for the estimation of litter size has been used in a range of mammals, with the recurring conclusion that the most reliable results can be obtained by counting only the dark scars (e.g. in the red fox, *Vulpes vulpes*: Elmeros et al. 2003, Ruette and Albaret 2011). This would suggest taking into account only the new scars of category 1 and category 2 identified in the females in my study. In Scandinavian brown bears, being aware of confounding and species-specific factors (such as potential confusions between different aged scar-sets when interpreting intermediate scar-phenotypes, the 2 and 3-year reproductive cycles, incidents of litter loss), I recommend that the estimates of mean litter size be based on the counts of new and old placental scars. This information on female reproductive output in the 2 consecutive breeding seasons before harvest is supposed to reflect more natural proportions and yield solid estimates. My approach is consistent with the method described in bear-literature (in the Hokkaido brown bear, *Ursus arctos yesoensis*: Tsubota et al. 1990; Mano and Tsubota 2002; American black bear, *Ursus americanus*: Hristienko et al. 2004; Japanese black bear, *Ursus thibetanus japonicus*: Yamane et al. 2009), however, I assessed the stained scar tissue.

Seasonal development of the endometrium, in concordance with the ovarian activities, was more commonly apparent in the uteri of autumn-harvested females with old placental scars in comparison to those with new scars. It generally missed in the uteri of spring-harvested females. The earliest evidence of endometrial bulges on a thickened uterine wall was found in 2-year-olds (14.3%; n=28), thereby revealing their sexual maturity and by association that they may already have been receptive. The earliest recorded age of primiparity was 3 years for the females in the southern and central core area, and 4 years for females in the north. Scar tissue evaluation suggests that the age of 3 years is also the earliest age for females to successfully raise a litter, as these primiparae showed no evidence of consecutive-year birthing. The estimated litters comprised 1-4 cubs, with 2-3 cubs being commonest. Mean litter size based on new placental scar counts was 2.45±0.11 (n=40), that based on new and old scar counts was 2.22±0.10 (n=74 scar-sets in 66 females). According to the Monte Carlo approximation, first-breeding 3-year-old mothers had significantly smaller litters than mothers at the age of 4 years. The estimates based on new and old scars suggest that an increase in litter size occurs at 3-5 years of age. Reconstructing population productivity from placental scar observations may be considered precarious when based on a harvest sample of females. The proportion of solitary females, and thus the rates of litter production are generally likely to be biased high. Keeping that in mind, the productivity came to 0.90±0.12 cubs per adult female and year (n=106) estimated from new, and 0.86±0.09 cubs per adult female and year (n=187 “scar”-sets in 106 females) estimated from new and old scar counts. I found a shift in litter production that occurred at 3-4 years of age, and evidence to suggest a continuing increase in productivity occurred at 4-5 years of age. The proportion of females with old scars was lower than the proportion with new until the age of 6 years. I conclude that first-time breeders are uncommon above the age of 6 years, and females may reach the prime years of adulthood by the age of 6-7 years. The presence of placental scarring from 2 consecutive years revealed incidents of litter loss in 8 of 66 females with placental scars. Total litter loss was more common when females raised singleton cubs in comparison to those raising multi-cub litters. I found evidence to suggest cub survival could as well be affected by maternal age and primiparity, but had insufficient data to ensure. Young, physically immature females may have difficulties sustaining a litter until den emergence. In the uterus of 4-year-old female, I found anecdotal evidence for a resorption of a singleton foetus in the winter prior to harvest. This presumed resorption-scar still has to be verified. The evidence of lactation in 12.5% (n=40) of the females that had new placental scars and were hunted in September, may indicate problematic incidents of cub orphaning due to autumn-harvest in Scandinavia.

ZUSAMMENFASSUNG

Die Analyse der Uteri (n=181 vollständige und 8 unvollständige) von skandinavischen Braunbärinnen (*Ursus arctos*) aus den Abschussjahren 1992 und 1997-2005, aus den 3 Kerngebieten weiblicher Fortpflanzung in Schweden, bot die einmalige Möglichkeit die Reproduktionserfolge von „12“ Jahren offenzulegen, und damit zu einem besseren Verständnis der Life-History von Braunbären beizutragen. Ich testete die Verlässlichkeit des Zählens plazentaler Narben in den gefärbten Uteri für die Schätzung weiblicher Reproduktionsparameter (wie Alter bei der Primiparität, Wurfgröße, Reproduktionsrate und Jungenmortalität) durch den Vergleich zwischen geschätzter und beobachteter Wurfgröße in einer Anzahl an Weibchen mit bekannter Reproduktionsgeschichte. Das Färben des Uterusgewebes erwies sich dabei als unentbehrlich für das Sichtbarmachen und Klassifizieren von 49.0% (n=98) oder zumindest 20.4% (n=98; bei bekannter Klassifizierung von orange-farbigen Narben) der neuen und 80.3% (n=66) der alten plazentalen Narben. Darüber hinaus versuchte ich die Verlässlichkeit der Methode unter Berücksichtigung folgender Informationen, bzw. Aspekte zu evaluieren: den gesammelten Laktationsnachweisen, dem Uterusstatus (Entwicklungszustand des Endometriums), dem Erscheinungsbild von plazentalen Narben in Abhängigkeit des Narbenalters und Umgangs mit den Proben, sowie den Ergebnissen der geschätzten Reproduktionsleistung im Vergleich zu der im Freiland beobachteten Leistung in der Literatur. Die plazentalen Narben blieben in den untersuchten Weibchen im Allgemeinen für 21 Monate postpartum erhalten. Gefärbte, neue plazentale Narben erschienen kräftig, für gewöhnlich dunkel pigmentiert, und zeigten ein vollständiges Narbenmuster. Gefärbte, alte plazentale Narben dagegen, erschienen verblasst und zeigten nicht mehr das vollständige Narbenmuster. Das Zählen plazentaler Narben zur Abschätzung der Wurfgröße wurde bereits bei einer Reihe von Säugetieren angewandt, mit dem wiederkehrenden Schluss, dass die verlässlichsten Ergebnisse durch das Zählen von ausschließlich dunklen Narben zu erzielen sind (z.B. beim Rotfuchs, *Vulpes vulpes*: Elmeros et al. 2003, Ruette und Albaret 2011). Die Konsequenz für diese Arbeit wäre, nur die neuen Narben der Kategorie 1 und Kategorie 2 in den Uteri der Bärenweibchen zu zählen. Werden jedoch hinderliche und artspezifische Faktoren (wie etwa potentielle Verwechslungen zwischen Narben-Sets unterschiedlichen Alters beim Interpretieren intermediärer Narbenphänotypen, die 2- und 3-jährigen Reproduktionszyklen, das Auftreten von Jungenmortalität) bei der Untersuchung von skandinavischen Braunbären nicht außeracht gelassen, so empfehle ich eine Schätzung der mittleren Wurfgröße auf Basis neuer und alter

plazentaler Narben. Diese Information über die weibliche Fortpflanzungsleistung in den letzten beiden Saisonen vor dem Abschuss kann, meines Erachtens, die natürlichen Verhältnisse besser widerspiegeln und eher zu soliden Schätzungen führen. Mein Ansatz entspricht damit der für Bären beschriebenen Methode (etwa für den Hokkaido Braunbär, *Ursus arctos yesoensis*: Tsubota et al. 1990; Mano und Tsubota 2002, Amerikanischen Schwarzbär, *Ursus americanus*: Hristienko et al. 2004; Japanischen Schwarzbär, *Ursus thibetanus japonicus*: Yamane et al. 2009), nur dass hier gefärbtes Narbengewebe evaluiert wurde.

Eine deutliche saisonale Entwicklung des Endometriums, in Abhängigkeit ovarieller Aktivität, war häufiger in den Uteri der im Herbst geschossenen Weibchen mit alten Narben zu erkennen, als in jenen mit neuen Narben. Sie fehlte allgemein in den Uteri der im Frühjahr geschossenen Weibchen. Die erstmaligen Aufwölbungen am Endometrium in einer verdickten Uteruswand bei den Zweijährigen (14.3%; n=28) lieferten Hinweise auf den Eintritt der Jungweibchen in die Pubertät und ihre mögliche Rezeptivität. Das Mindestalter der Bärinnen bei ihrer Primiparität lag im südlichen und zentralen Studiengebiet bei jeweils 3 Jahren, im nördlichen dagegen bei 4 Jahren. Da in den Uteri dieser primiparen Weibchen keine Anzeichen für Folgejahr-Geburten zu finden waren, schien es auch das Mindestalter für die erste erfolgreiche Wurfaufzucht zu sein. Die nachgewiesenen Würfe umfassten 1-4 Jungen, wobei 2-3 Junge am häufigsten waren. Die mittlere Wurfgröße betrug 2.45±0.11 (n=40) basierend auf der Zählung der neuen plazentalen Narben, und 2.22±0.10 (n=74 Narben-Sets in 66 Weibchen) basierend auf der von neuen und alten Narben. Gemäß der Monte-Carlo-Simulation brachten die 3 Jahre alten, primiparen Mütter signifikant kleinere Würfe hervor als die Mütter im Alter von 4 Jahren. Die Schätzungen basierend auf neuen und alten Narben weisen auf einen Anstieg der Wurfgröße im Alter von 3-5 Jahren hin. Die Schätzung der Produktivität einer Population anhand des Registrierens plazentaler Narben ist kritisch, wenn die Uteri einer Jagdstichprobe als Grundlage dazu dienen. Man kann davon ausgehen, dass der Anteil solitärer Weibchen nicht repräsentativ, und die ermittelte Reproduktionsrate damit stark verzerrt sein wird. Vorbehaltlich ihrer Bias-Anfälligkeit betrug die Produktivität 0.90±0.12 Junge pro adultem Weibchen und Jahr (n=106) nach Schätzung anhand neuer plazentaler Narben, und 0.86±0.09 Junge pro adultem Weibchen und Jahr (n=187 „Narben"-Sets in 106 Weibchen) nach Schätzung anhand neuer und alter Narben. Ich konnte eine deutliche Veränderung in der Produktivität der Weibchen im Alter von 3-4 Jahren nachweisen und fand Indizien für eine fortlaufende Produktivitätssteigerung unter den

Weibchen im Alter von 4-5 Jahren. Der Anteil der Weibchen mit alten Narben lag bis zum Alter von 6 Jahren unter jenem mit neuen Narben. Ich schließe daraus, dass Primiparae über das Alter von 6 Jahren hinaus selten werden, und die Weibchen ihre „Blütejahre“ etwa im Alter von 6-7 Jahren erreichen. Das Vorhandensein von plazentalen Vernarbungen aus 2 Folgejahren konnte die Wurfverluste von 8 aus 66 Weibchen mit Narben offenlegen. Der Verlust eines gesamten Wurfs trat häufiger bei Würfen mit einem einzelnen Bärenjungen auf als in den größeren Würfen. Ich fand zwar Hinweise, dass das Überleben der Jungen auch durch das mütterliche Alter und die Primiparität beeinflusst werden könnte, jedoch waren die Daten zur Bestätigung limitiert. Für junge, physisch unreife Weibchen könnte es schwierig sein einen Wurf bis zum Auftauchen aus dem Winterquartier durchzubringen. Im Uterus einer 4-jährigen Bärin fand ich einen anekdotischen Hinweis auf eine Resorption eines Einzelfötus im Winter vor dem Abschuss. Für diese mutmaßliche Resorptions-Narbe ist die Bestätigung allerdings noch ausständig. Der Milchnachweis in 12.5% (n=40) der Weibchen, die neue plazental Narben in ihren Uteri aufwiesen und im September geschossen wurden, könnte auf problematische Zwischenfälle bei der herbstlichen Bärenjagd in Skandinavien hinweisen, in Zuge derer Bärenjunge verwaisten.

INTRODUCTION

Knowledge of the reproductive performance of the brown bear (*Ursus arctos*) is essential for understanding the life-history of this species. An organism's life-history can be described as its lifetime pattern of growth, reproduction and mortality, formed by a long-term evolutionary process, but it may also result from an immediate response of an organism to the environment to which it is exposed (Begon et al. 1990). Life-history theory focuses on the phenotypic variation of demographic traits of populations (Stearns 1992), like that expressed in the allopatric brown bear populations dispersed throughout the Holarctic (Arctic, high to low temperate zone: Spady et al. 2007; already extirpated in tropical Mexico: Brown 1985, Mattson and Merrill 2002), and the maximization of phenotypic fitness under the influence of natural selection. Natural selection involves the selective pressure through humans that can induce altered, respectively suboptimal life-history strategies, to particularly mention the effects of selective harvesting on phenotypic evolution in exploited populations (Law 2003, Zedrosser 2006a, Bischof et al. 2009, etc.).

Human persecution is a principle reason for the decline and the extinction of the brown bear in many parts of the world (Servheen et al. 1999, Swenson et al. 2000). This is aggravated by human-caused environmental alteration, such as habitat fragmentation due to intensive land development. Hence, the brown bears' range diminished in the course of history, and nowadays in Europe, large and viable populations are only found in eastern and northern Europe (Swenson et al. 2000, Zedrosser et al. 2001). In Sweden conservation measures were implemented at the end of the 19th century (Swenson et al. 1995), and in 2005 the population size of brown bears was already estimated to be between 2350 and 2900 (Kindberg and Swenson 2006, Bischof et al. 2009), even with a legal annual harvest quota of approximately 5% (Bischof et al. 2008). Yet, conservation of large carnivore species like the brown bear is challenging, particularly that of small, isolated populations (e.g. in south-central and south-western Europe: Swenson et al. 2000, Zedrosser et al. 2001) as inbred individuals may fail to pursue changes in the optimum phenotype. Reproductive performance under investigation provide the basis for appropriate conservation measures of these species, and as a key issue in studies of population dynamics, it is moreover of indispensable need for management decision at population level. In order to maintain an adequate population size and well-balanced ecosystems in the bear habitats, to estimate the rates of increase and set sustainable harvest quotas, brown bear management is reliant upon long-term data of the age at

primiparity, body size and mass at maturity, litter size, inter-litter intervals, cub mortality, age at senescence, sex ratio, etc.. It enables to monitor the evolutionary development of these traits, and reveal the underlying causations of their progressing.

Brown bear populations are characterized by relatively low rates of increase (Bunnell and Tait 1981, Miller 1990). Individuals are sparsely distributed, long-lived (k-selected strategists), and usually difficult to monitor. Therefore, breeding data from field observations usually involve small sample sizes and gaps in observational coverage, despite high time investment and project costs. Measuring the levels of sex steroid hormones in the blood or faeces of brown bears steadily to monitor their reproductive profiles is feasible only to gain breeding data from captive animals. The steroid profiles can provide evidence for the timing of ovulation (e.g. in Japanese black bears, *Ursus thibetanus japonicus*: Yamane et al. 2009), and the timing of corpus luteum reactivation at implantation, thereby delimitating the period of delayed implantation (e.g. in Hokkaido brown bears, *Ursus arctos yesoensis*: Tsubota et al. 1987; Asiatic black bears: Sato et al. 2001). Ultrasonography can be applied on anesthetized animals to visualise the foetal growth during the post-implantation period (e.g. Tsubota et al. 1987). However, in wild populations substantial knowledge of the reproductive biology has to be obtained by methods such as: direct observations from the ground or a helicopter, the temporal immobilization of bears to take biometrical measurements, draw blood, and collect tissue and hair samples (Bischof et al. 2009), the search for cub traces or remains at den sites, the collection of fresh faeces, as well as through hunter observations. Biotelemetry enables to constantly track the marked subadult and adult individuals during the active period (Bischof et al 2009), and to assess their spatial distribution (Zedrosser et al. 2006a). Furthermore, infrared cameras could be applied to monitor bears, imaginably mothers with their neonatal cubs, in the den during winter. Although this might be an appropriate approach to investigate the in-den cub mortality in American black bears (Miller 1994), a method that involves a hibernal visit of brown bear females is too precarious for humans to be advisable (Zedrosser et al. 2006b). Another approach is reconstructing reproductive history in female bears from their dental cementum. Prolonged lactations, as well as lactation intervals can be determined by the cementum layering patterns of a premolar bear tooth. This method, though successful in some American black bear populations (e.g. Rogers 1975, Coy and Garshelis 1992, Hristienko et al. 2004: a tooth be read in conjunction with examining a reproductive tract), was unreliable for brown bear populations (Matson et al. 1999).

Because uteri of harvested female brown bears are collected in Sweden (Bischof et al. 2009), the estimation of litter size from placental scar counts can offer a valuable support for the assessment of female reproductive performance in wild-ranging populations. Placental scars provide a record of breeding events within a female's uterus for a certain period of time post-partum, and therefore a unique chance to close gaps in observational data post-mortem. An aspect of post-mortem analyses of uteri to pay attention to is that harvest samples of females are possibly demographically biased by the selectivity of harvest (e.g. McLellan and Shackleton 1988, McLellan et al. 1999, Bischof et al. 2008), in particular by the selection against females with cubs (Hristienko et al. 2004). Due to the legal protection of family groups (Bischof et al. 2008) solitary females are more vulnerable to be harvested than females accompanied by their young.

In the reproductive tract of female bears, the characteristic zonary endotheliochorial placenta of carnivores is interrupted on the mesometrial side. Instead, the zone forms a curved disc and the surrounding haemophagous region of tissue is ring-shaped (Mossman 1987). According to this zonary gross morphology of the chorion-endometrium attachment, a distinct implantation site is formed for each foetus in a female's uterus (Erickson et al. 1964, Kaufmann and Burton 1994). In the haemophagous region, or haemophagous organ (Renfree 1982) at the edges of the placenta, blood cells from maternal haemorrhage are phagocytised to provide iron and other nutrients for the developing foetus. The blood escaping into this tissue is mostly part phagocytised by neighbouring placental cells, but yet some blood is taken up by macrophages in the closely associated endometrium. In these endometrial macrophages, the blood is then converted into yellowish granules of hemosiderin, an unsolvable, intracellular iron-storage complex. This explains why distinct areas of the endometrium contain a high density of hemosiderin-laden macrophages and appear darker than the surrounding endometrial tissue (Mossman 1987 in Hristienko et al. 2004). At parturition, the placenta is rejected and these dark pigmented marks, the *placental scars* are left behind as evidences for the successful placentation at each implantation site in the female uterus (Wydoski and Davis 1961, Martin et al. 1976). Due to the slight loss of uterine tissue in the course of the separation of the placenta during parturition, the carnivore placenta is moreover classified as placenta semideciduata (Dyce et al. 1991).

Deno (1937, 1941) described the formation of placental scars and the involution within the uterus in mice, *Mus musculus*, and placental scars have already early been used as a measure

of fertility in rats, *Rattus norvegicus* (Davis and Emlen 1948). The method to count placental scars has thereupon been used in a wide range of mammals, including American black bears, *Ursus americanus* (Erickson et al. 1964, Kordek and Lindzey 1980, Hristienko et al. 2004), prairie voles, *Microtus ochragaster* (Martin et al. 1976), brown bears (Tsubota et al. 1990), Arctic foxes, *Alopex lagopus* (Strand et al. 1995), Spiny rats, *Niviventer coxingi* (Yu and Lin 1999), European hares, *Lepus europaeus* (Bray et al. 2003, Hackländer et al. 2004), red foxes, *Vulpes vulpes* (Elmeros et al. 2003, Ruette and Albaret 2011), American minks, *Mustela vison* (Elmeros and Hammershøj 2006), Asiatic black bears (Yamane et al. 2009) etc., for the estimation of litter size, to evaluate its accuracy for litter size estimations, and to improve the accuracy of the method by bleaching or staining the uterine tissue. Yet, placental scars are no evidence for a successful full-term pregnancy. Post-implantation resorptions of foeti or abortions of embryos or stillborn cubs may leave behind scars similar to that after a successful full-term gestation, which results in an overestimate of the true litter size (Wydoski and Davis 1961). Discrepancies between estimated and true litter sizes may also result from the inability to distinguish between recent and older sets of placental scars, because in some species placental scars from more than one breeding cycle are visible (Lindström 1981, Wandeler and Lüps 1993, Elmeros et al. 2003). At the same time, if scar tissue regenerates rapidly, it may result in an underestimation of true litter size. Tsubota et al. (1990) found that old scars sometimes disappear at different rates. These individual variations in the intensity of pigmentation of similar-aged scars were reported for several carnivore species (e.g. Strand et al. 1995, Elmeros et al. 2003, Elmeros and Hammershøj 2006). The embryogenesis and birth of monochorial twins may also result in an underestimation of true litter size.

Placental scars were found to persist for more than a year postpartum in American black bears (Erickson et al. 1964) and brown bears (Tsubota et al. 1990), necessitating an accurate classification. Martin et al. (1976) classified scars based on their shading of darkness, because their appearance fades over time. The hemosiderin-laden endometrial macrophages left behind after parturition migrate through the uterine endometrium towards the myometrium, and mesometrium, and finally deteriorate. Simultaneously new endometrial cells grow from the edges of the wound (Bray et al. 2003), implying that placental scars will become smaller in diameter. Tsubota et al. (1990) classified the scars in brown bears according to their diameter: 5-10mm in recent scars, and ~2mm in old scars. Yamane et al. (2009) classified the scars in Asiatic black bears additionally according to their colour: ≥5mm and reddish brown pigmentations in new scars, and <5mm and black pigmentations in old scars. A number of

authors focused on the intensity of the pigmentation in an attempt to differentiate between placental scars from the immediately previous parturition, earlier parturitions, or resorptions, but recommended only the counts of dark (i.e. new) scars for reliable litter size estimations (e.g. Ruette and Albaret 2011, Elmeros et al. 2003, Elmeros and Hammershøj 2006). In American black bears, Erickson et al. (1964) found older placental scars to fade more rapidly than new scars, when a uterus was exposed to formalin. Kordek and Lindzey (1980) used the bleaching effects from formalin to minimise the chance of misclassifications between old and new scars. Hristienko et al. (2004) recommended the use of a light box for the identification of very faint, difficult-to-age scars, although they did not recommend the count of these scars for litter size estimations. In the American mink, faint placental scars in the most regressed state were described to be reduced to small orange-pigmented spots in the uterine tissue (Elmeros and Hammershøj 2006). Although placental scars of light shade may originate from earlier parturitions, because their appearance fades over time (Erickson et al. 1964), they may as well represent prenatal mortalities as demonstrated in foxes (Lindström 1981, Strand et al. 1995). In red foxes, staining the uterine tissue clearly facilitated the identification of atypical scars, i.e. scars form earlier parturitions or scars from resorptions and abortions, however, this method did not allow their differentiation (Ruette and Albaret 2011). In bears the very small scars sites were discussed to be from resorptions or abortions of foeti that occurred in the course of placental formation (Hristienko et al. 2004), though they may just as well originate from earlier full-term gestations (Yamane et al. 2009).

Researchers have used different methods to obtain reliable litter size estimations. However, all the findings above can reflect how different the appearance of scars in diverse mammalian species is, how different their perception and interpretation by various authors may be, and even how dissimilar scars appear to be within the same species at times. – So, my question: "Can it indeed be possible to provide a standard for placental scar counts, leading to reliable estimations of the female reproductive performance"?

Unresolved issues, related theories and objectives of this thesis:

1 Reliability of litter size estimations based on placental scar counts – does staining improve the method?

1.1 Benefits of staining for placental scar counts

It is a well-described phenomenon that placental scars fade over time after parturition, because hemosiderin-laden endometrial macrophages migrate towards the outer uterine layers and mesometrium, and finally deteriorate (Martin et al. 1976). A staining method developed for rat (*Rattus rattus*) uteri by Salewski (1964), has proven beneficial for placental scar counts in European hares (Bray et al. 2003), and red foxes (Ruette and Albaret 2011). Its gainful effects were particularly apparent in placental scar tissue towards the end of scar persistency-time. Staining the uterine tissue during the course of scar counts resulted in more comparable and repeatable results at interpreting scars (Ruette and Albaret 2011), as well as in solid estimations of mean values of breeding parameters (Bray et al. 2003). In issue 1.1 I focus on the benefits of the staining method for placental scar counts in Scandinavian brown bears, comparing the number of visible scars before and after staining. Additionally, I analyse the benefits of staining to assess the characteristics of scar features throughout tissue regeneration by means of pre-post comparisons of my scar classification-efforts.

1.2 Estimation of litter size based on placental scar counts

Understanding the scar regeneration process and identifying characteristic features of different-aged scars is fundamental for litter size estimation based on scar counts. Indeed, in species with persistent placental scars from more than one breeding cycle, the difficulty of distinguishing between recent and older sets of placental scars is a frequently debated element of uncertainty (e.g. Lindström 1981, Wandeler and Lüps 1993, Elmeros et al. 2003). In bear species such as the American black bear (Hristienko et al. 2004), Asiatic black bear (Yamane et al. 2009), and brown bear (Tsubota et al. 1990), new and old placental scars can be identified in the uterus. New scars were confirmed to be from the immediately previous gestation, and old scars from the gestation of more than a year, occasionally even 2 years before the female was killed.

The process of scar tissue regeneration in female bears is most likely affected by local conditions in, respectively specific adaptations to their habitat. Alterations in length of hibernation and the time until weaning, etc. may modify the endocrine activities, uterus involution and uterus development in the females considerably enough to suggest investigations at population level, even though placental scars have been evaluated in closely related taxa. In chapter 1.2 I aim to calculate the time of persistency of placental scars in brown bears in Scandinavia, to improve the understanding of changes in the scar-appearance during regeneration and to systematically classify placental scars according to their age-dependent characteristic features.

Placental scars are an important source of information on bear reproduction. The reliability of placental scar counts for litter size estimations has been evaluated in a number of species like arctic foxes (Strand et al. 1995), red foxes (Elmeros et al. 2003), and American minks (*Mustela vison*, Elmeros and Hammershøj 2006), while that of stained scar counts has been assessed in European hares (Bray et al. 2003) and red foxes (Ruette and Albaret 2011). The guiding principle applied in all of these studies, the comparison between estimated and truly observed litter sizes, requires a sufficient number of females with evidence of breeding. In brown bears, corroborative evidence from confirmed family groups is usually scarce. Tsubota et al. (1990), in their study of Hokkaido brown bears, based their comparison on the available breeding evidence for 15 females with cubs, yearlings, or dependent 2-year-olds. To my knowledge, staining the uterine tissue in order to estimate female reproductive performance has not yet been applied in the brown bear. My litter size estimations based on scar counts in the stained uteri of Scandinavian brown bears with known reproductive history should clarify if it is possible to accurately distinguish between new and old placental scars. Therefore, it is tested if the method provides a solid standard for estimations of reproductive parameters. Available data from positive lactation of nursing bear mothers by the time of harvest can provide additional breeding evidence, and may thus support the results.

Placental scars per se are no evidence for a successful full-term pregnancy (e.g. Wydoski and Davis 1961). According to Hristienko et al. (2004) scar sites <3mm are believed to be from foeti that did not appear to develop to full term. But is it possible to distinguish between placental scars from successful full-term gestations and scars from abortions of embryos during the late gestation period? With progressing tissue regeneration new endometrial cells grow from the edges of the wound (Bray et al. 2003), thus old scars may appear smaller as

well. In addition, light-shaded scars may originate from earlier parturitions (Erickson et al. 1964), but they may as well represent prenatal mortalities (Lindström 1981, Ruette and Albaret 2011) as lesser blood cells from maternal haemorrhage are taken up and phagocytised in the endometrium. Is it, therefore, possible to distinguish between old placental scars and scars formed during resorptions of foeti? My investigations of potential scars from resorptions or abortions may show.

2 Estimation of female reproductive performance

In issue 2 the estimated reproductive performance through placental scar counts in Scandinavian brown bear females will be discussed with reference to the reported performance in the field and patterns found in the literature. Analyses of the uteri are expected to provide a backup of female breeding data, like the in-den litter size, and unobserved litter production and litter sizes in the field, at least for the breeding season before harvest. Data of the in-den litter size is particularly interesting, as it cannot be recorded by direct observations in the field. Data of the litter production and litter sizes are generally expected to reveal unobserved breeding events in the field. Evidence from observed family groups is usually relatively scarce in wild-ranging bears, even if bears are intensively monitored like in Scandinavia (525 individuals were followed throughout a period of more than 20 years: Bischof et al. 2009). In addition, the available uteri samples may provide evidence of female age at primiparity and of cub mortality.

2.1 Age at primiparity

A key event in life history is the timing of first birth, and may decisively affect a female's lifetime reproductive success. Age at primiparity is reported to be a reproductive trait particularly sensitive to local conditions for bears (Noyce and Garshelis 1994, Ferguson and McLoughlin 2000). Faster-growing and larger females usually reproduce earlier in life than smaller females (Stearns 1992), whereby body mass and growth primarily depend upon sufficient food supply (Rogers 1977, Bunnell and Tait 1981, Stringham 1990a, Zedrosser et al. 2006a). Considering that primiparous female bears may not have reached maximum skeletal size by the time of first breeding (Schwartz et al. 2003a, Zedrosser et al. 2006a), the trade-off between growth and reproduction (Festa-Bianchet et al. 1995), and between current and future reproductive success (Williams 1966) provide a solid theoretical basis for

relatively low performance in first-time breeders (Künkele 2000). If the timing of primiparity results from an immediate response of young females to environmental conditions in the habitat, knowledge about its timing cannot be easily transferred among brown bear populations. In this sense, the earliest age at primiparity is intended to be assessed for Scandinavian brown bear females by recording the earliest presence of placental scars in the available uteri samples. I hypothesise that young, not yet fully-grown females still have to invest intensively in their growth, which may occur at the cost of reproduction. Their reproductive output in terms of cubs per litter is likely to be lower than that of physically mature bear mothers. A female bear may promote compensatory growth by delaying sexual maturity to attain larger body size (Taylor 1994 in Zedrosser et al. 2006a). If delayed maturity leads to further growth, and larger body mass is correlated with increased reproductive performance, then delayed maturity leads to higher performance in the first breeding attempt (Stearns 1992). I presume that starting the reproduction earlier may result from favourable conditions in the habitat and thus be an opportunistic response of young females. Negative selection due to altered environmental conditions may however keep the number of sexually premature females in the population low.

Traits, such as the presence of corpora lutea, placental scars and cubs, can be indicative to determine if a female is sexually mature. Moreover, the condition of the genitalia may be used as evidence that a female is already receptive and may give birth to young in the following winter (Stringham 1990b). In ovulating females, one would expect an ovary-induced endometrial development, i.e. cell proliferation and growth during the period of delayed implantation, in order to prepare the uterine environment for implantations. The uterus is expected to be thick-walled, or thick-walled and convoluted from August to October (Yamane et al. 2009).

2.2 Litter size

The comparison of the litter sizes estimated from scar counts with the litter sizes confirmed by field-observation should help to assess the reliability of scar counts in stained uteri. To start from the premise that placental scar counts correspond with the placentation rate after successful implantation, at best the parturition rate, and abortions of embryos, stillborn cubs, as well as post-natal mortality of cubs may occur, I expect the estimated litter size based on placental scar counts to be higher than the litter size observed in the field. If, by contrast, scar

counts would lead to an underestimation of mean litter size, the method would prove to be poorly reliable. Despite the high ambitions to observe mothers with cubs-of-the-year, or to capture and mark mothers with yearlings in Scandinavia in early spring (Arnemo and Fahlman 2007, SBBRP 2013), there may be an informational lack on litter sizes due to gaps in observational coverage and offspring mortality prior to the spring observations. Placental scar counts may help to acquire the litter size at parturition. However, to confirm incidents of neonatal cub loss one is in turn reliant on litter size observations in the respective spring.

Estimations of the litter size by placental scar counts may be biased by the age distribution of the females examined. Zedrosser et al. (2009) found that young, primiparous brown bear mothers had smaller litters than multiparous females. This is because young females still have to grow, bearing high costs for their physical maturation. It is also supported by the frequent reports of larger females that produce more offspring and offspring of better quality than smaller females (e.g. Stearns 1992). According to the model by Schwartz et al. (2003b), the onset of primiparity and litter production in younger brown bears is a gradual process that builds to a maximum (around age 8) when the females reach the prime years of adulthood. Prime-aged females were estimated to have the highest fertility. With this current knowledge in mind, I expect a reduction of the estimated mean of litter sizes if uteri samples from young and primiparous females with placental scars are overrepresented in the sample collection. In addition, this may apply also to old females, because senescence effects may occur in female brown bears (Craighead et al. 1995a, Schwartz et al. 2003b). It is important to note that Schwartz et al. (2003b) modelled the age-specific probability of litter production, whereas the age-specific litter size in brown bears still needs to be disclosed in detail. I hypothesise that litter size as estimated by placental scar counts increases with age as long as the bear mothers have not reached their physical maturity. As soon as they have reached their full body size, litter size development is expected to occur independent of age. Depending on the time of persistence of placental scars, I may succeed to assess the mean litter sizes of earlier gestations beside the mean litter size for the cubs-of-the-year. In American black bears (Erickson et al. 1964) and brown bears (Tsubota et al. 1990), scars were found to persist for more than a year postpartum, although they faded and became smaller in diameter over time. Comparing the mean values of the estimated litter size, I presume that the mean litter size based on older scars is likely to be lower than the mean litter size based on new scars. The prediction is: Estimates of the litter size based on older placental scars are vulnerable to underestimate true litter size due to the progressing endometrial regeneration.

2.3 Population productivity

Population productivity, or reproductive rate, is the mean number of offspring raised per adult female per year. Per capita rates of reproduction and survival tend to be positively related to nutritional status and food supply as documented for several bear species, including the brown bear (Stringham 1980, 1985, 1986, 1990a; Bunnell and Tait 1981). Due to the variation in the productivity among brown bear populations (Ferguson and McLoughlin 2000, Nawaz et al. 2008), the population productivity in Scandinavia is of great interest for local management decisions. The productivity is determined by both, the mean number of cubs of the year, and the proportion of adult females without cubs of the year. Considering the 2- to 3-year reproductive cycle of female Scandinavian brown bears (Dahle and Swenson 2003a, Dahle and Swenson 2003b), the adult females without cubs of the year can theoretically be divided in those weaning their yearlings and mating again, those with dependent yearlings, and those not being reproductively active. Here a question arises: Is the proportion of females that are not reproductively active, the proportion with the 2-year reproductive cycle, and that with the 3-year cycle, in the sample of examined females, representative of all female bears in the Scandinavian population? Because it is not legal to harvest females with dependent offspring (Bischof et al. 2008), the harvest sample of females is likely biased in favour of solitary females, and females with a 2-year reproductive cycle are more vulnerable to be harvested than females with a 3-year cycle. My estimates of the productivity of the Scandinavian bear population, which are based on placental scar counts, i.e. a harvest sample of females, may underestimate true reproductive rates and therefore be lower than reported rates based on observational data.

2.4 Cub mortality

It is not unusual that young bears with their mothers experience mortality (Bunnell and Tait 1985, Swenson et al. 2001). Several factors have been proposed as important for cub survival, such as nutritional, social and disturbance factors. Among these factors, the cub loss in Scandinavia was best explained by social factors, i.e. sexually selected infanticide, in particular in the south (Swenson et al. 2001).

Brown bear females do not reproduce every year (Schwartz et al. 2003a, Schwartz et al. 2003b, Dahle and Swenson 2003a, Dahle and Swenson 2003b). Therefore, I should be able to

identify incidents of cub loss in the females in this study if I succeed to find placental scars from consecutive-year birthing. Mothers that give birth to a single cub are expected to have a higher probability to lose their litter than mothers of multi-cub litters, as predicted by the parental investment theory, because the defence of offspring should be related to the reproductive value of the offspring (Maynard-Smith 1984). Moreover, the risk of cub mortality is assumed to be higher for primiparous mothers than for multiparous mothers. Primiparous mothers may be less experienced in skills like foraging and parental care (Becker et al. 1998, Wang and Novak 1994). In addition, they may be less efficient defending their cubs against infanticidal males, have less knowledge of local dominance hierarchies, and less experience in avoiding potentially infanticidal males (Zedrosser et al. 2009).

The research questions are:

1 The reliability of the method of litter size estimations based on placental scar counts in the brown bear – does staining improve the ability to identify placental scars and categorize them according to their age?

1.1 The evaluation of the benefits of staining for placental scar counts

1.2 The evaluation of the accuracy of the litter size estimations based on placental scar counts

- How long do placental scars persist postpartum?
- Is the number of observed offspring in the field consistent with the number of placental scars counted in the uterus of female bears?
- Is it possible to identify scars from resorptions or abortions?

2 The estimations of female reproductive performance based on placental scar counts

2.1 The estimation of the age at primiparity

- Are there any primiparity-effects on the litter sizes?

2.2 The estimation of the litter size

- Does age at parturition, the study area or the year at parturition influence the litter size?

2.3 The estimation of the population productivity

- Does age at parturition, the study area or the year at parturition influence the population productivity?

2.4 The analysis of the cub mortality

- Is the risk of cub mortality dependent on age at parturition, the litter size, the study area or the year at parturition?

MATERIALS AND METHODS

Study population and study areas

The Scandinavian brown bear population is structured into 3 subpopulations based on genetic data (Manel et al. 2004), which also coincide with the 3 areas of female concentration. These core areas for reproduction are connected by male-mediated gene flow (Manel et al. 2004). I obtained samples from all 3 areas within the habitat range in Sweden (Figure 1), and based my analysis on the "inferred" spatial structure found by Manel et al. (2004). The southern core area was located in the counties of Gävleborg, Dalarna, and southwestern Jämtland in south-central Sweden. The central core area was located in northeastern Jämtland, Västernorrland, and Västerbotten in north-central Sweden. The northern study area was in Norrbotten County in northern Sweden (Bischof et al. 2008). Overall these three areas covered 292 000 km² and extended from ~60 to 69 degrees of latitude. They occurred within the southern, intermediate, and northern boreal vegetation zones with cool, humid climate, characteristic coniferous forests (Bischof et al. 2008), and clear-cut forestry as the primary land use (Nordisk ministerrådet 1984, Bernes 1994). The areas are primarily characterised by a rolling landscape, but there are also mountainous areas. The dominating tree species are Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), and birches (*Betula spp.*) (Zedrosser et al. 2006a). The vegetation period in the southern study area is ~150 to 180 days, but decreases to ~110 to 130 days in the north (Moen 1998). Because the snow-free period is shorter in the north, these areas are less productive and their carrying capacity is lower than in the southern areas (Dahle et al. 2006). Bear densities range from ~30 individuals/1000 km^2 in the south to ~11 individuals/1000 km^2 in the north (Støen et al. 2006a).

Bear hunting and sample collection

Brown bears in Sweden can be legally hunted from late August, usually August 21st, until the annual quota is filled. Family groups are protected regardless of the age of the dependent offspring. The harvest methods applied by hunters from 1986-2005 comprised stalking, still hunting, hunting with dogs and, before 2001, hunting over bait (Bischof et al. 2008). Successful hunters are obligated to present the carcass to an official inspector on the day of the harvest, and provide information about the harvest site and date, and the method of hunting. The sex and body mass of the bear are recorded, and various biological samples,

including the first premolar, skin, hair, reproductive organs and particular tissue, must be handed over to the authorities (Bischof 2008). The age of a killed bear is estimated by counting cementum annual layers on the first premolar (Matson et al. 1993, Craighead et al. 1970).

For this study, the reproductive organs of 259 females were available, provided by the National Veterinary Institute of Sweden. These females were legally hunted during the April to October in the years 1986, 1992, and 1997-2005. The main proportion (240 of 257 with known harvest date) of the females was shot from late August to October and only few females were shot prior to the start of the official hunting season (Table 1). Organ samples were soaked in water to avoid dehydration, and then stored frozen at -18°C until further analysis. Supplemental data, such as evidence from confirmed family groups or lactation, were collected as part of the field activities of the Scandinavian brown bear research project group.

Brown bear reproduction and reproductive physiology

Like many other species of the temperate zone, brown bears exhibit obligate reproductive seasonality and are physiologically limited to a single reproductive cycle annually (Spady et al. 2007). Oestrus and mating occur in late spring to early summer, implantation is obligatory delayed during the gestation period (Hamlett 1935, Wimsatt 1963, Kordek and Lindzey 1980), and parturition takes place during the hibernation period. In concordance with the change of season, the uterine tissue develops, i.e. endometrial cell proliferation, growth and convolution proceed under the influence of the sex steroid secretion of the ovaries (Yamane et al. 2009). Thereby, the photoperiod is suggested to act as a principle *zeitgeber* for the synchronisation of the endogenous circannual rhythms of the ovaries with the outer environment (Sato et al. 2001, Tsubota et al. 1998). Social factors (such as behavioural reproductive suppression in young females within a matrilinear assemblage: Støen et al. 2006b; pseudopregnancy in non-mated females after their exposure to a male: Sato et al. 2001), metabolic state, and nutrition can exert strong influence on ovarian cycles, leading to altered secretions of sex steroids in females (Nelson 2000) and affect their reproductive activities.

The breeding cycle starts with females entering into oestrus at the time when their ovaries contain matured, preovulatory follicles. As the follicles grow, they secrete oestrogens. In most species, in order to stimulate behavioural oestrus, individuals have to be primed with oestradiol (Nelson 2000). This increase of oestradiol is then followed by a temporally delayed increase of progesterone (Nelson 2000). Even though the bear oestrus cycle is poorly understood, it is known that serum oestradiol is elevated during the oestrus period, and that progesterone starts to increase gradually after oestrus (Tsubota et al. 1998). At the same time, endometrial cell proliferation takes place in the female uterus during the early mating season, as long as the serum progesterone level is low. Cellular growth in the endometrium is then stimulated by the slow increase of serum progesterone during the period of delayed implantation (Yamane et al. 2009). Mating behaviour coincides with the presence of preovulatory follicles, and males will seek out females in oestrus. Oestrus usually persists for 1 to 18 days, with a mean of 4 days (Spady et al. 2007). Brown bear females are seasonal polyoestrous (Craighead et al. 1995a). Longer oestrus periods, as well as re-entrance into oestrus provide a female more temporal flexibility in finding a mate before ovulation, thus being particularly important for bears with large home ranges, living in low-energy habitats (Spady et al. 2007). Although brown bears may show spontaneous pseudopregnancy, which implies that corpora lutea are formed independently of fertilisation (Tsubota et al. 1987), evidence is mounting that bear females are induced ovulators as demonstrated for American black bears (Boone et al. 2004). In unmated female brown bears, oestrus was observed to last longer than in mated females (Ishikawa et al. 2003), suggesting that copulation, or other sexual stimuli, are required to initiate a series of neural events and lead to the release of matured ova (Boone et al. 2004). Moreover, re-entrance into oestrus provides a female the chance to actively search for mates, to act promiscuous, and thereby confound paternities in order to prevent sexually selected infanticide (Bellemain 2006a). The successive waves of follicular development provide the basis for the serial oestrus periods and allow females to mate with several males (Steyaert et al. 2012).

In bears, like in other carnivores, after ovulation and mating the corpora lutea secrete progesterone throughout gestation in order to maintain pregnancy, or pseudopregnancy, in case the ova had remained unfertilised (Tsubota et al. 1987, Sato et al. 2001). However, female brown bears have an obligate delayed implantation, a phenomenon also referred to as embryonic diapause (Renfree & Calaby 1981). Fertilization in Hokkaido brown bears was found to occur already ~210 days before parturition (Tsubota et al. 1987). During the delay

period, the progesterone secretion of the temporary "dormant" corpora lutea remains low. In accordance with the proceedings in the ovaries, the fertilized ova initially develop to blastocysts, but then undergo a quiescent state and float freely in the lumen of the uterus (Sato et al. 2001). The uterine wall grows thicker as the uterine glands develop and cells in the endometrium grow after May through July-mating season. This uterine development from a regular to a thickened and convoluted uterine wall during the delay period is necessary to maintain the viability of the blastocysts and to prepare the uterine environment for implantation (Yamane et al. 2009). It is the corpus luteum dormancy state that allows a female to re-enter oestrus and encounter further males for additional matings (Spady et al. 2007). Because fertilised ova from distinct oestrus cycles may contribute to a single litter, corpus luteum dormancy thus also facilitates multiple paternities (Craighead et al. 1994). In addition, the pituitary hormone prolactin is required for maintenance of the corpora lutea, and thereby of gestation. The first annual hyperprolacticemia occurs photoperiod-induced under long daylight conditions (Sato et al. 2001), thus the timing of the mating period is constrained. Later in the season, under short daylight conditions, the second annual prolactin increase causes the termination of the embryonic diapause. At corpus luteum reactivation in late November to early December, a dramatic progesterone increase occurs, inducing the implantation of the previously inactive blastocysts (Sato et al. 2001), which occurs ~60 days before parturition (Tsubota et al. 1987).

The active gestation period with successive embryo development is indicated by a higher level of serum progesterone (Tsubota et al. 1987). It lasts for ~6 to 8 weeks and ends with the birth of 1 to 4 cubs during hibernation in the winter den between January and March (Pasitschniak-Arts 1993, Schwartz et al. 2003a). Considering that prolactin is a key hormone controlling the ovarian cycle and that the timing of mating is constrained, there are several approaches to explain the ultimate function and evolution of the embryonic diapause in the brown bear. It has been proposed that the short post-implantation gestation helps to limit the energetic costs of reproduction by shortening the in-utero embryogenesis, which in turn reduces the size of the neonatal cubs and reduces the initial costs of lactation (Spady et al. 2007). In addition, the seasonal delayed implantation effectively uncouples the timing of birth from the timing of oestrus and mating (Sandell 1990). Considering that the appropriate timing of birth is the key factor for the evolution of seasonal reproductive strategies, the most expensive phase of lactation should coincidence with the peak of food supply within seasonal habitats. In fact, lactation peaks around midsummer in the year of the cubs' birth (Steyaert et

al. 2012). Additionally, body fat reserves are essential for the reproductive success of female brown bears (Blanchard 1987, Stringham 1990a, Spady et al. 2007). Switching to lactation after a short in-utero development may help them to preserve muscle mass and protein during hibernation (Ramsay and Dunbrack 1986, Hissa 1997). Besides, the delay of implantation provides females with fertilised ova the opportunity to gain mass when environmental conditions are favourable and to delay parturition and initial nursing to the winter season. Robbins et al. (2012) found female brown bears in superior condition gave birth earlier, and thereby lactate longer in the den, than females in poorer condition. Delayed implantation may thus provide flexibility in timing of birth so that females are able to track environmental and body conditions long after conception to optimise their reproductive output (Robbins et al. 2012). If a female has not gained sufficient body fat reserves by the time she heads into the winter den, she still has the possibility to resorb the fertilized, but not yet implanted blastocysts before the more expensive gestation period. According to Mano and Tsubota (2002), embryo loss and neonatal mortality seem not to be common in brown bears.

By the time of their birth, the altricial cubs weigh ~350g to 500g depending on the litter size and maternal condition (Steyaert et al. 2012). Reports of mean litter size range from ~2.3 to 2.4 cubs per litter in Sweden (Swenson et al. 2001). The mother and her offspring leave their winter den on average in late April and they are active all through the snow-free period until November (Sandegren and Swenson 1997). Cub survival is significantly affected by social, as well as nutritional factors, but also human disturbance appears to be relevant, causing den site abandonment in winter (Swenson et al. 1997b). Swenson et al. (1997a, 2001) proposed that the major social agent for cub mortality in the Scandinavian brown bear population was sexually selected infanticide.

Because bear cubs are not weaned in the first breeding season after hibernation, and nursing and lactation for ~1.4 to 3.5 years (McLellan 1994, Schwartz et al. 2003a) inhibit postpartum oestrus, brown bear females can only re-enter oestrus and mate again in the second breeding season after parturition at the earliest. In the Scandinavian brown bear population, almost all females in southern core area exhibited a 2-year reproductive cycle, which corresponds to the minimum inter-litter interval for successively reproducing females (Dahle and Swenson 2003a, Dahle and Swenson 2003b). Those in the northern areas exhibited a 2- or 3-year reproductive cycle (Dahle and Swenson 2003a, Dahle and Swenson 2003b), with a mean inter-litter interval of 2.6 years (Swenson et al. 2001). However, females that lose their cubs

during the mating season can re-enter oestrus after 2-7 days (Steyaert et al. 2012). In Scandinavia, cub loss frequently occurs during the mating season, supporting the sexually selected infanticide hypothesis (Swenson et al. 2001). Female will leave lactational anoestrus, mate again, and produce a second litter within two consecutive years. The reproductive rate of brown bears, which is defined as the mean number of offspring raised per adult female per year, ranges from 0.23 in the Deosai National Park in Pakistan up to 0.96 in Scandinavia (Steyaert et al. 2012).

The mating system of brown bears can generally be classified as polygamous, as males compete for access to females in oestrus. Yet, the plasticity in reproductive strategies allows bears to appropriately respond to current biological aspects and human impacts (Steyaert et al. 2012). Hunting, for example, can lead to altered reproductive strategies in bear populations, as the loss of adult males causes immigration of new males, and these new immigrants may subsequently kill cubs in order to accelerate the females' reproduction (Swenson et al. 1997a, 2001). Consequently, not only will males seek for females in oestrus, but also females will actively seek for copulations with males (Dahle and Swenson 2003c).

The earliest recorded age of primiparity in female brown bears is 3 years (Zedrosser et al. 2004). In Scandinavia, the mean ages of female primiparity were 4.7 and 5.3 years for the south and north respectively (Zedrosser et al. 2009). Although primiparous females in the south were significantly younger than primiparae in the north, behavioural reproductive suppression evidenced for philopatric females in the south, could lead to a delay in the onset of the breeding activities (Støen et al. 2006b). The life expectancy in wild female brown bears can be at least 34 years (Schwartz et al. 2003b) and their reproductive longevity, around age 27, comes close to their physical longevity (Craighead and Mitchell 1982, Schwartz et al. 2003b). Craighead et al. (1995a), however, recognised that the females' primiparity, as well as senescence can affect litter production. Young 4 to 8-year-old, as well as old 21 to 25-year-old brown bear females had lower fertility than those in their prime-years of adulthood. The age of primiparity in Scandinavian brown bear females is however lower than that in North American, and Zedrosser et al. (2009) found only a marginal proportion (of 5% at the maximum) of first-time breeders among the 7-year-olds.

Sample preparation and examination

The ovaries, oviducts, mesometrium, as well as the connective tissue were removed from each uterus. Both uterine horns were opened longitudinally on the side opposite to the mesometrium, and the endometrium was then examined macroscopically for the presence of placental scars. Based on their macroscopic features I distinguished between new and old placental scars while recording their number. Dark, vibrant scars with a largely complete scar-pattern were presumed to be new, i.e. from the immediately previous winter. They were fairly matching the description of either category 1 or category 2 placental scars in a stained uterus in Table 2.1. Pale, incomplete scars were presumed to be old, i.e. from the winter before last, and were fairly matching the description of either category 3 or category 4 scars in a stained uterus in Table 2.1. In the unstained uteri, scar tissue evaluation was restricted to this differentiation "new" versus "old". In addition, I recorded all types of scars separately that were not matching the samples of either new or old placental scars in an unstained uterus. The atypical "scars" comprised the pale orange-coloured scars (lightly pigmented aggregations of orange granules), the even older looking scars (very faint and fragmentary scars believed to be from gestations that occurred ≥2 years prior to harvest), and all potential artefacts (marks with no recognisable bilateral symmetry).

Afterwards the uterine tissue was stained by use of Turnbull reaction (Salewski 1964, Yamada 1988) to make macroscopic features more obvious and enhance the detectability of old, faded or orange-coloured placental scars. Turnbull blue arises by the reaction of ferrous iron with potassium ferricyanide in acidic solution, and can therefore be used as a stain for the granules of hemosiderin, an unsolvable form of tissue iron, in the endometrium left over from gestation (Yamada 1988). The parts of the endometrium containing large numbers of hemosiderin-laden macrophages, i.e. "the placental scar", will subsequently appear more intensively than the surrounding tissue. Accordingly, the uteri were immersed into a 10% ammonium-sulphide (H_8N_2S) solution for 8 minutes, then rinsed with water, then immersed into a solution of equal parts of 1% chlorhydric acid (HCl) and 20% potassium-hexacyanoferrate ($K_4[Fe(CN)_6],3H_2O$) for further 8 minutes, and finally again rinsed with water (Salewski 1964, Bray et al. 2003, Ruette and Albaret 2011). After this procedure, the placental scars were once more macroscopically counted and classified. In an attempt to describe the appearance of placental scars in distinct stadia throughout tissue regeneration, I outlined characteristic time-related scar-features. I assigned 4 scar categories that documented

these changes in the number, density, and arrangement of hemosiderin-laden macrophages as they migrated towards the outer uterine layers and the mesometrium (Table 2.1) (Martin et al. 1976). At this stage artefacts (e.g. in Figure 9) were clarified and all of the observed placental scars, including the orange-coloured ones (e.g. in Figure 10), however not the even older looking ones, were classified according to the pattern, the intensity, and the colour of their pigmentation as described in Table 2.1. All uteri samples with scars were photographically documented (e.g. in Table 2.1, as well as in Figure 5, 6, 7, 8 and 10). To view the schematic representation, outlining characteristic scar-features of the 4 categories in order to make results more comparable and repeatable, see Table 2.2. According to Hristienko et al. (2004) scar sites with a very small diameter (<3mm) are a result of abortions or resorptions of foeti (e.g. in Table 3). In addition, I was looking for unimplanted blastocysts attached to endometrial folds in the uterus.

Statistical analysis

Choice of normal distribution methods versus non-parametric methods

The data in the study were generally realizations from discrete and bounded distributions. The data values could only take integer values and the range of possible values was limited. Starting from this premise, the distribution of sample means would converge to a normal distribution with increasing sample size. The number of observations was considerably high, so that the approximation of the distribution of sample means by a normal distribution was a reasonable approach. I therefore compared sample means by normal distribution methods, using paired and unpaired T-Tests, and described the data by mean values and standard errors of the mean (mean±SE).

I did however use non-parametric tests, whenever the assumption of normally distributed mean values was not valid. The cause did occur in the study when the number of observations was too low, i.e. when comparing many small groups or individual groups to answer specific questions.

Analysis of staining benefits (topic 1.1)

To test the benefits of staining for reliable placental scar counts, I used a paired-sample t-test comparing the observations of placental scars in the unstained and stained uteri as listed: the placental scar counts before with the placental scar counts after staining, regarding all scar-

categories; the sum of category 1+2 placental scars before with the sum of category 1+2 placental scars after staining, including samples with orange-coloured scars; the sum of category 1+2 placental scars before with the sum of category 1+2 placental scars after staining, excluding samples with orange-coloured scars; the sum of category 1+2+orange-coloured placental scars before with the sum of category 1+2 placental scars after staining, assuming orange scars became "Category$_{1+2}$" scars when stained; and the sum of category 3+4 placental scars before with the sum of category 3+4 placental scars, the "Category$_{3+4}$ scars" after staining.

To see whether the unstained orange-coloured scars were integrated in the "Category$_{1+2}$" or "Category$_{3+4}$" of placental scars after staining, I used Pearson correlation to test the linear relationship between the following variables: the sum of category 1+2+orange-coloured placental scars before staining and the counts of Category$_{1+2}$ scars after staining; and by contrast the sum of category 3+4+orange-coloured placental scars before staining and the counts of Category$_{3+4}$ scars after staining. I confirmed with a Kolmogorov-Smirnov-Test that the counts of orange-coloured scars before staining, the counts of category 1+2+orange placental scars before staining, as well as the counts of category 3+4+orange placental scars before staining were normally distributed. Thus, I used Pearson correlation although sample sizes were low. Along with the Pearson correlations the following T-Test pairs were compared testing two-tailed: the sum of category 1+2+orange-coloured placental scars before with the counts of Category$_{1+2}$ scars after staining; and by contrast the sum of category 3+4+orange-coloured placental scars before with the counts of Category$_{3+4}$ scars after staining; and the sum of category 3+4 placental scars before with the counts of Category$_{3+4}$ scars after staining. Moreover, I used a linear regression to analyse the relationship between the number of Category$_{1+2}$ placental scars after staining (the dependent outcome variable) and the sum of category 1+2 placental scars including the orange scars before staining (the predictor), and a second excluding the orange scars before staining.

Accuracy of litter size estimations (topic 1.2)

To understand if placental scars of the category 1 and 2 were consistent with the "new" scars formed during the parturition in the year of harvest, the relationship between the sum of category 1 and 2 scars counted in the uteri and the number of cubs-of-the-year observed in the field was analysed with a Spearman correlation. Due to the low number of cub-observations, the assumption of normal distribution was not valid, why I chose a rank-based test. I

calculated 2 options, one not regarding the orange scars before staining, and a second integrating the orange-coloured scars in the "Category$_{1+2}$" (the hypothesized category of "new" placental scars).

Analysis of age at primiparity and litter size (topic 2.1 and 2.2)

To estimate mean litter size, the mean of placental scar counts, which can be attributed to distinct scar-sets (Category$_{1+2}$ or Category$_{3+4}$), was calculated for all females with at least 1 placental scar in their uterus. The age-dependent differences in the mean litter size at primiparity were analysed with a t-test for independent samples. To investigate differences in age, study area and harvest year in the estimated litter sizes, I applied the Kruskal-Wallis-Test. Whenever reasonable, I pooled the individual ages in age-classes to increase the number of observations per group. Females from 9 to 20 years of age were summarised, as they are believed to be in their prime-age (Craighead et al. 1995a). Females > 20 years of age were summarised, as they are supposed to show a lower reproductive performance than prime-aged females (Craighead et al. 1995a). The litter sizes of young females, age 3 and age 4, were compared with the litter size of females >4 years of age to analyse age-specific shifts in litter sizes. Yet, the number of observations per group was generally low, the variances of all groups were not identical, and many small groups were compared. For this reason I chose a nonparametric, rank-based test for more than 2 groups of sample data to analyse the distribution of these populations. Whenever the sample sizes were too low to allow the use of normal distribution methods, my approach was to simulate the distribution of the test statistic under the null hypothesis by randomly drawing from the original data. Here I applied the Monte Carlo approximation, based on 10000 sampled tables. Along with the *p*-values of the Monte Carlo approximation, I presented the 99% confidence interval (C.I.) for the true *p*-value. I used the Mann-Whitney-Test to compare the estimated litter sizes, as well as population productivity of primiparous 3-year-old and primiparous 4-year-old females. The Mann-Whitney-Test was, moreover, used for the comparison of the estimated litter sizes of 3-year-old and 4-year-old breeders (that could have been primiparae as well as multiparae), 3-year-old and ≥4-year-old females, 3-year-old and ≥5-year-old females, and 4-year-old and ≥5-year-old females to investigate age-specific shifts in litter size in young breeders. Besides, the proportions of 3 to 4-year-old females in the sample of females with Category$_{1+2}$ scar counts and the sample of females with Category$_{3+4}$ scar counts were compared using a Chi-Square-Test, because the proportion of young breeders could affect mean litter size (see Zedrosser et al. 2009).

Analysis of population productivity (topic 2.3)

Mean population productivity was calculated as the mean number of placental scars per potentially adult female and year, including those females without scars in the respective year. Here, again mean values were presented along with the standard error of the mean (SE), and again age, study area and harvest year dependent differences in the estimated population productivity were analysed by Kruskal-Wallis- and Mann-Whitney-Test. To test for age-specific shifts in litter production in young breeders, I compared the estimated productivity of 3-year-old and 4-year-old females, 4-year-old and 5-year-old females, and 5-year-old and 6-year-old females. The Monte Carlo approximation, based on 10000 sampled tables, was used whenever sample sizes were small. Proportions of breeding bear females in relation to the females' age were analysed by logistic regression model, choosing backwards stepwise Wald elimination-method. The dependent variable was non-breeding or breeding (0=non-breeding, 1=breeding) and the continuous independent variable was female age from 3 to 24 (when estimates were based on new scars, or new and old scars), or 3 to 23 years (when estimates were based on old scars).

Analysis of cub mortality (topic 2.4)

Females with evidence of consecutive-year birthing (uteri that had 2 scar-sets, i.e. $Category_{1+2}$ and $Category_{3+4}$ scars) were considered to have lost their first litter in the year before harvest. Females with $Category_{3+4}$, but no $Category_{1+2}$ scars were considered to have successfully raised cubs. However, I did not regard mothers with $Category_{1+2}$, but no $Category_{3+4}$ scars, as these females might not have bred in the year before the last. For the analysis, I took into account bear mothers with a harvest-age $\geq$5 years, since the earliest evidence of old placental scars in the uteri examined was in 4-year-old females. The rate of litter loss was calculated as the proportion of females with 2 scar-sets (cub loss) among the females with either 2 scar-sets (indicating cub loss) or an "old" scar-set (indicating cub survival). The corresponding rate of cub loss was calculated as the proportion of $Category_{3+4}$ placental scars in the uteri with 2 scar-sets among the $Category_{3+4}$ placental scars in the overall uteri examined. To analyse possible variations in the estimated litter loss across maternal age at loss (ranging from 4 to 23 years), I used a stepwise logistic regression. The dependent variable was cub survival or total cub loss (0=no cub loss, 1=total cub loss) and the continuous independent variable was female age. I entered the independent covariates into the logistic regression model and removed by backwards elimination choosing backwards stepwise Wald-Method. To evaluate if there were associations between the estimated litter loss and the breeding experience of the females (in

the age-classes 4 and 5-23 years), the litter loss and litter sizes (1-4 cubs), as well as the litter loss and study areas (south, central, north) I used a Fisher's Exact Test.

All statistical analyses were performed using the software Statistical Package for the Social Sciences (SPSS 20., SPSS Inc., Chicago , IL). Results were considered to show a trend when $p < 0.100$, to be significant when $p \leq 0.050$, and to be highly significant when $p \leq 0.001$. All tests were performed two-tailed, unless otherwise specified.

RESULTS

Sample collection

Of the overall 259 samples of female brown bears, 181 samples contained the completely collected uteri eligible for placental scar counts. Even so 30.1% (78 of 259) of the overall uteri samples were incomplete, or seriously damaged, and only 8 of these samples were, among others, used when assessing the status of the uterus (i.e. the thickness of the wall and endometrial development). To be more specific 17.8% (46 of 259) of the uteri samples of 3- to 24-year-old, potentially birth-experienced females of the overall samples were not available for placental scar counts (Table 4). The uteri samples were in a non-homogeneous condition, showing indications of decay or partial dehydration.

The age of 177 of the 181 females was known (Table 5), and 2 females of unknown age were classified as juvenile according to their immature uterus, and 2 as breeding adults based on the presence of placental scars and uterus size. I found evidence that 34.8% (63 of 181) of the uteri derived from sexually immature cubs (<1 year), yearlings (1 year), and subadult 2-year-old females, because they were more or less small and pale flesh-coloured, their horns were narrow, and their wall was thin with a not yet fully developed homogenous endometrium. Already in the 2 to 3-year-old females, I observed an incipient uterus development in concordance with the change of season and their reproductive state. These females, that had entered puberty, had both a slightly thickened to thick uterine wall and bulges on the internal mucosa. In the adult females, I observed either a thickened uterine wall with bulges on the endometrium, or a uterine wall of regular thickness with or without crinkled, no longer swollen endometrial folds, however without endometrial bulges (e.g. in Figure 2, 3 and 4).

The uteri of 116 females that did not appear entirely immature were stained, and then examined for scars straightaway to test the benefits of the method. Additional 6 uteri had been stained 2½ years prior to their examination, so the staining was no longer effective by the time the placental scar counts were performed (Table 4). Placental scars were observed in the recently stained uteri of 66 females (37.7%; n=175 including juvenile unstained uteri), and in the formerly stained uteri of additionally 4 of those 6 females (n=181 including juvenile unstained uteri). The numbers of complete uteri samples by female age-class and study area, as well as the proportion of uteri with placental scars by female age are shown in Table 5. The numbers of complete uteri samples in the harvest years of 1992 and 1997 to 2005, as well as the proportion of uteri with placental scars by year are shown in Table 6.

1 Reliability of litter size estimations based on placental scar counts – does staining improve the method?

1.1 Analysis of the benefits of staining for placental scar counts

Macroscopic observation of uteri prior to staining

The uterine horns of adult females showed a considerable variation in their length. Notably longer horn-pairs along with large ovaries were found in females with an advanced age of 12 (~20.0cm from bifurcation to cranial end), 17, 19 and 24 years, but fairly long horns in relation to age were observed in younger females too (e.g. ~13.0cm from bifurcation to cranial end in a 5-year-old; ~15.0cm in an 8-year-old). In general, the uterus bicornis was symmetric, except for 4 uteri (2.2%; n=181) of younger females (age 3-6) with dissimilar horn lengths. Placental scars, if present, were found in mature uteri of any length. Bulges on the endometrium in a thickened uterine wall were evident in the uteri of young females entering puberty, that had already mature uteri however no placental scars, i.e. in sexually mature but nulliparous females. Overall I examined 114 uteri samples, 106 complete and 8 incomplete, for endometrial development. The earliest evidence of endometrial bulges in these samples was in 4 of the 2-year-old females (14.3%; n=28), of which 25 were harvested during late August to October. In the residual 3 females, harvested earlier in the season, I found still immature uteri, and my findings were consistent with the slight head-circumference (<50cm) and low body mass (<50kg) of these females by the time of their harvest. In one of the sexually mature 2-year-olds, the ovarian activity was additionally ascertained by the presence of corpora lutea that were recognisable under the ovarian surface.

I recorded no endometrial development in the uteri of 2 of 14 females 3 years of age. One of them had an apparently immature uterus, which corroborated that not all of the 3-year-olds were sexually mature as well. Moreover, endometrial bulges in a thickened uterine wall were evident in the uteri of adult females with placental scars, as well as in adult females without placental scars. The combined scar-age and endometrium-thickness information revealed that bulges on the endometrium in a thickened uterine wall were present in 66.7% (18 of 27) of the females with old placental scars, but just in 31.4% (11 of 35, among those 1 with slighter endometrial development) of the females with new scars that were harvested during August to October. One of the females with new placental scars that showed endometrial development was confirmed as non-lactating. Noticeable were the endometrial bulges in a thickened uterine wall recorded in the uterus of a 3-year-old female, as she had small ovaries and was harvested in May. All of the other females with a thick-walled uterus and endometrial bulges were harvested during the hunting season. Placental scars were observed at different distances from the bifurcation of the uterus bicornis and the cranial ends of the horns respectively, inter alia in close vicinity to the bifurcation, however none was observed directly at the cranial ends. Due to the longitudinal section of the uterine horns on the antimesometrial side, the placental scars appeared as bisected rings. They were defined by more or less complete circle-lines externally, and a to some extent present pigmentation inside these circle-lines. The most vibrant, almost black-coloured placental scars in the 181 uteri examined for scars were rather reminiscent of 2 half-moons than of a bisected ring, as they were completely filled with dark pigmentation. Lateral shadows or some irregularly distributed pigmented spots, i.e. aggregates of hemosiderin-laden macrophages, flanking the placental scars, were either present or missing. The evidence from the more or less stereotypic patterns suggested that scars have faded over time and that just remnants of their characteristic pattern were left behind with progressing tissue regeneration. Prior to staining, it was difficult to identify the very faded and incomplete placental scars with a nearly concealed scar-pattern, and to differentiate them from artefacts (e.g. Figure 9). Besides the variation in scar-patterns, I observed a palette of scar-colours, from bluish black and black to different shades of grey, from brown, reddish-brown and orange-brown to orange, as well as from yellowish-brown to pale yellow, among placental scars in different uteri. I even found up to two different shades of colour within individual uteri (e.g. Figure 11 picture above). To my perception, the placental scars with a dark, vibrant appearance frequently had a larger dimension than the faded ones. The black to dark grey scar-pigmentation provided an indication that the placental scars were formed during the immediately previous gestation period. The obscure remnants of bisected rings

together with a pale yellow colouration were deemed to be placental scars in their most regressed state, formed during earlier gestation periods. The 28 orange-brown to orange placental scars (e.g. in Figure 10), I found in the unstained uteri of 15 females, can be described as lightly pigmented aggregations of orange granules. In the unstained uteri, I interpreted these puzzling scars as old. In total I found 102 placental scars in the uteri of 46 (n=175 including the juveniles) females before staining. In a single uterus of a 4-year-old bear female from the southern study area I observed an extraordinary small, but dark pigmented antimesometrial scars site, which was deemed to be a resorption or an abortion of a foetus that occurred in the winter before the female was harvested (Table 3). In this uterus, endometrial development was evident as well. The search for unimplanted blastocysts, particularly in the uteri showing endometrial development, remained unsuccessful. The classification of scars, in new, old, orange-coloured and resorptions or abortions, according to the morphological features (mainly the more stereotype scars-patterns!) described above is presented in Table 7. To view examples of the presumed stadia of scar regeneration in the unstained uteri see Figure 5, 6, 7 and 8. Artefacts found in an unstained uterus are presented in Figure 9; orange-coloured placental scars in an unstained uterus are presented in Figure 10.

In addition I recorded my findings in the formerly, no longer effectively stained uteri of 6 females. I could identify 7 scars in 4 of those uteri (Table 7).

Benefits of staining for placental scar counts

For the analysis of the benefits of staining I only considered the samples of recently stained uteri (n=116) with an *active* staining. Placental scars were detected in 67 of the 116 uteri either before or after staining. I detected 102 placental scars in the uteri of 46 females before, and 164 placental scars in 66 uteri after staining. In 2 uteri I overestimated the number of placental scars prior to staining, and after staining 4 marks (2.4%; n=164) that might have been placental scars were confirmed as artefacts. In 37 unstained uteri I underestimated the number of placental scars, and I found that 66 (40.2%) of the finally observed 164 placental scars became visible only when staining the uteri. The pre-post comparison revealed a difference of in total 70 “scars” (66 placental scars and 4 artefacts) in the uteri of 35 of 116 females. The mean of placental scar counts before staining was 0.88±0.11 (n=116), whereas the mean after staining was 1.41±0.13 (n=116). Statistical analysis confirmed a highly significant increase of scar counts in the stained uteri in comparison to the unstained (t-test paired: t=-5.28; df=115; $p<0.001$; n=116; Figure 13). The mean difference of placental scar

counts before and after staining was 0.53±0.10 (n=116). To view examples that illustrate the enormous benefits of staining for placental scar counts see Figure 14 and 15.

The procedure of staining allowed me to classify placental scars more precisely according to the presumed time-related changes of the colour, intensity, and in particular, the pattern of their pigmentation as described in the *four-category scheme* in Table 2.1. I detected 44 placental scars of the category 1 in the uteri of 16 bears, 54 placental scars of the category 2 in the uteri of 24 bears, 43 placental scars of the category 3 in the uteri of 18 bears, and 23 placental scars of the category 4 in the uteri of 17 bears. Hereafter I summarized all category 1 and 2 placental scars, that were deemed to be *new* scars, to the "Category$_{1+2}$" and all category 3 and 4 placental scars, that were deemed to be *old* ones, to the "Category$_{3+4}$" in the further analysis (Table 8).

Excluding the females that simultaneously had Category$_{1+2}$ and Category$_{3+4}$ placental scars, I found a scar distribution of 53.1% (76 of 143) and 46.9% (67 of 143) between the left and the right uterine horn. I registered a maximum of 2 placental scars per horn and parturition. In a single female, I found 3 scars from the same parturition within her left horn, but only 1 scar in her right horn.

Classification of orange-coloured placental scars

Orange-coloured placental scars (Figure 11 and 12) appeared atypical and could not be classified accurately prior to staining, while the same scars could be classified according to the *four-category scheme* after staining. To learn in which of these categories the 28 orange-coloured scars I found in the unstained uteri of 15 females were integrated when stained, I compared the placental scar counts in each of those females before and after. In 11 uteri the number of unstained orange placental scar was equal to the number of Category$_{1+2}$ placental scars after staining. Nine of these uteri had just Category$_{1+2}$ placental scars when stained, thus misclassifications can be ruled out. The 2 other uteri simultaneously had Category$_{1+2}$ and Category$_{3+4}$ placental scars, but the number of unstained orange scars matched the number of Category$_{1+2}$ scars. In 4 of the 15 uteri I detected a proportion of the stained Category$_{1+2}$ placental scars in form of unstained orange scars. Despite the full number of scars remaining undetected prior to staining, I can rule out that the orange placental scars became Category$_{3+4}$ scars after staining, because they were absent.

The full number of Category$_{1+2}$ placental scars observed in stained uteri was best predicted by adding the orange placental scars to the Category$_{1+2}$ before staining (Pearson correlation: r=0.48; p=0.072; n=15; t-test paired: t=-1.74; df=14; p=0.104; n=15) (Figure 16). Here, the means of scar counts were 2.00±0.17 (mean±SE$_{Cat1+2+orange}$; n=15) before, and 2.27±0.12 (mean±SE$_{Cat1+2}$; n=15) after staining. As expected, there was no correlation evident for the sum of Category$_{3+4}$ and orange placental scars in unstained, and Category$_{3+4}$ placental scars in stained uteri (Pearson correlation: r=0.09; p=0.764; n=15; t-test paired: t=9.54; df=14; p<0.001; n=15). The means of scar counts in this place were 1.87±0.17 (mean±SE$_{Cat3+4+orange}$; n=15) before, and 0.13±0.09 (mean±SE$_{Cat3++4}$; n=15) after staining. According to Kolmogorov-Smirnov-Test, the counts of category 1+2+orange placental scars before staining (z=1.16; p=0.134; n=15) and the counts of category 3+4+orange placental scars before staining (z=1.22; p=0.100; n=15) were normally distributed, thus the assumption of normally distributed mean values appears reasonable when assessing orange scars, even with low sample sizes. Considering all uteri samples with an active staining, the number of Category$_{1+2}$ placental scars counted in the stained uteri was predicted more exactly by the linear relationship between the sum of unstained Category$_{1+2}$ and orange placental scars, and the number of stained Category$_{1+2}$ placental scars (Regression: y=0.92x+0.21; R^2=0.72; F=287.37; p<0.001; n=116) (Figure 17). Whereas the linear regression for the relationship of Category$_{1+2}$ placental scar counts before and after staining was less precise when orange scars were not included in the category before staining (Regression: y=0.82x+0.48; R^2=0.45; F=92.52; p<0.001; n=116) (Figure 18). In a few instances, the pre-post comparison of evidence pictures could confirm the statistical results (e.g. in Figure 11 and 12).

Visibility and classification of Categroy$_{1+2}$ and Category$_{3+4}$ placental scars

As long as the unstained orange-coloured placental scars were recorded separately, Category$_{1+2}$ placental scars were identified in the uteri of 21 females before, but in 40 after staining (n=116). This corresponded to 52 placental scars before staining and 98 after staining. In 1 unstained uterus I misclassified 2 placental scars (2.0% overestimation; n=98). Thus, I found that 48 (49.0%) of the finally observed 98 Category$_{1+2}$ placental scars became just visible and could be categorised accurately when stained. The increase of Category$_{1+2}$ scar counts in the uteri due to staining was highly significant (t-test paired: t=-4.54; df=115; p<0.001; n=116; Figure 19). The means of scar counts were 0.45±0.09 (mean±SE$_{Cat1+2}$; n=116) before, and 0.84±0.12 (mean±SE$_{Cat1+2}$; n=116) after staining, resulting in a mean difference of 0.40±0.09 (n=116) scars between these paired observations.

Moreover, I calculated the staining benefits for the counts of $Category_{1+2}$ placental scars excluding the 15 samples with orange-coloured scars. I identified 50 $Category_{1+2}$ scars in 20 uteri before, and 64 in 25 uteri after staining (n=101). Staining of the uteri revealed an overestimation of 2 (3.1%; n=64) and an underestimation of 16 (25.0%; n=64) placental scars within the $Category_{1+2}$. The increase of $Category_{1+2}$ scar counts due to staining was significant (t-test paired: t=-2.05; df=100; p=0.043; n=101). The means of scar counts were 0.50±0.11 (mean±$SE_{Cat1+2,excl.orange}$; n=101) before, and 0.63±0.12 (mean±$SE_{Cat1+2,excl.orange}$; n=101) after staining (Figure 20), resulting in a mean difference of 0.14±0.07 (n=101) scars between these pre-post observations.

Taking into account that orange placental scars were classified as $Category_{1+2}$ scars after staining, the number of placental scars in the stained uteri was significantly higher by 20.4% (n=98) (t-test paired: t=-2.51; df=115; p=0.014; n=116; Figure 21). I counted 80 either $Category_{1+2}$ or orange placental scars in the uteri of 35 females before and 98 $Category_{1+2}$ placental scars in 40 females after staining. In 1 unstained uterus I misclassified 2 scars (2.0% overestimation; n=98). Therefore, 20 out of the finally observed 98 $Category_{1+2}$ placental scars could just be counted when the uteri were stained. The means of scar counts were 0.69±0.11 (mean±$SE_{Cat1+2+orange}$; n=116) before, and 0.84±0.12 (mean±SE_{Cat1+2}; n=116) after staining, resulting in a mean difference of 0.16±0.06 (n=116) scars between these pre-post observations.

For comparative purpose, I combined the data from scar tissue evaluations with the data from the assessment of the uterus status. I found that in the simultaneous presence of new scars and endometrial bulges in a thickened uterine wall, new scars were frequently (in 8 of 11 uteri) concealed prior to staining.

$Category_{3+4}$ placental scars were observed in the unstained uteri of 13, and stained uteri of 34 females (n=116), which corresponded to 22 scar counts before staining and 66 after staining. In the uteri of 2 females 5 $Category_{3+4}$ placental scars (7.6%; n=66) were misclassified before staining, as they were classified as $Category_{1+2}$ scars when stained. In the uteri of further 2 females 4 scars (6.1%; n=66) that were interpreted as $Category_{3+4}$ placental scars were confirmed as artefacts when stained. This revealed an overestimation of 9 scars (13.6%; n=66) in 4 uteri. So I found that 53 (80.3%) of the finally observed 66 $Category_{3+4}$ placental scars became visible only, and could be categorised accurately and distinguished from

artefacts only when stained. The results confirm a highly significant increase of $Category_{3+4}$ placental scar counts in the stained uteri when compared to those in the unstained (t-test paired: t=-3.79; df=115; p<0.001; n=116; Figure 22). The means of scar counts were 0.19±0.06 (mean±SE_{Cat3+4}; n=116) before, and 0.57±0.09 (mean±SE_{Cat3+4}; n=116) after staining, resulting in a mean difference of 0.38±0.10 (n=116) scars between these paired observations.

1.2 Accuracy of litter size estimations based on placental scar counts

Persistency of placental scars postpartum

In the 70 uteri with present placental scars checked against each other (n=181), I found evidence to suggest females could have scars from at least 2 breeding seasons in their uterus. This was apparent from the obvious changes in the intensity and arrangement of the scar-pigmentation identified between placental scars of the category 2 and category 3, whereas these changes seemed to proceed more continuous between either Category 1 and 2, or Category 3 and 4 scars. The field-data from observed cubs traveling with their mothers in the year of harvest, as well as in the year before, confirmed that the placental scars were either formed during the immediately previous gestation period or during the gestation period of more than one year prior to the females' harvest (Table 9). Taking into account that cubs are born in January through March, I can conclude that placental scars were detectable 7-9, and even 19-21 month postpartum. In a single uterus, I identified extremely faded and incomplete remnants of 2 yellowish placental scars that were dated older than category 4. This sighting of even older scars, formed through a parturition 29-31 month prior to the August of the female's harvest, was corroborated by the evidence from 2 confirmed cubs two years prior to the harvest date (Table 9).

Correlation between $Category_{1+2}$ placental scars and the number of observed cubs in the year when the mother was harvested

The correlation between the number of $Category_{1+2}$ placental scar in the uteri and the number of observed cubs accompanying their mothers in the year when the females were harvested was highly significant (Spearman's correlation: R=0.90; p<0.001; n=14) (Table 9). Accordingly, placental scars of the $Category_{1+2}$ corresponded to the "new" placental scars formed during the parturition in the year of harvest.

One of the 14 uteri tested had an old, no longer effective staining. In this uterus 2 lightly pigmented aggregations of orange granules, i.e. orange-coloured scars were observed. Under the assumption that orange scars of this phenotype correspond to the new scars after staining, a Spearman's correlation was calculated for the relationship between the sum of $Category_{1+2}$ and orange placental scars counted in the uteri and the observed litter size in the year of their harvest. The estimated litter size from placental scar counts of these phenotypes was even more precise. There was a highly significant positive correlation between new $Category_{1+2}$ placental scars including the orange scars, and the litter size observed in harvest year (Spearman's correlation: R=1.00; $p<0.001$; n=14) (Figure 23).

The comparison of placental scar data with the lactation data available for 5 females showed that all nursing females, which were supposed to have had dependent offspring in the year of harvest, had $Category_{1+2}$ placental scars in their uteri. The regular thickness of the uterine wall and the absence of endometrial bulges, recorded for 4 of these autumn-harvested females, were consistent with the scar-classification efforts.

Correlation between $Category_{3+4}$ placental scars and the number of observed cubs in the year prior to the mother was harvested

In the uteri of 4 females that were confirmed to have had cubs in the year prior to harvest, I was able to identify placental scars of the $Category_{3+4}$. Including 2 females that were not reproductively active in the year before they were shot, and 1 female with placental scars older than $Category_{3+4}$, but no $Category_{3+4}$ scars in her formerly stained uterus, the known litter size (0-3) was consistent with the number of placental scar in the uteri of 7 bear females (Table 9). Thus, $Category_{3+4}$ scars were considered to be "old" placental scars, formed during the parturition in the year before harvest.

Confusions with resorptions or abortions

The resorption or abortion of a foetus found in the uterus of a single female (n=181) looked different in comparison to the old placental scars. It did not appear incomplete or faded like the old scars, but in comparison to the new scars, it was a small compact looking scar-site. The reproductive history of this particular female was unknown, but the resorption-scar was estimated to be from the previous winter, because of its intensive, dark pigmentation and complete appearance (Table 3). In this uterus, endometrial development was evident as well.

2 Estimation of female reproductive performance

2.1 Age at primiparity, autumn-body mass and head circumference of breeders

I examined the uteri of 177 females with known age, and among those 171 with an active staining. According to the age of the youngest females with new placental scars (n=2 from Jämtland and Västernorrland), the earliest recorded age at primiparity was 3 years. The harvest data shared that the female from Västernorrland was not lactating when shot in September. The youngest females with old placental scars (n=2 from Dalarna and Jämtland) were 4 years old, and did not have new scars in their uteri simultaneously. Among the 171 females, placental scars were recorded in the uteri of 8.0% (n=25) of the 3-year-old females, however only new; in 43.8% (n=16) of the 4-year-old females, in 76.9% (n=13) of the 5-year-old females and in 88.9% (n=9) of the 6-year-old females I recorded new as well as old placental scars. In the age classes of the 4 to 6-year-old females, the proportion of females with old placental scars was lower than the proportion of females with new (Table 10). From the age of 7 on the proportion of females with old to that with new placental scars altered between the age-classes depending on the sample collection (Table 10).

The mean litter size of primiparous 3-year-old females, estimated from new placental scar counts in 3-year-old, and old placental scar counts in 4-year-old females was 1.25±0.25 (median=1; n=4). The mean litter size for primiparous 4-year-old bears was estimated from new placental scar counts in 4-year-old females that did not have old scars simultaneously, and was 2.20±0.20 (median=2; n=5). I did not regard the old placental scars found in 5-year-old females, as these females could already have had their first, unrecognised litter at the age of 3. According to the Monte Carlo approximation, the number of cubs of the first litter was significantly higher for the first-breeding 4-year-old bear mothers than for those starting to reproduce at age 3 (Mann-Whitney-Test: U=2.00; Monte Carlo sig. *p*=0.043; 99% C.I.=0.038 – 0.049; n=4+5=9). Including the females that did not give birth to cubs within the last 2 seasons (with 0 placental scars), the mean population productivity for primiparous 3-year-old bear females was 0.12±0.06 (n=41), that for primiparous 4-year-old females was 0.69±0.27 (n=16). The comparison of the population productivity between the 3 and 4-year-old bear females revealed a statistically significant increase in productivity from the age of 3 to the age of 4 (Mann-Whitney-Test: U=249.50; *p*=0.028; Monte Carlo sig. *p*=0.014; 99% C.I.=0.011 – 0.017; n=41+16=57).

The known autumn body mass of 23 of 103 females with new placental scars that gave birth to their cubs in the same year when they were shot varied from 83-173 kg (Figure 24). Some of the 2-year-old nulliparous females had a higher autumn body mass (85-110kg) than some of the bear mothers with cubs of the year. The two 2-year-old females with endometrial bulges in a thickened uterine wall and known body mass had 82 and 85kg each. The known head circumference of the 37 of 166 females with new placental scars varied from 54.0-72.0cm (Figure 25). The head circumference of all four 2-year-old females with bulges on the endometrium was 53.5, 62.0, 63.0, and 65.0cm.

2.2 Litter size

For the litter size estimations, I considered all females with at least 1 placental scar in the uterus. I observed a maximum of 4 placental scars per female for one breeding season, however 4 scars were also the maximum observed in females having evidence of consecutive-year birthing. The range of scars formed during the same parturition was 1 to 4, with 2 to 3 estimated cubs being commonest. For the frequency of litter sizes based on new and old placental scar counts, see Table 11. The mean number of new placental scars was 2.45±0.11 (n=40) per female. The mean number of old placental scars was 1.94±0.15 (n=34) per female. In total I calculated a mean of 2.22±0.10 placental scars (n=74) for 66 females with an active uterus staining. The mean litter size calculated from new placental scars that were either found in the uteri with an active or an old staining was 2.43±0.11 (n=42) (Table 12).

I found no significant differences, but a tendency for differences in the estimated litter sizes from new placental scar counts between the age-classes of 3, 4, 5, 6, 7, 8, and 9 to 20 years (Kruskal-Wallis-Test: χ^2=10.24; df=6; Monte Carlo sig. *p*=0.093; 99% C.I.=0.085 – 0.100; n=2+5+8+6+3+2+13=39 excluding uteri with old staining; Kruskal-Wallis-Test: χ^2=9.23; df=6; Monte Carlo sig. *p*=0.147; 99% C.I.=0.138 – 0.156; n=2+5+8+6+3+3+14=41 including uteri with old staining), and a significant difference between young breeders at the age of 3 and 4 (Mann-Whitney-Test: U<0.01; Monte Carlo sig. *p*=0.050; 99% C.I.=0.044 – 0.056; n=2+5). For these tests, I took into account the uteri of all females with new placental scars and known age in the year of harvest. The median and the mean numbers of new placental scars per age-class in the bear females are shown in Table 13.

The comparison of the estimated litter sizes from new and old placental scar counts between the age-classes of 3, 4, 5, 6, 7, 8, 9 to 20, and 21 to 23 years revealed a tendency for differences in litter sizes (Kruskal-Wallis-Test: χ^2=12.69; df=7; Monte Carlo sig. p=0.065; 99% C.I.=0.058 – 0.071; n=4+11+11+9+5+5+26+2=73). I considered 73 sets of either new or old placental scars found in the uteri of 65 females with known age in the year of harvest. For all bears with old placental scars, I took into account an age-reduction of 1 year. Also, there was a trend towards differences in litter sizes between the age-classes excluding the 2 females older than 20 years (Kruskal-Wallis-Test: χ^2=10.57; df=6; Monte Carlo sig. p=0.096; 99% C.I.=0.088 – 0.103; n=4+11+11+9+5+5+26=71). For females 3 years, 4 years, and 5 to 23 years of age, I found significant differences in litter sizes (Kruskal-Wallis-Test: χ^2=7.72; df=2; Monte Carlo sig. p=0.018; 99% C.I.=0.014 – 0.021; n=4+11+58=73). The 3-year-old females had significantly smaller litters than the ≥4-year-old females (Mann-Whitney-Test: U=46.50; Monte Carlo sig. p=0.026; 99% C.I.=0.022 – 0.030; n=4+69=73), and ≥5-year-old females (Mann-Whitney-Test: U=34.50; Monte Carlo sig. p=0.013; 99% C.I.=0.010 – 0.016; n=4+58=62), but the litter size of the 4-year-olds was not significantly smaller than that of the ≥5-year-olds (Mann-Whitney-Test: U=234.00; Monte Carlo sig. p=0.148; 99% C.I.=0.139 – 0.158; n=11+58=69). The medians and means of estimated litter sizes based on new and old placental scar counts in the different age-classes are shown in Table 14. The proportions of young breeders, age 3 to 4 years, did not statistically differ in the sample of females with new scar counts and the sample of females with old scar counts (Pearson Chi-Square: χ^2=0.35; df=1; p=0.382; one-tailed; n=73) (Table 15).

Comparing the mean litter sizes between the 3 study areas, I found no significant differences for females in the south, the central, and the north, as evident from new placental scar counts (Kruskal-Wallis-Test: χ^2=3.20; df=2; p=0.202; n=20+14+6=40; Kruskal-Wallis-Test: χ^2=3.69; df=2; p=0.158; n=22+14+6=42). The median and mean numbers of new placental scars in the 3 areas are shown in Table 16. Also, there were no significant differences in litter sizes between the southern, the central, and the northern area, when estimates were based on new and old placental scar counts (Kruskal-Wallis-Test: χ^2=1.57; df=2; p=0.457; n=38+25+11=74). For the test I took into account 74 sets of either new or old placental scars found in the uteri of 66 bear females with known harvest area. Uteri with an old staining were excluded. The median and mean numbers of new and old placental scar counts in the 3 study areas are shown in Table 17.

Analysing litter size development throughout the years, I found no significant differences in litter sizes among the years of harvest from 1997 to 2005, as evident from new placental scar counts (Kruskal-Wallis-Test: χ^2=5.30; df=8; Monte Carlo sig. p=0.779; 99% C.I.=0.768 – 0.789; n=2+2+3+4+1+7+3+10+8=40 uteri with active staining; Kruskal-Wallis-Test: χ^2=5.17; df=8; Monte Carlo sig. p=0.789; 99% C.I.=0.778 – 0.799; n=2+2+3+4+1+9+3+10+8=42 including uteri with old staining). For the medians and means of new placental scar counts in the respective years of harvest, see Table 18. The results were still not significant excluding the harvest year of 2001 with sample-size n=1 (Kruskal-Wallis-Test: χ^2=4.44; df=7; Monte Carlo sig. p=0.771; 99% C.I.=0.760 – 0.782; n=2+2+3+4+7+3+10+8=39 uteri with active staining; Kruskal-Wallis-Test: χ^2=4.24; df=7; Monte Carlo sig. p=0.789; 99% C.I.=0.778 – 0.799; n=2+2+3+4+9+3+10+8=41 including uteri with old staining). When the estimates were based on new and old placental scars observed in the uteri of 66 females, mean litter sizes were not significantly different between the years of "parturition" as well (Kruskal-Wallis-Test: χ^2=7.81; df=9; Monte Carlo sig. p=0.563; 99% C.I.=0.550 – 0.576; n=4+4+3+8+4+5+10+12+16+8=74 sets of scars). Thereto, I considered the respective year of harvest for 40 females with new scar-sets, and the respective year before harvest for 34 females with old scar-sets. The 8 females with placental scarring from 2 consecutive years were considered twice. For the medians and means of estimated litter sizes based new and old placental scar counts in the years from 1996 to 2005 see Table 19.

2.3 Population productivity

Population productivity was calculated as the number of cubs per potentially adult female ≥3 years at parturition (n=106) and year. The adult females that displayed either new placental scars or old were reproductively active mothers within the last 2 breeding seasons before harvest (61.3%; n=106). Whereas the adult females without placental scars either had a 3 year reproductive-cycle, or were not reproductively active within the last 2 breeding seasons before harvest (38.7%; n=106).

The mean population productivity, or reproductive rate of the 3 to 24-year-old females based on new placental scar counts in the uteri with an active staining was 0.90±0.12 cubs per female and year (n=106). The mean productivity calculated from new placental scars that were either found in the uteri with an active or an old staining was 0.88±0.12 cubs per female and year (n=112). Comparing the estimated reproductive rates of the bear females in the age-

classes 3, 4, 5, 6, 7, 8, 9 to 20, and 22 to 24 years, significant and highly significant differences were ascertained (Kruskal-Wallis-Test: χ^2=20.98; df=7; Monte Carlo sig. *p*=0.002; 99% C.I.=0.001 – 0.003; n=25+16+13+9+6+4+31+2=106 excluding uteri with old staining; Kruskal-Wallis-Test: χ^2=22.38; df=7; Monte Carlo sig. *p*=0.001; 99% C.I.<0.001 – 0.002; n=25+16+13+9+6+5+35+3=112 including formerly stained uteri). The differences remained significant excluding the old females, 22 to 24 years of age, with sample size n=3 in the latter test (Kruskal-Wallis-Test: χ^2=20.52; df=6; *p*=0.002; n=25+16+13+9+6+5+35=109). Means of productivity by age-class based on new scar counts are shown in Figure 26, the means and medians in Table 20. More detailed analysis revealed a significant difference in productivity between 3 and 4-year-old bear females (Mann-Whitney-Test: U=148.50; *p*<0.036; Monte Carlo sig. *p*=0.034; 99% C.I.=0.029 – 0.039; n=25+16=41), and a trend towards a difference between 4 and 5-year-old females (Mann-Whitney-Test: U=68.50; *p*=0.084; Monte Carlo sig. *p*=0.085; 99% C.I.=0.078 – 0.092; n=16+13=29), whereas there was no more significant difference between 5 and 6-year-old females (Mann-Whitney-Test: U=48.50; Monte Carlo sig. *p*=0.529; 99% C.I.=0.516 – 0.541; n=13+9=22). The results confirmed that the reproductive rates for first-time breeders, at least for the 3-year-olds, were lower than for older females. The factor age of females from 3 to 24 years entered into a logistic regression showed no significant correlation with the proportion of females that were breeding (Wald: B= 0.06; Exp(B)=1.06; df=1; *p*=0.182; n=106).

The mean population productivity of the 3 to 23-year-old females, based on old placental scar counts, was 0.81±0.12 cubs per female and year (n=81). For each of the females with 0-4 old placental scars I took into account an age reduction of 1 year from the females' age at harvest to obtain their age at parturition. The estimated reproductive rates from old scar counts varied significantly between the age-classes of 3, 4, 5, 6, 7, 8, 9 to 19, and 21 to 23 years (Kruskal-Wallis-Test: χ^2=14.13; df=7; Monte Carlo sig. *p*=0.034; 99% C.I.=0.030 – 0.039; n=16+13+9+6+4+5+26+2=81). More detailed analysis showed that the reproductive rate of the 3-year-old females was significantly lower than that of 4-year-olds when considering the asymptotic *p*-value, and that this is suggestive when considering the Monte Carlo significance (Mann-Whitney-Test: U=69.00; *p*=0.050; Monte Carlo sig. *p*=0.060; 99% C.I.=0.054 – 0.066; n=16+13=29). The reproductive rate of the 3-year-old females was however significantly lower than that of females ≥4 years of age (Mann-Whitney-Test: U=320.00; *p*=0.008; Monte Carlo sig. *p*=0.008; 99% C.I.=0.005 – 0.010; n=16+65=81). Means of productivity by age-class based on old scar counts are shown in Figure 27, the means and

medians in Table 21. Applying logistic regression, I found significant differences in the proportions of breeding females among individual ages from 3 to 23 years (Wald: B=0.11; Exp(B)=1.11; df=1; p=0.040; n=81).

In an attempt to diminish the effects of the 2 to 3-year reproductive cycles of female brown bears in Scandinavia, population productivity was moreover estimated from new and old placental scar counts. To start from the premise that each uterus can give insight in the reproductive history of the last 2 consecutive breeding seasons, I took into account a sum of 187 scar-sets, which included 106 scar-sets with new scars from the harvest year, and 81 scars-sets with old scars from the year prior to harvest in the uteri of 106 females. For the old scar-sets, I considered the females' age to be one year less than their age at harvest. The mean population productivity based on new and old scar counts was 0.86±0.09 cubs per female and year (n=187). The estimated reproductive rates from new and old placental scar counts varied highly significantly between the female age-classes of 3, 4, 5, 6, 7, 8, 9 to 20, and 21 to 24 years (Kruskal-Wallis-Test: χ^2=25.26; df=7; Monte Carlo sig. p<0.001; 99% C.I.<0.001 – 0.001; n=41+29+22+15+10+9+57+4=187). Comparing the estimated reproductive rates between the 3 and 4-year-old females, results confirmed a significant increase in productivity among these first-time breeders (Mann-Whitney-Test: U=417.00; p=0.003; n=41+29=70). Means of population productivity by age-class based on new and old scar counts are presented in Figure 28, the means and medians are presented in Table 22. Entering the factor age of females in a logistic regression, it confirmed a significant correlation with the proportion of breeding females when considering 3 to 24-year-old females (Wald: B=0.08; Exp(B)=1.08; df=1; p=0.017; n=187).

There were no significant differences in the estimated reproductive rates of the adult bear females ≥3 years at parturition between the 3 study-areas, no matter if based on counts of new placental scars (Kruskal-Wallis-Test: χ^2=0.14; df=2; p=0.933; n=53+33+20=106) or old (Kruskal-Wallis-Test: χ^2=0.32; df=2; p=0.853; n=43+25+13=81). Also, I found no significant differences in the reproductive rates between the harvest years of 1992, and 1997 to 2005, as evident from new scar counts (Kruskal-Wallis-Test: χ^2=2.44; df=9; Monte Carlo sig. p=0.991; 99% C.I.=0.989 – 0.993; n=1+7+5+4+11+3+17+13+24+21=106). The medians and means of productivity in the 3 study areas based on new placental scar counts are shown in Table 23, those based on old scar counts in Table 24. The medians and means of productivity in the

harvest years 1992, and 1997 to 2005 based on new placental scar counts are shown in Table 25.

2.4 Analysis of cub mortality

Females with both "new" ($Category_{1+2}$) and "old" ($Category_{3+4}$) placental scars in their uterus were considered to have lost their earlier litter in the year prior to harvest. Such evidence of placental scarring from 2 consecutive years was found in 12.1% (8 of 66) of the females with placental scars, and indicated total litter loss in 23.5% (8 of 34) of the females with old placental scars. Four of the females with evidence of consecutive-year birthing, were 5 years old by the time of their second parturition, suggesting that these females had lost their earlier litter at the age of 4. For a 12-year-old female from the southern study area data from field observations showed the emergency of her earlier litter, a singleton cub after den. All of the 8 bear mothers with 2 scar-sets just had 1 old scar for their earlier litter, but either 2 (n=5) or 3 (n=3) new scars for their litter in the subsequent year. These findings indicated the birth of a single cub in the year prior to harvest and its loss. The sum of placental scars observed within two subsequent years was not exceeding the amount of 4 in each uterus. The foetal resorption-scar from the previous winter, observed on the "developed" endometrium of a 4-year-old female from the southern area, indicated an incidence of in-utero litter loss.

The differences in the proportion of females which had lost their earlier litter and thus had both new and old placental scars, in relation to the proportion of successful breeders with exclusively old placental scars, were not significant between the ages 5 to 24 years (Wald: B=-0.16; Exp(B)=0.85; df=1; p=0.175; n=32). In addition, I analysed the cub mortality of 4 and ≥5-year-old females. At age 4 the estimated litter loss was 66.7% (n=6 litters), at ages ≥5 the litter loss was only 15.4% (n=26 litters). The difference was significant (Fisher's Exact Test: p=0,023; n=32). This corresponded to a cub loss of 40.0% (4 of 10 cubs) among the 4-year-olds and 7.5% (4 of 53 cubs) among the ≥5-year-olds (Table 26).

I also tested the estimated cub mortality as a function of the number of bear cubs a mother lost. Total litter loss was more common when females raised singleton-cubs (66.7%; n=12 litters), in comparison to females raising multi-cub litters (0.0%; n=20) (Table 27). The difference was highly significant, when estimates were based on the evidence of placental scarring from 2 consecutive years (Fisher's Exact Test: χ^2=15.92; p<0.001; n=32). Analysing

females in the south, the central and the north, the estimated cub mortality did not statistically differ between these 3 core areas for reproduction (Fisher's Exact Test: χ^2=0.39; p=1.000; n=32) (Table 28).

When examining the uteri (n=106 complete+8 incomplete=114) for evidence of endometrial development, in 11 of 35 females with new scars that were harvested during August to October I observed an obvious endometrial development. The coincidence of 1-4 new scars and endometrial bulges in the uterus of the 3 to 14-year-old females points out at litter losses. One of these females, with 1 new placental scar, 3-years-old, and from the central study area, was confirmed as *not lactating* by the time of her harvest.

In 5 females evidence for lactation coincided with the presence of new placental scars. This combined information indicates that these females had dependent cubs when they were shot.

DISCUSSION

Sample collection

This study is based on the examination of complete uteri samples of 181 brown bear females available for scar tissue evaluation, and intact incomplete uteri samples of further 8 females, that were involved assessing the additional reproductive parameter endometrial development. Even so, 17.8% (n=259) of the obtained uteri samples of potentially birth-experienced 3 to 24-year-old females had to be excluded from scar counts, because they were collected incomplete. Particularly parts towards the cranial ends of the uterine horns were frequently missing in these samples, most notable if the uterine horns were both or either long-horned and thin-walled. Erickson et al. (1964) registered a mean horn length of 18.0cm in non-breeding adult American black bears, whereas Aune et al. (1994) reported a mean horn length of 14.7cm in adult female brown bears. As there were still to some extent decayed, partially dehydrated samples among the complete uteri, my examinations largely excluded measurements of the length (e.g. uterine horn length) or width (e.g. placental scar diameter) of morphological features. Yet, for one of the largest uteri examined, I recorded a horn length of ~20.0cm from bifurcation to cranial end. Although the locations of placental scars as observed by Erickson et al. (1964) suggested that implantations occur most frequently at about a third

of the distance from both the bifurcation and the cranial ends of the uterine horns, I detected placental scars at different distances from the bifurcation and cranial ends. My observations involved scar sightings close to the bifurcation, but missed their sightings directly at the cranial ends near the ostium uterinum tubae. Because the samples were collected from wild females this may best explain their non-homogeneous quality. Besides, the carcass-inspection and collection of biological samples after a successful harvest was reorganized throughout the years of sampling, i.e. from 1986-2001 the samples were collected by the Swedish Hunter Association, while thereafter the task was assigned to the National Veterinary Institute (Bischof et al. 2008). Even though decay has complicated the uterus analyses, the comprehensive collection of 259 reproductive tracts provided a unique source to gain long-term reproductive data of a species difficult to follow (Table 1). The scar analysis was moreover complicated by an old staining in the uteri of 6 bear females. Here, the benefits that result from the colouration of the hemosiderin-laden macrophages were no longer apparent by the time of my observations. These findings confirmed the advice given by Bray et al. (2003) that the staining solutions are corrosive, making it important to rinse the uteri with water after the staining procedure, to keep them wet, and to carry out scar tissue evaluations right afterwards. That way the staining is still active by the time of observation. I was able to successfully assess placental scar tissue within a few days after staining, which came in useful for comparative analyses.

No placental scars were observed in the uteri of 67 females (37.0%, n=181) of age ≤2 years, thereby identifying these females as nulliparous. However, 4 (14.3%, n=28 including 2 incomplete samples) of the 2-year-olds in fact have already been sexually mature. The earliest evidence of a thickened uterine wall and bulges on the endometrium in the samples examined was in 2-year-old females that did not show any indications of placental scars. Yet, the development of their endometrium in concordance with the change of season clearly indicated the endocrine activity of their ovaries. This makes sense, as 2-year-old females must be receptive, because I found placental scars in the uterine horns of 3-year-old females. The uteri of juvenile individuals are easy to distinguish from those of adult females based on their morphology, like the dimension of the uterine horns and the development of the endometrium (see *Macroscopic observations of uteri*). Misidentification is probably rather caused by the inconsistent or vague terminology presented in the literature, particularly if the reproductive state of female bears had to be revealed through field-observations. For example, females termed as “subadult” may either be premature females (i.e. weaned, but not sexually mature:

Taylor 1994) or sexually mature, nulliparous females (if based on the observed age of first reproduction in the field: e.g. Zedrosser et al. 2006a; Zedrosser et al. 2009; Schwartz et al. 2003b). However, the latter could already be receptive or have mated, and as such be considered "breeders". My findings suggest that invasive methods have a clear advantage over field observations in relation to the identification of "breeders". Of the juvenile females with known age, at least 5 were dependent offspring when they were killed in the autumn hunting season. The highest sample sizes were attained from yearlings, 2 and 3-year-old females. Among the potentially sexually mature females at the age of 3 to 24, the proportion of females with placental scars in the uterus increased rapidly. The breeding activity of the ≥6-year-old females remained high until the age of 24 years (Table 5). In brown bears, reproductive longevity in females approximates physical longevity (Pasitschniak-Arts 1993; Schwartz et al. 2003b), which in the wild is about 25 to 30 years (Zedrosser 2006c). Although placental scar counts are a suitable method to assess age-specific litter sizes, and to confirm produced litters, the sampling effort varied among ages (Table 5) and may thus be considered a source of bias assessing reproductive performance. For females 14 to 24 years of age, my observations were limited to 1-2 uteri per age-class. Moreover, the sample collection lacked females older than 24 years to verify findings of reproductive senescence. The relative low abundance of females older than 12-13 years of age in my sample of females is consistent with the reports of Schwartz et al. (2003b), that female survival decreases rapidly after ~12 years of age. As family groups were protected, the harvest sample of females was likely biased in favor of solitary females, i.e. young, solitary females that had not given birth. Also females that exhibited a 2-year reproductive cycle were more vulnerable to be hunted than those exhibiting a 3-year cycle. The sampling effort furthermore varied between the study areas, as shown in Table 5, and the harvest years, as shown in Table 6. The harvest year of 1986 was not represented in the sample of intact and complete uteri, so my data collection starts with the year 1992.

1 Reliability of litter size estimation based on placental scar counts – does staining improve the method?

1.1 Benefits of staining for placental scar counts

Macroscopic observation of uteri prior to staining

Stretched uteri with long, but rather thin-walled horns were observed mainly in multiparous females, as suggested by female age and evidence of placental scarring. The longest horns were found in females with an advanced age. Age-dependent involution has been described for multiparous domestic mammals, i.e. that reproductive tracts of older animals are frequently larger (together with a rather thin wall thickness), show asymmetries, discolouration of the endometrium, and so on (Dyce et al. 1991). With few exceptions, the bicornuate uteri of the brown bear females were built symmetrically. It is interesting to note that I found the uteri with a distinct horn length only in young females, 3-6 years of age. These young females may have given birth to a single cub, so that the nidation and subsequent growth of the embryo took place in one of the 2 uterine horns. Thereby one horn was stretched, but not the barren horn.

Bulges on the endometrium are present during the period of delayed implantation, in order to prepare the uterus for implantation. In Asiatic black bears without dependent offspring, the thickness of the endometrium was reported to be larger from August-October, than from May-July (Yamane et al. 2009). In the autumn-harvested females in my sample, shot during August-October, endometrial bulges in a thickened uterine wall were more commonly present in the females with old placental scars than in the females with new scars. Instead, in autumn-harvested females with new scars, I could more commonly notice a “regular” uterine wall with no obvious endometrial development. Similar results were published for the American black bear (Hristienko et al. 2004). Even so, 31.4% (n=35) of the females in my sample simultaneously had endometrial bulges in a thickened uterine wall, as well as new placental scars. In these instances, I either misidentified new placental scars and old ones, or endometrial bulges and no longer swollen endometrial folds that remained behind from a previous season, or the females had lost their litter shortly after parturition. Litter loss would have allowed them to re-enter oestrus, and under the hormonal influence of progesterone and oestradiol-17β (Yamane et al. 2009) cell proliferation and growth would have taken place in the endometrium. Yamane et al. (2009) demonstrated the relevance of the corpora lutea, as

well as the season of the year for the regular development of the endometrium. Based on the presence of endometrial bulges and corpora lutea, recognisable under the ovarian surface, I was able to ascertain endocrine ovarian activity, as well as the maintenance of corpora lutea already in a few (14.3%; n=28) of the 2-year-old females. My results show that Scandinavian brown bear females may breed already at age 2, i.e. they reach puberty and get into oestrus, being receptive for the first time. By this means, successful conception and implantation can occur at the minimum age of 2 years in Scandinavia. This proposed successful implantation and gestation was indeed confirmed through the presence of placental scars in 3-year-old females, i.e. 3 being the minimum age of first litter production in Scandinavia (see *Sample collection*). In female grizzly bears the age of first confirmed conception is 3 years, and there were even indices for follicular activity occurring as early as age 2 (Aune et al. 1994). The age of first confirmed litter production of female brown bears from Austria (Zedrosser et al. 1999) and Croatia (Frkovič et al. 2001) is 3 years. Moreover, it was surprising to observe endometrial bulges in one female harvested in May, although cell proliferation has been described to take place in May, but cell growth does not take place until the period of delayed implantation (Yamane et al. 2009).

In order to document the characteristic age-dependent scar-features that enable to distinguish between placental scars formed during recent and earlier gestation periods, their appearance throughout tissue regeneration was described in detail (for the description of scars in the stained uterus see Table 2.1). In accordance with Hristienko et al. (2004), placental scars were deemed to be "new", i.e. from the immediately previous gestation period, if they had a vibrant appearance and a complete scar-pattern. Placental scars were deemed to be "old", i.e. from the gestation period of more than 1 year prior, if they were faded and the scar-pattern was incomplete. I observed a variation of scar colourations from different shades of black, grey, brown, and orange to yellowish. In some uteri up to 2 different shades of colour were detectable, whereby the question arises if these different coloured scars originated from the same litter or not. In general, dark, vibrant colours provide an indication of new scars, and pale, faded colours of older ones. But, as scar colours may as well alter in relation to the handling of the samples (see *Classification of orange placental scars*), I recommend to focus on the age-dependent variations of the more stereotypical scar-pattern described in Table 2.1. Only the faint yellowish colouration of very old placental scars formed during a gestation period of more than 2 years ago, along with the obscure remnants of the bisected ring-pattern, can provide a reliable colour-indication. In the American mink, for comparison, pale scars in

the most regressed state were described to be reduced to small orange-pigmented spots (Elmeros and Hammershøj 2006). However, faint yellowish scars in the uteri of female brown bears seem to be the exception (see *Persistency of placental scars postpartum*). In the brown bear, the orange scars found in the unstained uteri lead to different results than described in the American mink. And, the orange scars were of particular interest as they could not be accurately aged as long as unstained (see *Classification of orange placental scars*).

Scar sites with a very small diameter (<3mm) may provide an indication for aborted or resorbed foeti in American black bears (Hristienko et al. 2004). Also Yamane et al. (2009) described the presence of a small black placental scar in Asiatic black bears, and speculated that it was the result of an abortion, that occurred in the course of placental formation during the immediately previous gestation period. As an alternative explanation, Yamane et al. (2009) proposed that this scar might have been formed during an earlier full-term gestation. In the uteri of 114 females ≥3-years of age, I was able to identify a single scar site in one female matching these descriptions (see Table 3). Under unfavorable environmental conditions female bears have the opportunity to resorb the fertilized, but not yet implanted blastocysts before the active gestation period starts due to delayed implantation. Therefore, one would assume that post-implantation resorption of embryos is less likely to occur in bears than in other carnivores without delayed implantation. Mano and Tsubota (2002) suggested for Hokkaido brown bears that embryo loss during the active gestation period is not common, whereas prenatal mortalities in members of the *Canidae*-family, e.g. in red fox (Vos 1994), and Arctic fox (Angerbjörn et al. 2004) without delayed implantation, were documented to be more likely. Yet, occasional instances of prenatal mortality in Scandinavian brown bears seem to occur. In central Sweden, human disturbance is a major cause of den abandonment by brown bears in winter, and pregnant females that changed dens prior to parturition lost young more often than those that did not move (Swenson et al. 1997b). The female in my sample showing indications of prenatal litter loss was 4 years of age and from the southern study area. She may therefore has been less experienced in finding a secrete winter-hideaway in an area where hibernal disturbances are more common, or she may have had difficulty reaching sufficient autumn body mass to sustain the unborn litter.

I was not able to find any unimplanted blastocysts in the uterine lumen of females with a thickened uterine wall and bulges on the endometrium. The uteri of these females showed all indications of a coming-up implantation, thus there might have been freely floating

blastocysts in the lumen. A proportion of these females may not have been pregnant, as once ovulation occurs the corpus luteum is also formed, maintains and functions in its manner in pseudopregnant bear females (Sato et al. 2001). In this case, the endometrium would have developed under the hormonal influence of the ovaries, and been apparently thickened from August-October (Yamane et al. 2009), regardless of whether or not a blastocyst existed. Second, even if to a lesser extent, the endometrium possibly would have been thickened in females without corpus luteum. According to Yamane et al. (2009) the endometrium in Asiatic black bears also develops corpus luteum-independent, in concordance with the changes of the seasons. Besides, it is reasonable to suppose that freely floating blastocysts can only be collected by flushing the fresh uteri – an option not applicable for my frozen carcass-material.

Benefits of staining for placental scar counts

The benefits of staining for placental scar counts were remarkable, because the pattern and colour defining placental scars were intensified and emphasised. To remember, I found that 40.2% of the finally observed 164 placental scars only became visible when staining the uteri. My results show that staining improved the visibility of concealed placental scars, which principally concerned the faded and incomplete old placental scars in the brown bear females in this study. Erickson et al. (1964) reviewed in Hristienko et al. (2004) observed placental scars to persist for more than one year in the American black bear, but to fade with progressing time after parturition. Staining is also advantageous to prevent overestimations of placental scars, even if overestimations in the bear females examined rarely occurred. In some instances, I had difficulties discriminating between the obscure remnants of old placental scars and artefacts prior to staining. This is evident as 2.4% (n=164) of the unstained marks, which might potentially have been recorded as placental scars, were confirmed as artefacts when stained.

Staining of the uteri allows to classify the scars more precisely in order to demonstrate different scar-stadia in the course of tissue regeneration. I distinguished between 4 scar-phenotypes and assigned them to 4 scar-categories (Table 2.1), although afterwards it seemed to be more reasonable to pool the phenotypes according to the season of scar-formation. The obvious difference I perceived in the appearance of category 2 and category 3 placental scars supported that scars of these categories originated not from the same year, but from 2 consecutive years. I recorded new ($Category_{1+2}$) placental scars in the complete uteri of 40,

and incomplete uteri of 3 further females. The presence of new placental scars in the presumed legally protected bear mothers can either be explained due to cub mortality before autumn hunting season, or because they were harvested even though they had dependent offspring (see *Cub mortality and cub orphaning in Sweden*). Among the old ($Category_{3+4}$) placental scars, detected in the uteri of 34 females, category 4 scars beyond no doubt were deemed to be old scars (23 in 17 uteri). There is, however, a possibility that scars of the category 2 and 3 phenotype (Table 8) were not assigned to the appropriate year of parturition. This may be explained in two different ways: placental scars from neighbouring scar-categories might have been confounded, which just makes an impact if category 2 and 3 were concerned, or placental scars might have regenerated at different rates, thus new scar might have been perceived as old ones or vice versa. The latter possibility has also been reported by Elmeros and Hammershøj (2006) who evaluated scar counts in the American mink.

For the American black bear, Erickson et al. (1964) reported that multiple ovulations were largely confined to one ovary, but that the transmigration of the ova from one uterine horn to the other seemed to occur commonly. Therefore, multiple implantations were divided between the both horns. The scar-distribution between the left and right uterine horn of the brown bear females in my study confirmed these findings. In general, I recorded a maximum of 2 scars per horn, but I found a single uterus with a 3 to 1 scar distribution either.

Unstained orange-coloured scars appeared as lightly pigmented aggregations of orange granules. They were difficult to detect and often interpreted as old scars, and some even remained unobserved. The post-staining observations of the uteri with the formerly orange scars revealed scars with a vibrant pigmentation and a for the most part complete scar pattern which corresponded to the one of new scars. Results from the statistical analysis clearly suggest that the unstained orange scars of this phenotype (e.g. in Figure 11 and 12) corresponded to “new placental scars”. A reasonable explanation for the orange scar-colour can be found in Hristienko et al. (2004), describing the appearance of the scars for American black bears to be strongly influenced by the handling of the samples. If the uteri are removed from the carcass within 2 hours and frozen rapidly, placental scars are coral-coloured. If the uteri remain in the carcass for longer, the scars become progressively darker, turning dark-brown after a few hours and bluish-black after 12 hours. Hristienko et al. (2004) assumed that the change in colour is caused by anaerobic microbial production of hydrogen sulphide in the carcass. To my perception, unstained orange scars appeared very light-coloured, thus they

could only be observed if their pigmentation was still intensive and their characteristic pattern was still complete. So, despite the fact that orange colour may not dependent on the scar's age, but rather on the handling of the samples, unstained orange scars seem to correspond to the new ones. One of the females provided an indication that the orange colour may not depend on the scar's age. Within her unstained uterus I counted 1 cranially located orange scar beside 2 black scars, however within her stained uterus all 3 scars appeared equally black (Figure 11). This pre-staining difference, but post-staining similarity in the colour of the scars, suggests that all the scars were of the same age, but stored in 2 different milieus (aerobic and anaerobic). The plastic bags containing the carcass samples were frequently damaged, and the water soaking the carcasses sometimes leaked. Thereby the samples were partly immersed in water and partly surrounded by air. Each time the carcasses were defrosted the microbes worked in their different milieus, at different rates.

It was surprising to realise that even among the new placental scars in the bear females, a considerable proportion remained unidentified prior to staining (for explanations see *Conclusions*). Testing the Turnbull reaction-based staining method in the red fox, Ruette and Albaret (2011) could reveal only a slight elevation of new placental scars (2 new scars in 2 uteri; n=75 uteri total) after this staining-procedure. In my study, taking into account the unstained orange-coloured scars, at least 20.4% (n=98) of the "new" Category$_{1+2}$ scars could only be counted when stained. Disregarding the proportion of uteri with orange scars, which I identified, but not accurately categorised before staining, the staining benefits were revealed in 25.0% (n=64) of the finally observed scars. For the accurate classification of new scars staining was even required in almost a half of the cases (n=98). The most remarkable elevation of scar counts, however, was revealed by the pre-post comparison of the numbers of old placental scars. In 80.3% (n=66) of the finally observed old scars staining was required to make scars visible (in 66.7%), accurately classify them (~7.6) and set them apart from artefacts (~6.1%).

1.2 Litter size estimations based on placental scar counts – accuracy of the method

Persistency of placental scars postpartum

In general, I found placental scars in Scandinavian brown bears to persist for at least 21 month postpartum, reflecting breeding data from the last 2 breeding seasons. By this means the differentiation between new and old sets of placental scars is obligatory for litter size

estimations. Occasionally even older scars, formed during the gestation period of more than 2 years prior to the females' harvest, seem to be visible. The occurrence of these "very old" scars in the sample collection was, however, too scarce (2 in 1 uterus; n=70 uteri), and their appearance was too obscure to take them into account for litter size estimations. The large variation of the sample quality from wild-ranging bears could thereby complicate the discrimination of "very old scars" from artefacts, as very old scars appear yellowish and dehydrated areas of the uterine tissue too. My results are based on the different appearance of the new and old placental scars (as described in Table 2.1), as well as the very old (extremely faint, yellowish) scars and were confirmed by a number of concrete field observations of the bear mothers with their cubs in the year of harvest, the year before harvest, as well as 2 years before harvest (Table 9). Similar times of persistency for placental scars were reported in the Hokkaido brown bear (Tsubota et al. 1990), as well as the American black bear (Hristienko et al. 2004).

In theory, if all adult females are equally vulnerable to hunting, placental scar counts in females with a 2-year reproductive cycle could reveal the number of cubs in the year of harvest based on new scar counts, the number of cubs in the year prior to harvest based on old scar counts, as well as the proportion on non-breeding females within the cycle in case of scar absence. In females with a 3-year reproductive cycle, the proportion of females without scars in their uteri comprises about a third of the females that were actually breeding within the cycle, i.e. the mothers that had to raise their still dependent yearlings (thus scars were largely no longer visible after the 2nd hibernation of the family group), as well as the non-breeding females.

Correlation between Category$_{1+2}$ and Category$_{3+4}$ placental scars and the observed number of cubs

Reconstructing reproductive history from placental scar counts has already been demonstrated for the brown bear (e.g. Hensel et al. 1969, Tsubota et al. 1990, Mano and Tsubota 2002), and suggested that there can be differences in the regeneration of placental scars among individuals. Therefore, some authors (e.g. Mano and Tsubota 2002) recommended using this approach with caution, however they did not apply a Turnbull reaction-based staining method to enhance the detectability of the scars and evaluate its success. For the evaluation of the reliability of "stained" scar-phenotypes in their function as age-predictors, I was dependent on evidence from confirmed family groups observed in the field. The direct comparison of

counted placental scars and observed cubs could yield a clear result: the number of $Category_{1+2}$ scar was 100% consistent with the number of dependent cubs in the year of harvest (n=14 adult females, among those were 5 females with scars). In addition, $Category_{1+2}$ placental scars were identified in 5 females with evidences of lactation at their official inspection (*see Materials and methods: Bear hunting and sample collection*), which confirms that these females have had dependent offspring in the year of their harvest. Three of these females were from the southern study area, where 95% of the litters were weaned as yearlings, and most females exhibit a 2-year reproductive cycle (Dahle and Swenson 2003a; Dahle and Swenson 2003b). Here the coincidence of $Category_{1+2}$ scars and milk evidences the nursing of dependent cubs-of-the-year. To test the accuracy of scar tissue evaluations in wild brown bears, de facto, involved small sample sizes. Nevertheless, the available information on confirmed offspring traveling with their mothers and lactations, evidently supported that $Category_{1+2}$ scars are the "new" placental scars, formed during the immediately previous gestation period, i.e. in the winter prior to harvest.

Because of the insufficient evidence from confirmed family groups in the year before harvest, I was not able to statistically test the reliability of the $Category_{3+4}$ scars, and the direct comparison between the number of scars and cubs was restricted to a few observations. In all instances the number of $Category_{3+4}$ scars matched the number of cubs accompanying these mothers in the year before harvest (n=4 uteri with $Category_{3+4}$ scars). In additional 3 uteri the definite absence of $Category_{3+4}$ scars was confirmed by known litter size zero. Based on these findings, along with the observational experience, I could acquire throughout the scar-observations in the entire sample-material (n=116 actively stained uteri), $Category_{3+4}$ scar are deemed to be "old" placental scars formed during the gestation period of more than one year prior to harvest.

Distinction of resorptions and abortions

In carnivores, scar sites from resorptions or abortions have been described in two different ways, one describing them as pale placental scars (e.g. in the red fox: Lindström 1981; Elmeros et al. 2003; Ruette and Albaret 2011), and a second as very small scar sites <3mm (e.g. in the American black bear: Hristienko et al. 2004), where foeti do not develop to full term. The pale appearance of scar sites from resorptions can be attributed to the reduced uptake of blood cells from maternal haemorrhage by the local endometrial macrophages, as a result of the untimely termination of gestation. The small diameter can be explained by the

small size of the foeti by the time of their resorptions. In one female, I found a single scar site with an extraordinary small diameter being consistent with the latter explanation. As the scar site did not appear to be incomplete or faded in any degree it was unlike the older placental scars, and therefore believed to be from a resorption of a foetus that occurred in the previous winter (Table 3). Due to its location in the uterine horn exactly opposite the line of mesometrial attachment, the placental scar-like colour (similar to that of new placental scars) and the not in the slightest irregular scar-pattern, I did not consider this scar an artefact, although I cannot rule out this option. Besides, this female was not lactating and came into oestrus in the year of harvest, which was apparent from the endometrial development in her uterus.

2 Estimation of female reproductive performance

2.1 Age at primiparity, autumn-body mass and head circumference of breeders

The earliest age of primiparity for Scandinavian brown bear females was 3 years, as evident from the debut of new placental scars in the 3-year-olds and old scars in the 4-year-olds in my study. The first appearance of old placental scars, in the absence of new scars, in two 4-year-old females is an indication that the age of first successful breeding could as well be 3 years. These observations suggest that the females were nursing dependent cubs, and thereby inhibiting their re-entry in oestrus (McNeilly 1988) and consecutive-year birthing. Even though the assumption of lactational anoestus seems reasonable, the females may simply not have been reproductively active in the year after an initial breeding-year with cub loss. My results are consistent with the age of first confirmed reproduction for brown bears in Austria (Zedrosser et al. 1999; Zedrosser et al. 2004) and Croatia (Frkovič et al. 2001). Swenson et al. (2001) reported that the mean age of female primiparity in Scandinavia was 4.5 years in the south and 5.4 years in the north, and the mean age for earliest successful breeding was 5.2 years in the south and 5.4 years in the north (see *Materials and methods: Brown bear reproduction and reproductive physiology*). Zedrosser et al. (2009) described the earliest records of primiparity at age 4 in the south, and not at age 3 as evident from my scar counts. Because bears likely loose litters prior to field observations, placental scar studies are an advantageous method to reveal the true age of first litter production. Successfully raising cubs at the early age of 3 has been established for female brown bears in more southern parts of Europe, however not for those in Scandinavia, where the vegetation-period is by far shorter

(*see Material and methods: Study population and study area*). This limits the bears' time to forage, while sufficient food supply and quality largely influence reproductive potential (e.g. Bunell and Tait 1981; Stingham 1986, 1990a; Rogers 1987; Miller 1994; Zedrosser et al. 2006a; Zedrosser et al. 2009), such as the timing of first reproduction (Ferguson and McLoughlin 2000). Even within Scandinavia primiparae in the south were significantly younger compared to those in the north (Zedrosser et al. 2009), and Swenson et al. (2001) suggested that northern females may prioritize growth to be able the store more fat, which they need for the >1-month longer hibernation period in the north. This may explain my two records of 3-year-olds producing their first litter in the southern study area and two in the central area, but none in the northern area. The presence of endometrial bulges in the uterus in the absence of milk, in one of the 3-year-old breeders from Västernorrland, however, shows that not all premature mothers succeed to raise their debut litter successfully (*see Cub mortality and cub orphaning in Sweden*).

I found that only few 2-year-old females reached their sexual maturity (14.3%; n=28 based on the endometrial development) and few 3-year-olds produced their first litters (8.0%; n=25 based on new scars and 12.5%; n=16 based on old scars). At the age of 4 the proportion of females being reproductively active increased strongly, but was still beneath 50.0% (n=16). At the age of 5 years, however, placental scar observations suggested that the majority (≥76.9%; n=13 with unknown breeding-activity at age 3) of the females had already been reproductively active. As the proportion of females with old scars was still below the proportion with new until the age of 6 years, but relative proportions were age-independent at the age ≥7, I considered almost all potentially breeders among the Scandinavian bear females to lead a reproductively active life by the age of 6 to 7 years. Zedrosser et al. (2009) found the proportion of females first giving birth at the age of 6 years to be 11.0% and 19.0%, and at the age of 7 years to be 0.0% and 5.0%, for the south and north respectively. My findings from scar observations can support the assumption that first-time breeders >6 years are uncommon.

Primiparity is a key event in the life history of all animals (Stearns 1992). Starting reproduction at an earlier age could theoretically provide females the chance to increase their life-time reproductive fitness. Although only a few females were available for my analysis, there is suggestive evidence that primiparous females at the age of 3 years had smaller litters (1.25±0.25; n=4) than those starting litter-production at the age of 4 (2.20±0.20; n=5). According to Stearns (1992), delayed maturity leads to further growth, and larger body mass

is correlated with higher reproductive performance. Delayed maturity can lead to larger litters and increased breeding success in the first breeding attempt, which is consistent with the suggestions from my scar counts. In comparison, Zedrosser et al. (2009) calculated the mean litter sizes for primiparous females among all age-classes to be 1.92±0.61 (n=27 for the ages 4-6) in the south, and 2.22±0.73 (n=18 for the ages 5-7) in the north. Based on scar counts, I found similar litter sizes to those observed in the field already in younger females. Considering the observational time lag between scar and cub counts and that scars are no evidence for successful breeding, it seems plausible that scar counts may lead to a higher estimate of litter size and successful litter production than field observations. Particularly, in young primiparous females, increased cub mortality in the pre-mating period, attributed to the poor condition of the mothers and their insufficient milk production (Zedrosser et al. 2009), provides "one" reasonable explanation for diverging numbers of scars and cubs (see *Cub mortality in Sweden*). The findings show that scar tissue evaluations are a gainful method to assess the age of primiparous females in wild brown bears, because placental scarring can disclose unobserved breeding events in the field and young females appear to be strongly represented in a harvest sample of females.

Body mass is a deciding factor for the timing of sexual maturity and first litter production in brown bears, as the reproductive output is positively related to the body mass in the previous autumn before giving birth (Rogers 1976, Stringham 1990a). With abundant food supply supporting sufficient body mass, age at primiparity tends to be earlier in bears (Rogers 1977, Stringham 1990a). Due to the presence of bulges on the endometrium in a thick-walled uterus, my study confirms that follicular activity can occur as early as age 2, in females with a known autumn body mass of 82 and 85kg. Stringham (1990a) evaluated the seasonally adjusted body mass (average of spring and autumn mass) of successfully breeding grizzly bear females in North America to range between 95 to 200kg. Before entering hibernation, brown bears add 20 to 40% of their spring body mass in form of fat reserves for hibernation (Zedrosser 2006c). However, I had no data available for the autumn body mass of females that were successfully giving birth in the subsequent winter. For my Scandinavian females with cubs of the year the body mass in the subsequent autumn ranged between 83 and 173kg (Figure 24). It is hardly surprising that the autumn body mass of some 2-year-old nulliparous females was exceeding the mass of some mothers with cubs of the year that had to bear the high costs for lactation. As Hellgren (1998) reported, lactating females may lose 27% of their body mass during hibernation, whereas non-lactating females lose 20% of their body mass. The small head

circumference of some young females with first endometrial development (e.g. 53.5cm for a 2-year-old), or with new placental scars (e.g. 54cm for a 5-year-old) suggests that these females had not reached their maximum skeletal size and still had to allocate energy for their physical maturation by the time of their sexual maturation and their primiparity. My assumption is supported by literature reports (e.g. Zedrosser et al. 2006a, Zedrosser et al. 2009).

2.2 Litter size

I found evidence for litters comprising 1 to 4 cubs (Table 11). The factors constraining the reproductive potential of the k-selected brown bears are their large adult body size and their trait of capital breeding (i.e. reproduction is financed using stored capital, i.e. body fat, Stephens et al. 2009). Initial growth requires sufficient intake of fat-rich milk, and bear cubs are not weaned before the second or third hibernation together with their mothers in Scandinavia (Dahle and Swenson 2003a, Dahle and Swenson 2003b). In addition, birth and initial lactation occur during the period of hibernation, a period without food-intake. During this time, brown bear mothers are dependent entirely on the stored adipose tissue to meet their own metabolic needs, as well as provide sufficient milk to sustain their neonatal cubs until den emergence (Zedrosser 2006b). Even though female reproductive output is generally limited to 4 cubs per litter, Scandinavian brown bear females are able to make up for cub losses that occur during their reproductive cycles previous to weaning by "re-production". In case of cub loss during the mating season, bear mothers have already paid high costs for lactation. They may start the new breeding season with a considerable deficit, though they may under favourable conditions, to some extent be able to compensate the loss by their consecutive-year breeding attempt. This offers one explanation for the maximum of 4 observed placental scars also for 2 consecutive seasons. If bear mothers would otherwise lose their cubs prior to the cost-intensive period of lactation (lactation peaks around midsummer: Steyaert et al. 2012), they may still have the potential to produce a full-size litter in the following year. Yet, particularly young primiparous bear mothers are affected negatively by cub losses during the premating season. As they still have to allocate energy to growth and reproduction, they may anyway have difficulties raising a litter in their first breeding attempts (e.g. Zedrosser et al. 2009). A very reasonable approach to explain the maximum of 4 scars for 2 subsequent seasons in my study is the higher probability of cub mortality for singleton cubs (see *Cub mortality in Sweden*), together with the low frequency of quadruplets. Based on

scar tissue evaluations, I found incidents of cub mortality in the uteri of 8 females, and in each one cub mortality concerned a singleton litter. I suggest that counts of >4 cubs within 2 subsequent seasons may yet be realistic if physically matured bear mothers, in good condition, loose litters with more than a singleton-cub, possibly earlier in the year (e.g. through human disturbance in winter: Swenson et al. 1997b).

The mean litter size was higher when estimated by counts of new placental scars in comparison to counts of old scars (Table 12). Despite staining the uteri, not all old placental scars may have been visible. In the Hokkaido brown bear, Tsubota et al. (1990) considered new as well as old placental scars to determine the mean number of implantations, and then compared the estimated, scar-based litter size with the observed litter size in the field. However, in 1 of 7 females with yearlings, they were not able to detect old scars, and in 1 of 2 females with 2-year-old offspring, they were not able to detect very old scars. This suggests that in some individuals the old placental scars may disappear more rapidly. Tsubota et al. (1990), however, did not stain their samples. Besides, if a total litter goes unrecognized by scar counts and litters of all sizes are equally likely to go unrecognized, this cannot explain a reduction of mean litter size. Similar findings in the American mink showed that the uterine tissue at each scar site may regenerate at different rates (Elmeros and Hammershøj 2006). If only some cubs of a multi-cub litter go unrecognized, this would indeed explain the lower mean litter size estimated through old scar counts. Yet, in minks scar tissue regenerates at higher rates (estimates are reliable up to 3 month postpartum, Elmeros and Hammershøj 2006) than in the brown bear. In my bear females, I found placental scars to be frequently present for at least 21 months postpartum and staining the uteri was obligate. I observed scars of the same set that looked inhomogeneous before but homogeneous after staining (e.g. Figure 11). New placental scars were more likely to be detected before staining than old (see *Benefits of staining*), but the staining-effect equalised the detectability of new and old scars. I was able to detect every single old scar either corresponding to the category 3 or 4 phenotype. Nevertheless, as old scars may be a source of bias, some authors recommend considering only dark, i.e. new placental scars, for litter size estimations (e.g. in the red fox: Elmeros et al. 2003, Ruette and Albaret 2011). Testing the accuracy of new scar counts for a small sample of females, my results confirmed new scars as reliable indicators for cubs (see *Correlations between Category$_{1+2}$ placental scars and the number of observed cubs in the year*). The suggestion to consider only the reliable new scars does not rule out an overestimation of the previous litter by confounding old scars with new ones. Kordek and Lindzey (1980) described

the probability of scar misidentification in American black bears to be marginal, due to the differences in their brightness. This also appears reasonable for the brown bear. Though scar tissue may have regenerated at lower rates during hibernation, there was a time lag of a year in scar-regeneration, which is supposed to be reflected by the apparently different phenotypes of old and new placental scars. An overestimate of the number of new scars at old scar's cost may still have been possible, because the evidence from confirmed family groups in the field was limited. After staining the uteri, however, I was able to verify a phenotypic gap between such different-aged scars. Therefore, I would suggest that simultaneously present new and old scars are usually easy to distinguish applying the Turnbull-reaction based staining method. Difficulties to distinguish between some of the new and old scars-sets were rather apparent between individuals than within an individual (for explanations see also *Conclusions*). If the pacing of scar-regeneration in the individuals appears to be modified by litter size, I would speculate that females with larger litters and higher reproductive investment show lower rates of scar-regeneration. In concordance with a prolonged uterus involution, placental scars may then appear vibrant and complete for a prolonged time. In this instance, litter size estimations from new scar counts could possibly overestimate true litter size and those from old scar counts could underestimate true litter size. Placental scars also are supposed to regenerate slower in multiparous females than in primiparous females, as each gestation harms a female's uterine tissue (see *Macroscopic observations of uteri prior to staining*). The difference in the estimated litter sizes from new versus old scar counts may to some extent be explained by the inaccurate counts of old scars, but it could have been affected by sample collection. If young primiparous females or females with litter loss are overrepresented in either the sample with the new scars or the old scars, this is supposed to lead to a reduced litter size for the respective sample. Zedrosser et al. (2009) reported young primiparous bear mothers to have smaller litters than multiparous. The proportions of 3 to 4-year-old mothers in my samples, however, were fairly balanced, so this was no likely reason to explain the difference in the estimates. According to the parental investment theory (Maynard-Smith 1984), mothers that give birth to a singleton are supposed to have a higher probability to lose their litters than mothers of multi-cub litters. My results support this assumption (see *Cub mortality in Sweden*). Yet, the proportion of females with litter loss in the sample of new scar counts remained unidentified and I was not able to compare the respective proportions between both samples.

Due to insufficient field-data from mothers observed with cubs in the year before harvest, testing the reliability of old scar counts was not possible. I thus compared my results of scar counts to the literature. Even if I would have considered old placental scars as new ones, but assigned them to one litter, the mean litter size should be accurate. The year of parturition and females' age at parturition would then, however, be mistaken. According to Zedrosser et al. (2009), the mean number of observed cubs per litter in Scandinavia from 1987-2006 was 1.92 (primiparous) and 2.38 (multiparous) for females in the south, and 2.22 (primiparous) and 2.49 (multiparous) for females in the north. As documented by Swenson et al. (2001), the mean litter size in Scandinavia from 1987-1998 was ~2.3 for the south, and ~2.4 for the north. These calculations largely excluded the provinces Jämtland, Västerbotten and Västernorrland. According to Wydoski and Davis (1961), the estimated litter size should be higher than the observed litter size, because placental scar counts rather correspond to the implantation rate, at best to the parturition rate, and abortions of foeti, stillborn cubs, as well as postnatal mortality of cubs may occur. In support of this argument, my estimated mean of cubs per litter based on new and old scar counts (2.22±0.10; n=74) may seem a bit too low in relation to the reported values. It is thus more reliable to estimate litter sizes based on new placental scar counts (2.45±0.11; n=40) than on old scars (1.94±0.15; n=34). However, my discovery of a presumable resorption-scar (Table 3) suggests that I was able to distinguish between scars from full-term gestations and resorptions. Factors such as den abandonment of cubs due to human disturbance (Swenson et al. 1997b), bear mothers in poor condition not being able to bear the costs of lactation (e.g. Zedrosser et al. 2009), or opportunistic abandonment of singleton cubs (e.g. Tait 1980), may affect early cub survival. Because such factors would commonly cause total litter loss, instead of litter reduction, they do not obligatory affect mean litter size based on field observations. If, however, neonatal mortality was more common in smaller litters, and these litters remained unobserved in the field, the mean of the observed litter sizes may have overestimated the mean litter size at parturition. My results comprise females of all age-classes, but it is important to consider that the relatively large proportion of females of age 3 to 4 years (20.3%; n=74) may have decreased the mean litter size due to their lower fertility. In addition, my calculations of mean litter size involved primiparous females younger than those observed in the field (see *The age at primiparity*). As thereby arises, the number of placental scars need not exceed the number of observed cubs in the field in order to consider scar counts reliable.

When estimates were based on new and old placental scars, the mean litter sizes were 2.26±0.12 (n=38) for females in the southern study area and 2.45±0.31 (n=11) for females in the northern area (Table 17), and thereby reasonably conform to the reported values for these areas (Table 29). Nevertheless, litter estimates in the north were based on a small sample of females and may not reflect natural proportions. Besides, in the north of Sweden, more than half of the females exhibited a 3-year reproductive cycle (Swenson et al. 2007). As family groups are legally protected and placental scars are usually no longer perceivable in the third year after parturition, successfully breeding females with a 3-year reproductive cycle ought to be not represented in the results from scar counts.

I found suggestive evidence that the estimated litter sizes generally differed in relation to female age at parturition, no matter if the estimates were based on new scars only, or on the counts of new and old scars. My results revealed that primiparous females at age 3 had smaller litters of cubs than females at age 4. The results from new and old scar counts moreover suggested that litter sizes increase steadily in young initial breeders at the age of 3 to 5 years. Although my predictions are based on low sample sizes, the proposed effects of primiparity on female reproductive performance are consistent with the findings of Zedrosser et al. (2009) for brown bears in Scandinavia. Zedrosser et al. (2009) reported that primiparous mothers gave birth to fewer cubs than multiparous. However, in Zedrosser et al. (2009) the youngest primiparous females were 4 years old, and not 3 years like in this study. Besides, Zedrosser et al. (2006a) described female brown bears to reach 90% of their asymptotic body size (i.e. the threshold size for females to reach sexual maturity: Kingsley et al. 1988) at ~4 to 5 years of age in Scandinavia. The timing of physical maturation thereby fits to the shifts in litter size occurring at the age 3-4 years and the age 4-5 years.

2.3 Population productivity

Reconstructing reproductive activity of brown bears based on counts of placental scars may be considered precarious due to the strong dependency of the results on the method of sample collection. Results may reflect the samples' property instead of the natural proportions. The scar count-method is applicable to estimate the productivity in carnivore species with annual reproductive cycles, like the red fox (Ruette and Albaret 2011). In Scandinavian brown bears, however, the 2 to 3-year reproductive cycles, and the selectivity of harvest resulting from these cycles, may lead to skewed results of the reproductive rate. The sample collection of

females was likely biased against females with dependent young and older females, but in favour of solitary females and young females. Family groups are legally protected (Bischof et al. 2008) and older female have already dispersed from their maternal home ranges, may due their experience be more efficient in avoiding dangerous confrontations, and not least are less abundant as their survival decline rapidly above the age of 12 (Schwartz et al. 2003b). Females with 2-year cycles are expected to be overrepresented in the harvest sample. Notwithstanding that the most common cycle in Scandinavia is the 2-year cycle, females being solitary each second year are more vulnerable to be hunted than those being solitary only each third year. Thus, relative proportions of females with cubs, yearlings, 2-year-old offspring, and no offspring in my sample may not be consistent with the natural proportions in the Scandinavian brown bear population.

Considering both, new as old scar-sets helps to diminish the effects of the 2-year reproductive cycle. The presence of either new or old scars revealed that more than 60.0% of the ≥3-year-old females were reproductively active within the last 2 breeding seasons before harvest. The remaining females either had a 3-year reproductive cycle or were inactive within the cycle. Alternatively, some of them may not have been sexually mature yet.

Based on counts of new placental scars I found a mean population productivity of 0.90±0.12 cubs per adult female and year (n=106), which was quite close to the reported productivity of ~0.96 of a highly productive Scandinavian subpopulation (reviewed in Steyaert et al. 2012). With the above reservations on mind, I would expect the estimated productivity based on counts of new scars to be biased towards lower rates. Contrary to this expectation, the estimated productivity is high enough to speculate about confusions between new and old scars in the course of their counts, about incidents of cub mortality in the pre-hunting season (most likely in the in-den or mating period), or about occasional culling of bear mothers with dependent cubs (see *Cub mortality*). If otherwise the proportion of females with "cubs" was overrepresented in my sample, this would explain an increase in the estimated reproductive rate based on new scar counts. In addition, it appears likely that my results include some subadult females ≥3 years of age (actually evidenced for 1 of the 3-year-olds), whereas by definition the reproductive rate should only concern adult females. Because of these sexual maturity speculations, I decided to include all female ≥3 years of age in my calculations, and focus on the age-dependent changes in population productivity.

The Scandinavian brown bear females started litter production at the age of 3 years (see *The age at primiparity*), and their fertility largely increased from age 3 to age 4. Estimates based on new scars also suggested a continuing increase of population productivity from age 4 to age 5. Although my estimated shift in litter production occurred at an earlier age (young primiparous females: 3-5 years, prime-aged females: ≥6-7 years, see *The age at primiparity*), my results were consistent with the findings by Craighead et al. (1995a) that young females (4-8 years) had a lower litter production than prime-aged females (9-20 years). Schwartz et al. (2003b), who modelled shifts in litter production in female brown bears, found one initially occurring at 4 to 5 years of age, and a later occurring at 28 to 29 years of age. Yet, the study of Craighead et al. (1995a) described grizzly bears in Yellowstone and the study of Schwartz et al. (2003b) combined female reproductive data from Sweden, Alaska, Canada and the USA. In the Alaskan bear population, by comparison, the mean age of primiparity is substantially higher that in the Scandinavian (Steyaert et al. 2012). I lacked sufficient information for older females when productivity estimates were based on new or old scars exclusively, which is recognisable in Figure 26 and Figure 27. Combining the information from counts of new and old scars in order to increase sample sizes in the single age-classes and diminish the effects of the 2-year reproductive cycles, logistic regression confirmed that the proportion of breeding females in the population was age-dependent. Aside from Schwartz et al. (2003b), also Craighead et al. (1995a) found that old females (21-25 years of age) had a lower fertility than the prime-aged. My estimated productivity seemed to show no variation in relation to the female age once the females reached their prime years of adulthood, but I had too few uteri available from females older than 20 years to analyse possible senescence effects. The estimated productivity of the Scandinavian brown bear was equal in the southern, central and northern core area for female reproduction in Sweden, both when based on new scars (Table 23) and old scars (Table 24).

2.4 Cub mortality and cub orphaning in Sweden

The occurrence of total litter loss was shown in 8 of 66 females. For females with old scars (n= 34) I was able to document their "potential" breeding success. The reported annual cub loss in Sweden was 35.0% in the south, but only 4.0% in the north from 1988-1998 (Swenson et al. 2001). My estimated rate of litter loss of 23.5% (n=34) for the entire Scandinavian bear population thus seems to be rather high, particularly as some females may stick (at least) to their alternated-year breeding cycle after their loss (e.g. Swenson et al. 1997a). However,

protection of females with cubs from hunting can contribute to biased results based on scar tissue evaluations. In addition, sample size was low.

Most critical times for brown bear cub survival are either from parturition to shortly after leaving the den, by the time the bear mothers start lactating their offspring, or in the mating season, if bear mothers encounter newly immigrating males (Swenson et al. 2001, Zedrosser et al. 2009). Cub loss in the pre-mating season can for the most part be explained by previous year's food conditions. High population density can cause a deterioration of available food resources, in particular for younger, subdominant conspecifics (Zedrosser et al. 2009). Sexually premature females still have to allocate energy to growth and reproduction, have to make a greater effort to reach the threshold autumn body mass required to successfully raise a litter, and are less capable to store sufficient fat reserves for hibernation in comparison to physically mature females (e.g. Swenson et al. 2001; Zedrosser et al. 2009). The production of small, highly altricial bear cubs is not very costly for bear mothers (Oftedal and Gittleman 1989; Spady et al. 2007). These costs, however, increase rapidly when the fast-growing cubs are nursed with milk (because lactation is the energetically most expensive period for females: Oftedal 1984; and it peaks around midsummer: Steyaert et al. 2012). Thus, for mothers in poor condition the most critical time to lose offspring is the initial period of lactation. Disturbance can be an important factor for early cub mortality (Swenson et al. 2001). Swenson et al. (1997b) showed that human disturbance was a major cause of den abandonment by brown bears in winter in central Sweden, and that having to change den prior to parturition increases the probability of early litter loss. Still, the neonatal in-den mortality rate for bears was reported to be low (5% in American black bears: Miller 1994; and "uncommon" in Hokkaido brown bears: Mano and Tsubota 2002). This is hardly surprising in species with an obligate delayed implantation, because females in poor physical condition, that had difficulties gaining sufficient body fat reserves until hibernation, may rather resorb the unattached blastocysts. Tsubota et al. (1990) found corroborative evidence for such pre-implantation blastocyst loss (in 6 of 10 solitary females) in the Hokkaido brown bear. Reviewing literature, I was not able to find quantitative recordings for post-implantation embryo loss or neonatal mortality in the brown bear. Instead, I found anecdotal evidence for in-utero litter loss, i.e. a "presumed" resorptions-scar from the previous winter on the developed endometrium of a 4-year-old female in my study. The accurate identification of scars from resorptions and scars from full-term gestations would contribute to close this informational gap. For the 4-year-old primipara from the southern area, which was located in

central Sweden, poor physical condition or disturbed hibernation seems plausible to explain her post-implantation embryo loss. Cub loss in the mating season is best explained by social factors, as it mainly appears to be caused by infanticidal males (Swenson et al. 2001; Zedrosser et al 2009). Swenson at al. (2001) identified social aspects, i.e. sexually selected infanticide, as the leading cause for brown bear cub mortality at least in some parts of Scandinavia. For one female, 11 years old by the time of litter loss, the emergency of her litter was confirmed by field-observations. Being able to rule out in-den mortality in this prime-aged female from the southern area, with high annual cub loss through sexually selected infanticide (Swenson et al. 2001), litter loss during the mating season appears plausible.

Whilst the risk of cub mortality was not generally dependent on maternal age at parturition, the results indicate that females at age 4 had a higher risk to lose a litter than the older. The physical immaturity and the inexperience of young primiparous females seem to contribute to lower cub survival (66.7% litter loss; n=6 at age 4 versus 15.4% litter loss; n=26 at age ≥5). My results are consistent with the findings of Dahle at al. (2006), who described yearling offspring size to be positively related to the size of the offspring's mothers, and the size of the offspring to be positively associated with early survival. Moreover, primiparous mothers may be less experienced in skills like foraging and parental care (Becker et al. 1998, Wang and Novak 1994), and in times when food is scarce, their access to food may be constrained by their lower dominance status. Primiparous females may be less efficient defending their cubs against infanticidal males, have less knowledge about local dominance hierarchies, and less experience in avoiding potentially infanticidal males (Zedrosser et al. 2009). In one 3-year-old female with a new placental scar, the absence of milk in the nipples in the presence of endometrial bulges by the time of harvest suggests that this premature mother lost her singleton-cub in the pre-mating or early mating season.

The risk of cub mortality in Scandinavian brown bears was clearly dependent on the litter size. My results based on scar counts showed that mothers of a singleton cub were more vulnerable to lose their litters than mothers of multi-cub litters. The higher singleton cub mortality in Scandinavia could as well be ascertained through field observations in the respective years (Zedrosser et al. 2006b). A well-founded approach to explain litter size dependency of cub mortality in Scandinavia, in particular in areas with high rate of sexually selected infanticide, is the "parental investment theory" (Zedrosser et al. 2006b). According to this theory, females adjust their defence intensity to the reproductive value of their offspring

(Maynard-Smith 1984, reviewed in Zedrosser et al. 2006b). For bear mothers it is potentially very dangerous to defend their offspring against males. Females may be wounded or killed in the course of these conflicts (Zedrosser et al. 2006b). The litter size dependent variation in maternal defence may therefore explain the higher singleton cub mortality found in my study. Alternatively, some authors proposed that it could be advantageous for bear mothers to "opportunistically abandon" singleton cubs in the pre-mating season or early mating-season, in order to re-enter oestrus, mate again and increase the future reproductive fitness (e.g. Tait 1980). This suggestion is as well consistent with my findings based on scar counts, because each unsuccessful mother (n=8) had lost a singleton-cub in the year prior to harvest, but then gave birth to a larger litter in her consecutive-year breeding attempt.

Supplementary, I tried to assess litter losses before the end of the mating season based on the coincidence of new placental scars and endometrial development. As suggested by the development of the endometrium, I recorded 11 females (n=35) that may have re-entered oestrus and have prepared for the next implantation already within the year of litter loss. Unlike the findings based on consecutive-year scarring, in these females I observed litters with more than a singleton-cub. However, I examined no fresh uteri samples, thus dehydration and decay may have caused misidentifications of endometrial bulges in a thickened uterine wall, and crinkled, no longer swollen endometrial folds remaining from a previous season, observed in multiparous females (in multiparous females uteri may appear stretched and rather thin-walled due to age-dependent involution: see *Macroscopic observations of uteri prior to staining*).

The problem of cub orphaning in brown or black bears has frequently been observed to be human-induced, by disturbance in winter den or harvest. In the American black bear, the most critical time for cub orphaning was the spring-harvest (Hristienko et al. 2004). But, could harvest lead to cub orphaning in Scandinavian brown bears as well? Even though family groups are legally protected, Bischof et al. (2008) argued that mothers traveling with cubs may be more vulnerable to be harvested, when hunted with dogs. If bear mothers send their cubs up in a tree, hunters may not recognize the offspring and therefore kill the legally protected bear mothers (Bischof et al. 2008). The positive lactation data available for 5 of my females (12.5%, n=40) that were hunted in September and had new placental scars in their uterus (with regular wall-thickness), may indicate that the problematic issue of cub orphaning due to harvest was also apparent in Scandinavia.

Conclusions

Staining the uterine tissue is obligate for reliable and extensive placental scar counts in brown bears. It improves the identification of scars hardly or no longer visible in the unstained uteri, which particularly concerned old and orange-coloured placental scars. Likewise, it clearly facilitates the accurate classification of different-aged scars, whenever puzzling intermediate scar-phenotypes are observed.

Placental scars of category 1 are obviously "new" scars that can easily be attributed to the harvest year. Thus, their number represents the number of cubs-of-the-year in the year of harvest. Placental scars of category 4 are "old" scars formed during a gestation in the year prior to harvest. Their number reveals the number of newborns in the year prior to harvest. The changes in the appearance of category 2 and category 3 placental scars are generally obvious enough to accurately discriminate between the two categories. Even if down-regulation of metabolic activities in bears during hibernation occurs, the time lag of a year in scar-tissue regeneration provokes distinct scar-stadia.

To combine the information from scar tissue evaluations with the evidence of endometrial development can be helpful to discriminate between new and old placental scars. If females are still lactating, ovulation is inhibited and their uterus does not prepare for a lying ahead implantation (e.g. Yamane et al. 2009; Hristienko et al. 2004), thus their uterine wall should have about regular thickness. I conclude that in the absence of endometrial bulges, new placental scars can be expected. If females are not lactating, their endometrium will prepare for a lying ahead implantation following oestrus and mating, and their uterine wall will be apparently thickened by August (Yamane et al. 2009). I conclude that in the presence of endometrial bulges, old placental scars can be expected. If, however, females lost their litter, new placental scars can also coincide with endometrial bulges in a thickened uterine wall. In these instances, I noticed that the new placental scars, identified in the stained uteri, were frequently concealed prior to staining (e.g. Figure 4). It can be attributed to the anew cell-proliferation and growth taking place in the endometrium, while the hemosiderin-laden macrophages migrate towards the outer uterine layers. One could argue that I misidentified these, "actually old", placental scars. By implication, confusions between new and old scar-set cannot entirely be ruled out when interpreting intermediate scar-phenotypes.

An explanation for puzzling intermediate scar-phenotypes is the different speed of endometrial regeneration in individuals. If scar tissue regenerates at high rates, new scars could tend towards category 3-phenotype. An old scar-set could vice versa tend towards category 2-phenotype if tissue regeneration occurs at reduced pace. An untimely termination of gestation or lactation implicates an earlier re-entry in oestrus associated with the typical endocrine activity of the ovaries, endometrial cell-proliferation and growth (Yamane et al. 2009), following unsuccessful gestation or lactational anestrus. In addition to the length of lactation, I suggest that litter size and maternal age (see *Litter size*), as well as the length of hibernation may affect scar tissue regeneration.

The inconsistent handling of samples during field-collection influenced the appearance of the uteri, placental scars inclusively. My study, however, clearly shows that tissue staining can equalise the handling-dependent variability of even-aged scars.

Although I was just able to ascertain the reliability of new scar counts for successful litter size estimations, I recommend basing mean litter size estimations on new and old scar counts. The difficulty is to attribute the litters to the appropriate year of parturition, than to distinguish between scars from different litters. The estimated litter sizes from new and old scar counts were not considerably lower than the observed mean litter sizes. In the field, singleton cub mortality may go unrecognized if occurring early in the season, leading to more optimistic results. Instead, the relative large proportion of young females in a harvest sample of females can lead to a lower mean litter size estimated by placental scar counts in comparison to the observed in the field. As supported by the results from litter size estimates, young primiparous bear mothers had smaller litters of cubs than older mothers.

Reconstructing population productivity from placental scar observations is difficult when based on a harvest sample of females. Even though our estimated rates from new scar counts were quite close to the reported rates, the proportion of solitary females and thus the rates of litter production are generally likely to be biased high.

The examination of uteri, involving endometrium and scar tissue evaluation, is a valid method to reveal the female age at puberty and age of primiparity, to estimate the female age at first successful reproduction, to confirm incidents of neonatal mortality, litter-reduction, litter-loss

and cub orphaning through harvest, and possibly that of prenatal mortality as well. My presumed resorption-scar still has to be verified.

Reproductive information can be used to gain insight in a bear's life history. The information gained from this study helps to understand the reproductive performance of Scandinavian brown bear females in their 3 core areas of reproduction (Figure 1). It could for instance reveal the early start-up of female breeding activity in Scandinavia. It was remarkable to observe the onset of puberty already in the 2-year-olds, and primiparity already in the 3-year-olds! By the age of 3 years females as well seemed to be able to successfully raise their first litters. Scar counts, however, can also reveal their practical relevance for effective and sustainable bear management decisions, as for instance in the issue of cub mortality. The present study may, in this sense provide a standard for future estimations of female reproductive performance based on placental scars counts in the brown bear (see Table 2.1, 2.2, 3 and 30).

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APPENDIX

Table 1 Sample of female brown bears harvested in Sweden from April to October 1986-2005. The reproductive organs of these females (n=259) were collected by the Swedish Hunter Association (1986-2001) and the National Veterinary Institute of Sweden (after 2001).

		Month of harvest							
Harvest year	Number of females	April	May	June	July	Aug.	Sept.	Oct.	N.a.
1986	1						1		
1992	1						1		
1997	22	1				4	17		
1998	20					1a	18	1	
1999	23		3	1		8	9	2	
2000	27		1	1		11	12	2	
2001	14					6	6		2
2002	35		1		2	9	23		
2003	29					9	20		
2004	47	1	1			10+1[a]	24	10	
2005	40		2	1		20	15	2	
Total	259	2	8	3	2	77+2[a]	146	17	2

N.a. Information not available.

[a] Shot prior to official hunting season.

Table 2.1 The 4 categories of placental scars, identified in the completely collected and stained uteri (n=116) of female brown bears, are defined by features representing the time-related stadia of scar deterioration after parturition. The colour of placental scars may be strongly affected by the handling of the samples in the course of their collection in Sweden, 1992-2005.

Example picture	Category	Pigmentation of scar tissue		
		Intensity	Colour	Pattern
	1	very intensive: very high density of hemosiderin-laden macrophages	vibrant: commonly dark, almost black to bluish-black, also brown, reddish-brown, orange-brown to orange	a bisected antimesometrial ring: defined by a distinct, broad circular line externally, more or less completely filled with pigmentation internally (2 "half-moons"); frequently flanked by lateral shadows or pigmented spots
	2	intensive: density of hemosiderin-laden macrophages is still high	still vibrant: frequently dark, from dark grey to brown and reddish-brown shades	a bisected antimesometrial ring: defined by a distinct, broad circular line externally, but less completely filled with pigmentation internally; frequently flanked by lateral shadows or pigmented spots

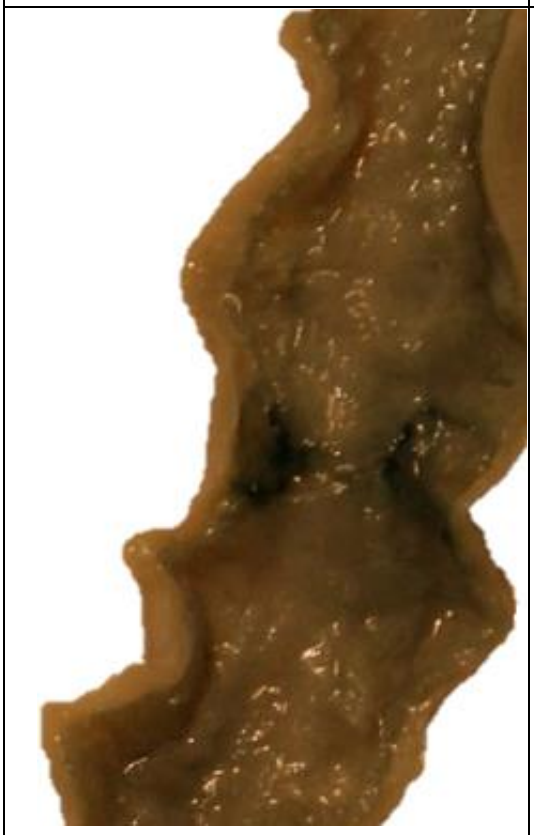
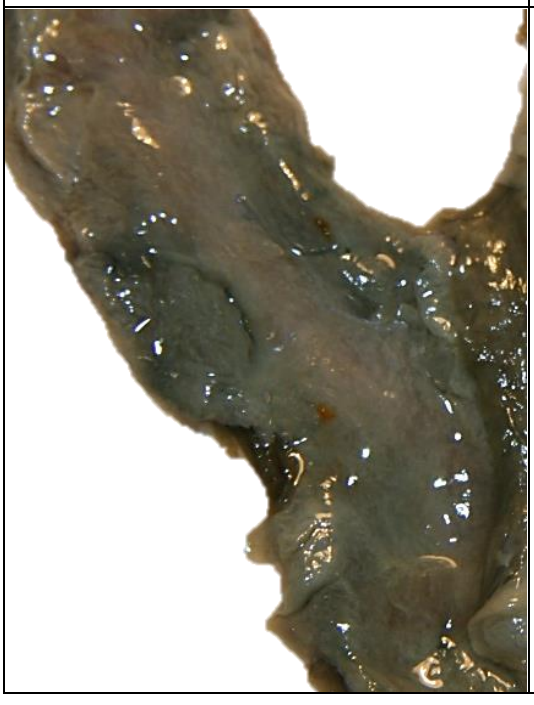
Example picture	Category	Pigmentation of scar tissue		
		Intensity	Colour	Pattern
	3	more or less slight	faded: from lighter grey to brown and yellowish-brown shades	no longer a complete bisected antimesometrial ring: with a thin, partly incomplete circular line externally, not filled with pigmentation internally; absence of lateral shadows
	4	very slight	very faded: also yellowish shades	a barely visible bisected antimesometrial ring: with remnants of the circular line externally, absence of pigmentation internally; absence of lateral shadows

Table 2.2 Schematic representation of the 4 categories of placental scars in the completely collected and stained uteri (n=116) of harvested female brown bears in Sweden, 1992-2005.

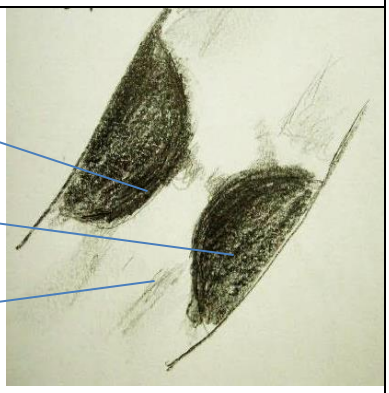
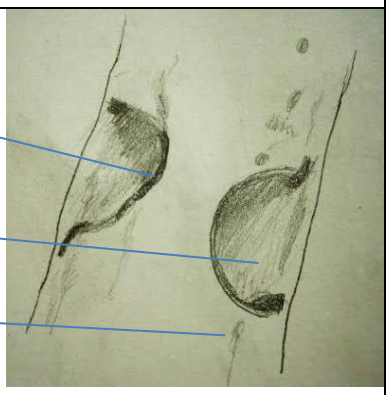
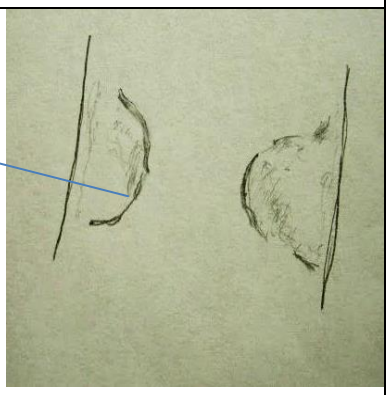
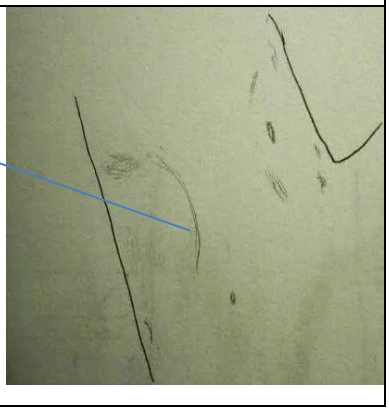
Scar category	Schematic representation of the pigmentation of scars tissue
1	bisected antimesometrial ring filled with pigmentation lateral shadows 
2	bisected antimesometrial ring less completely filled with pigmentation lateral shadows and pigmented spots 
3	bisected antimesometrial ring with incomplete circular lines 
4	just remnants of the circular lines visible 

Table 3 A solitary case of a potential resorption or abortion of a foetus, in the winter before harvest, found in the left horn of a completely collected and stained uterus (n=116) of a 4-year-old female brown bear from the southern study area in Sweden, 1992-2005.

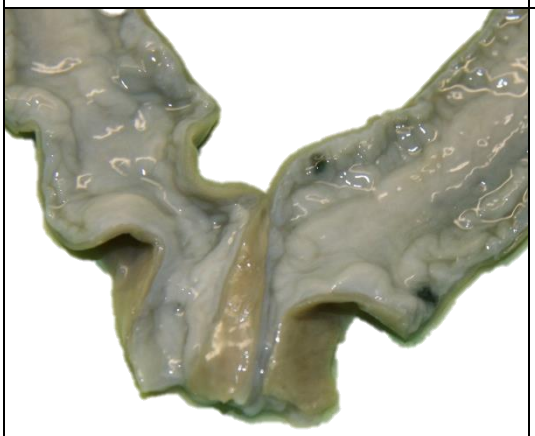
Example picture	Category	Pigmentation of scar tissue		
		Intensity	Colour	Pattern
	resorption or abortion of a foetus in the winter before harvest	intensive: high density of hemosiderin-laden macrophages	vibrant: almost black	scar sites < 3mm, deemed to be aborted or resorbed foeti (Hristienko et al. 2004)

Table 4 Sample collection and staining of uteri: the study is based on the complete uteri samples of 181 female brown bears harvested in Sweden during 1992-2005, and in particular on 116 uteri with an active staining during placental scar counts.

Age-class (years)	Samples of females						
	Overall sample size	Incomplete uteri	Complete uteri		Staining of complete uteri		
	n	n	n	%	Active[a]	Old[b]	No[c]
0-2	99	32	67	37	10	0	57
3-24	160	46	114	63	106	6	2
Total	259	78	181	100	116	6	59

[a] Uteri that were examined right after staining.

[b] Uteri that had been stained 2½ years prior to their examination.

[c] Unstained uteri.

Table 5 Numbers of complete uteri samples by age-class and study area, and proportions of uteri with placental scars (PS) by age from 0-24 years of harvested female brown bears in Sweden, 1992-2005.

Age (years)	Study areas n females			Total		% females with PS
	North	Central	South	n uteri with PS	n females	
0	1	1	3	0	5	0.0
1	3	7	24	0	34	0.0
2	5	5	16	0	26	0.0
3	7	8	10	2	25	8.0
4	0	6	10	7	16	43.8
5	4	2	7	10	13	76.9
6	2	3	4	8	9	88.9
7	0	3	3	5	6	83.3
8	0	0	5	5	5	100.0
9	0	0	5	5	5	100.0
10	0	2	1	3	3	100.0
11	2	3	3	7	8	87.5
12	2	2	2	5	6	83.3
13	1	1	1	1	3	33.3
14	0	1	0	1	1	100.0
15	0	1	0	1	1	100.0
16	1	0	0	1	1	100.0
17	0	0	2	1	2	50.0
18	1	0	1	1	2	50.0
19	0	1	1	2	2	100.0
20	1	0	0	1	1	100.0
22	0	0	2	2	2	100.0
24	0	0	1	1	1	100.0
Unknown, juvenile	0	1	1	0	2	0.0
Unknown, adult	0	1	1	1	2	50.0
Total (w.f.st.)	30	48	103	70 (66)	181 (175)	38.7 (37.7)
Total ≥3 years (w.f.st.)	21	34	59	70 (66)	114 (108)	61.4 (61.1)

(w.f.st.) Without the formerly stained uteri.

Table 6 Amount of complete uteri by age-class, total numbers of complete uteri, as well as numbers and proportions of uteri with placental scars (PS) of harvested female brown bears in Sweden, during 1992-2005. Females aged 0-2 years were deemed nulliparous. Females aged 9-20 years were deemed prime-aged adults, whereas those older than 20 years were pooled as post-prime adults. Females younger than 9 years, here summarised to provide an overview, were tested age by age in the analyses of litter size and population productivity.

	Age-class					n total		%
Harvest year	0-2	3-8	9-20	21-24	Unknown, adult	Uteri with PS	Females	≥3-year-olds with PS
1992	0	1	0	0	0	0	1	0.0
1997	3	3	4	0	1	5	11	62.5
1998	5	1	4	0	0	3	10	60.0
1999	9	4	0	1	1	4	15	66.7
2000	6	8	2	1	0	8	17	72.7
2001	4	2	1	0	0	1	7	33.3
2002	9	12	8	0	0	12	29	60.0
2003	7	9	5	0	0	7	21	50.0
2004	13	18	7	0	0	18	38	72.0
2005	11	16	4	1	0	12	32	57.1
n total	67	74	35	3	2	70	181	61.4
% total	37.0	40.9	19.3	1.7	1.1	38.7	100.0	

Table 7 Observations of new, old, and orange-coloured placental scars (PS) in the uteri of 175 females examined prior to staining, and in the no longer effectively stained uteri of 6 females of overall 181 harvested female brown bears in Sweden (1992-2005). Also extraordinarily small scar sites deemed as resorptions or abortions were recorded.

	Unstained uteri (n=175)		Formerly stained uteri (n=6)	
Scar-appearance	With PS	n PS	With PS	n PS
New	21	52	1	2
Old	13	22	2	3
Orange-coloured	15	28	1	2
Total	46[a]	102	4	7
Resorption, abortion	1	1	0	0

[a] In the uteri of 2 females I simultaneously found new and old PS, and in the uterus of 1 female I simultaneously found new and orange PS.

Table 8 Numbers of uteri with placental scars (PS) of the 4 categories, placental scar counts (PSC), and means±SE (standard error of means) in the 4 categories of placental scars among the overall 181 harvested female brown bears in Sweden (1992-2005). Due to the similar appearance of the placental scars in those categories, category 1 and category 2 were summarised to "Category$_{1+2}$", and category 3 and category 4 to "Category$_{3+4}$".

Scar category	Uteri with PS	n PS	Mean PSC±SE
1	16	44	2.75±0.14
2	24	54	2.25±0.15
3	18	43	2.39±0.18
4	17	23	1.35±0.15
1+2	40	98	2.45±0.11
3+4	34	66	1.94±0.15
Total[a]	66[a]	164	2.48±0.10
Total[b]	74[b]	164	2.22±0.10

[a] One uterus simultaneously had category 3 and category 4 placental scars, 8 uteri had both, Category$_{1+2}$ and Category$_{3+4}$ placental scars.

[b] Number of scar-sets (Category$_{1+2}$ or Category $_{3+4}$) found in the uteri of 66 females with placental scars.

Table 9 The time of persistence of placental scars (PS) postpartum, in 181 harvested female brown bears in Sweden during 1992-2005, was confirmed by the observations of cubs traveling with their mothers (n=14) in the field in the year of harvest, the year before harvest and 2 years before harvest. Observations of scars that were formed during a gestation period of more than 2 years prior to harvest were possible, but uncommon.

		Placental scar counts			Observed cubs		
Female id	Staining	Cat.$_{1+2}$ "New"	Cat.$_{3+4}$ "Old"	"Very old"	In harvest-year	In year before	2 years before
00BD99	+	3	0	--	3	0	2
03WW10	+	3	0	--	3	n.a.	n.a.
02WW05	old	2	--	--	2	1	n.a.
97SS06	+	2	1	--	2	1	0
V1240/04	old	0	--	--	0	2	0
02WW11	old	0	--	--	0	2	0
03BD01	old	0	0	2	0	0	2
V0862/05	+	0	3	--	0	3	0
00WW13	+	0	3	--	0	3	0
02WW10	+	0	4	--	0	n.a.	n.a.
V1196/04	+	0	2	--	0	2	0
03WW11	+	0	3	--	0	n.a.	n.a.
03WW19	+	0	0	--	0	n.a.	n.a.
02WW04	old	2[a]	0	--	2	0	0

+ Uteri with effective staining.

Cat. Category of placental scars.

[a] Orange-coloured placental scar.

-- Not detectable due to old staining or exceedance of the persistence-time.

n.a. Information not available.

Table 10 Proportion of breeding females[a] with either new or old placental scars (PS), or new and old placental scars simultaneously by female age, among the overall 181 harvested female brown bears in Sweden during 1992-2005. N=177 is excluding 4 of 181 females with unknown age, and as the uteri of 6 of these females had an old staining, I was just able to record the new scars. N=171 is excluding 6 of 177 females with a formerly stained uterus.

Age (years)	% females with new PS	n=177	% females with new PS	% females with old PS	% females breeding within last 2 seasons	n=171
0	0.0	5	0.0	0.0	0.0	5
1	0.0	34	0.0	0.0	0.0	34
2	0.0	26	0.0	0.0	0.0	26
3	8.0	25	8.0	0.0	8.0	25
4	31.3	16	31.3	12.5	43.8	16
5	61.5	13	61.5	46.2	76.9	13
6	66.7	9	66.7	33.3	88.9	9
7	50.0	6	50.0	50.0	83.3	6
8	60.0	5	50.0	50.0	100.0	4
9	40.0	5	40.0	60.0	100.0	5
10	0.0	3	0.0	100.0	100.0	3
11	50.0	8	42.9	42.9	85.7	7
12	50.0	6	60.0	40.0	80.0	5
13	0.0	3	0.0	33.3	33.3	3
14	100.0	1	100.0	0.0	100.0	1
15	0.0	1	0.0	100.0	100.0	1
16	100.0	1	100.0	0.0	100.0	1
17	0.0	2	0.0	100.0	100.0	1
18	0.0	2	0.0	100.0	100.0	1
19	100.0	2	100.0	50.0	100.0	2
20	100.0	1	100.0	0.0	100.0	1
22	0.0	2	0.0	100.0	100.0	1
24	0.0	1	0.0	100.0	100.0	1

[a] Placental scar counts allow to reveal the breeding activity of females within the last 2 breeding seasons, which implies that the breeding activity of females with a 3-year reproductive cycle cannot be completely assessed by this method.

Table 11 Frequency of litter sizes, of harvested female brown bears in Sweden from 1992-2005, based on the counts of new and old placental scars (PS) in 66 of 181 complete uteri samples with an active staining during scar tissue evaluations.

	Females with			
n cubs per litter	new PS	old PS	Total	%
1	3	13	16	21.6
2	18	11	29	39.2
3	17	9	26	35.1
4	2	1	3	4.1
Total	40	34	74[a]	100

[a] In 8 of the 66 uteri with placental scars (PS) I found 2 sets of placental scars.

Table 12 Litter size estimations, in harvested female brown bears in Sweden from 1992-2005, based on new, on old, as well as on new and old placental scars counts (PSC) in 66 of 181 complete uteri samples with an active staining during scar tissue evaluations. In addition, the litter size based on new placental scars found in 42 of 181 uteri with an active or an old staining is registered in parentheses.

	Litter size based on					
	New PSC		Old PSC		New and old PSC	
n cubs per litter	n scars	n females	n scars	n females	n scars	n scar-sets
1	3	3	13	13	16	16
2	36 (40)	18 (20)	22	11	58	29
3	51	17	27	9	78	26
4	8	2	4	1	12	3
Total	98	40 (42)	66	34	164	74[a]
Mean±SE	2.45±0.11 (2.43±0.11)	40 (42)	1.94±0.15	34	2.22±0.10	74[a]

[a] In 8 of the 66 uteri with placental scars (PS) I found 2 sets of placental scars.

SE Standard error of the mean.

Table 13 Median and mean number of new placental scars (PS) by female age-class in the complete uteri of 39 of 181, respectively 41 of 181 harvested female brown bears in Sweden, 1992-2005.

	New PS after staining				New PS total[a]			
Age-class	Median	Mean	±SE	n	Median	Mean	±SE	n[a]
3	1.00	1.00	0.00	2	1.00	1.00	0.00	2
4	2.00	2.20	0.20	5	2.00	2.20	0.20	5
5	2.50	2.38	0.26	8	2.50	2.38	0.26	8
6	3.00	2.83	0.31	6	3.00	2.83	0.31	6
7	2.00	2.33	0.33	3	2.00	2.33	0.33	3
8	3.00	3.00	0.00	2	3.00	2.67	0.33	3
9-20	2.00	2.54	0.18	13	2.00	2.50	0.17	14
Total	2.00	2.44	0.12	39	2.00	2.41	0.11	41

[a] New placental scars in all stained uteri, including the 2 formerly stained.

SE Standard error of the mean.

Table 14 Median and mean of the estimated litter sizes by female age at parturition, when based on counts of new and old placental scars (PSC) in the actively stained uteri of 65 of 181 harvested female brown bears in Sweden, 1992-2005. In total 66 of 181 females had placental scars, however, 1 female with unknown age could not be considered.

Age-class	New and old PSC after staining			
(years)	Median	Mean	±SE	n scar-sets
3	1.00	1.25	0.25	4
4	2.00	1.91	0.25	11
5	2.00	2.18	0.23	11
6	2.00	2.44	0.29	9
7	2.00	2.40	0.24	5
8	3.00	2.80	0.20	5
9-20	2.00	2.19	0.18	26
21-23	3.00	3.00	0.00	2
Total	2.00	2.21	0.10	73[a]

[a] In 8 of the 65 uteri with placental scars (PS) I found 2 sets of placental scars.

SE Standard error of the mean.

Table 15 The proportions of 3 to 4-year-old breeders and ≥5-year-old breeders recorded in the sample of uteri with new scars, as well as in the sample of uteri with old scars. Of the overall 181 harvested female brown bears in Sweden during 1992-2005, I considered 65 females with placental scars and known age.

	Females with				
Age-class	New PS		Old PS		PS total
(years)	%	n	%	n	n
3- 4	17.9	7	23.5	8	15
≥5	82.1	32	76.5	26	58
Total	100.0	39	100.0	34	73[a]

[a] In 8 of the 65 uteri with placental scars (PS) I found 2 sets of placental scars.

Table 16 Median and mean numbers of new placental scars (PS) by study area, in the uteri with new scars of 40 of 181, respectively 42 of 181 harvested female brown bears in Sweden, during 1992-2005.

Study area	New PS after staining			n females	New PS total[a]			n females[a]
	Median	Mean	±SE		Median	Mean	±SE	
South	2.00	2.30	0.15	20	2.00	2.27	0.14	22
Central	2.50	2.43	0.17	14	2.50	2.43	0.17	14
North	3.00	3.00	0.37	6	3.00	3.00	0.37	6
Total	2.00	2.45	0.11	40	2.00	2.43	0.11	42

[a] New placental scars in all stained uteri, including the 2 formerly stained.

SE Standard error of the mean.

Table 17 Median and mean numbers of new and old placental scars (PS) by study area, in the stained uteri with scars of 66 of 181 harvested female brown bears in Sweden, 1992-2005.

	New and old PS				
Study area	Median	Mean	±SE	n scar-sets	n females 3-4 years old[b]
South	2.00	2.26	0.12	38	9
Central	2.00	2.04	0.17	25	4[a]
North	2.00	2.45	0.31	11	2
Total	2.00	2.22	0.10	74[a]	15[c]

[a] In 8 of the 66 uteri with placental scars (PS) I found 2 sets of placental scars.

[b] Female age at parturition (for females with old PS: 1 year less than their age at harvest).

[c] One female was with placental scars, but unknown age.

SE Standard error of the mean.

Table 18 Median and mean numbers of new placental scars by harvest year, as observed in the uteri with new scars of 40 of 181, respectively 42 of 181 harvested female brown bears in Sweden, 1992-2005.

	New PS after staining				New PS total[a]			
Harvest year	Median	Mean	±SE	n females	Median	Mean	SE	n females[a]
1997	2.00	2.00	0.00	2	2.00	2.00	0.00	2
1998	2.50	2.50	0.50	2	2.50	2.50	0.50	2
1999	2.00	2.33	0.33	3	2.00	2.33	0.33	3
2000	3.00	2.75	0.25	4	3.00	2.75	0.25	4
2001	3.00	3.00		1	3.00	3.00		1
2002	3.00	2.57	0.37	7	2.00	2.44	0.29	9
2003	3.00	2.67	0.33	3	3.00	2.67	0.33	3
2004	2.50	2.50	0.27	10	2.50	2.50	0.27	10
2005	2.00	2.13	0.23	8	2.00	2.13	0.23	8
Total	2.00	2.45	0.11	40	2.00	2.43	0.11	42

[a] New placental scars in all stained uteri, including the 2 formerly stained.

SE Standard error of the mean.

Table 19 Medians and means of estimated litter sizes by year of parturition, based on the counts of new and old placental scars (PSC) in the stained uteri with scars of 66 of 181 harvested female brown bears in Sweden, from 1992-2005.

	New and old PSC			
Year of parturition	Median	Mean	±SE	n scar-sets
1996	1.50	1.75	0.48	4
1997	1.50	1.50	0.29	4
1998	2.00	2.00	0.58	3
1999	2.00	2.13	0.30	8
2000	3.00	2.75	0.25	4
2001	2.00	2.20	0.58	5
2002	2.50	2.50	0.27	10
2003	2.00	2.25	0.22	12
2004	2.00	2.31	0.22	16
2005	2.00	2.13	0.23	8
Total	2.00	2.22	0.10	74[a]

[a] In 8 of the 66 uteri with placental scars (PS) I found 2 sets of placental scars.

SE Standard error of the mean.

Table 20 Median and mean productivity by age-class, based on new placental scar counts (PSC) in the stained uteri of 106 of 181 harvested female brown bears in Sweden, during 1992-2005. Productivity was calculated as the number of cubs, i.e. new scars (0-4), per potentially adult (≥3-year-old) female and year. In addition, the reproductive rates based on new scar counts in 112 of 181 uteri with an active or an old staining are registered in parentheses.

Age-class (years)	Productivity based on new PSC			n females
	Median	Mean	±SE	
3	0.00	0.08	0.06	25
4	0.00	0.69	0.27	16
5	2.00	1.46	0.37	13
6	2.00	1.89	0.51	9
7	1.00	1.17	0.54	6
8	1.50 (2.00)	1.50 (1.60)	0.87 (0.68)	4 (5)
9-20	0.00	1.06 (1.00)	0.24 (0.22)	31 (35)
22-24	0.00	0.00	0.00	2 (3)
Total	0.00	0.90 (0.88)	0.12 (0.12)	106 (112)

SE Standard error of the mean.

Table 21 Median and mean productivity by age-class, based on old placental scar counts (PSC) in the stained uteri of 81 of 181 harvested female brown bears in Sweden, during 1992-2005. Productivity was calculated as the number of cubs, i.e. old scars (0-4), per potentially adult (≥3-year-old) female and year. Considering old scar counts, female age at parturition is 1 year less than the age at harvest.

	Productivity based on old PSC			
Age-class (years)	Median	Mean	±SE	n females
3	0.00	0.19	0.14	16
4	0.00	0.77	0.30	13
5	0.00	0.56	0.29	9
6	0.50	0.83	0.40	6
7	1.00	1.25	0.75	4
8	2.00	1.60	0.68	5
9-19	0.50	0.92	0.23	26
21-23	3.00	3.00	0.00	2
Total	0.00	0.81	0.12	81

SE Standard error of the mean.

Table 22 Median and mean productivity by age-class, based on new and old placental scar counts (PSC) in the stained uteri of 106 of 181 harvested female brown bears in Sweden, during 1992-2005. Productivity was calculated as the number of cubs per potentially adult (≥3 years) female and year. Overall I considered 187 either new or old scar-sets with 0 to 4 placental scars each, i.e. 106 scar-sets with new scars from the harvest year, and 81 scars-sets with old scars from the year before harvest.

	Productivity based on new and old PSC			
Age-class (years)	Median	Mean	±SE	n scar-sets
3	0.00	0.12	0.06	41
4	0.00	0.72	0.20	29
5	0.50	1.09	0.26	22
6	2.00	1.47	0.36	15
7	1.00	1.20	0.42	10
8	2.00	1.56	0.50	9
9-20	0.00	1.00	0.17	57
21-24	1.50	1.50	0.87	4
Total	0.00	0.86	0.09	187

SE Standard error of the mean.

Table 23 Median and mean productivity by study area, based on new placental scar counts (PSC) in the stained uteri of 106 of 181 harvested female brown bears in Sweden, during 1992-2005. Productivity was calculated as the number of cubs, i.e. new scars (0-4), per potentially adult (≥3-year-old) female and year.

	Productivity based on new PSC			
Study area	Median	Mean	±SE	n females
South	0.00	0.87	0.16	53
Central	0.00	0.94	0.22	33
North	0.00	0.90	0.33	20
Total	0.00	0.90	0.12	106

SE Standard error of the mean

Table 24 Median and mean productivity by study area, based on old placental scar counts (PSC) in the stained uteri of 81 of 181 harvested female brown bears in Sweden, during 1992-2005. Productivity was calculated as the number of cubs, i.e. old scars (0-4), per potentially adult (≥4 years of age in harvest year) female and year.

	Productivity based on old PSC			
Study area	Median	Mean	±SE	n females
South	0.00	0.93	0.19	43
Central	0.00	0.68	0.19	25
North	0.00	0.69	0.29	13
Total	0.00	0.81	0.12	81

SE Standard error of the mean.

Table 25 Median and mean productivity by harvest year, based on new placental scar counts (PSC) in the stained uteri of 106 of 181 harvested female brown bears in Sweden, during 1992-2005. Productivity was calculated as the number of cubs, i.e. new scars (0-4), per potentially adult (≥3-year-old) female and year.

Harvest	Productivity based on new PSC			
year	Median	Mean	±SE	n females
1992	0.00	0.00		1
1997	0.00	0.57	0.37	7
1998	0.00	1.00	0.63	5
1999	1.00	1.00	0.58	4
2000	0.00	1.00	0.43	11
2001	0.00	1.00	1.00	3
2002	0.00	1.06	0.35	17
2003	0.00	0.62	0.33	13
2004	0.00	1.04	0.28	24
2005	0.00	0.81	0.25	21
Total	0.00	0.90	0.12	106

SE Standard error of the mean.

Table 26 Litter loss and corresponding cub loss in relation to the maternal age in the year of loss, as estimated from placental scar counts in the stained uteri of 32 of 181 harvested female brown bears in Sweden, during 1992-2005. All females that had new and old placental scars from 2 subsequent years in their uterus were supposed to have lost their earlier litter. All females with old, but no new placental scars were considered to have successfully raised cubs.

	Litter loss		Cub loss	
Age (years)	Rate	n litters	Rate	n cubs
4	0.667	6	0.400	10
5	0.333	3	0.200	5
6	0.333	3	0.200	5
7	0.000	2	0.000	5
8	0.000	3	0.000	8
9	0.000	3	0.000	6
10	0.000	3	0.000	6
11	0.500	2	0.250	4
12	0.000	1	0.000	2
14	0.000	1	0.000	1
16	0.000	1	0.000	2
17	0.000	1	0.000	2
18	1.000	1	1.000	1
21	0.000	1	0.000	3
23	0.000	1	0.000	3
4	0.667	6	0.400	10
5-23	0.154	26	0.075	53
Total	0.250	32	0.127	63

Table 27 Litter loss in relation to female litter size of 1 to 4 cubs, as estimated from placental scar counts in the stained uteri of 32 of 181 harvested female brown bears in Sweden, from 1992-2005. All females that had new and old placental scars from 2 subsequent years in their uterus were supposed to have lost their earlier litter. All females with old, but no new placental scars were considered to have successfully raised cubs.

	Litter loss	
Litter size	Rate	n Litters
1	0.667	12
2	0.000	10
3	0.000	9
4	0.000	1
Total	0.250	32

Table 28 Litter loss in the southern, central and northern study area, as estimated from placental scar counts in the stained uteri of 32 of 181 harvested female brown bears in Sweden, from 1992-2005. All females that had new and old placental scars from 2 subsequent years in their uterus were supposed to have lost their earlier litter. All females with old, but no new placental scars were considered to have successfully raised cubs.

	Litter loss	
Study area	Rate	n litters
South	0.235	17
Central	0.300	10
North	0.200	5
Total	0.250	32

Table 29 Comparison of the estimated litter sizes based on new and old placental scar counts (PSC) in the stained uteri of 66 of 181 harvested female brown bears in Sweden (1992-2005), and the reported litter sizes based on cub observations in the field (Swenson et al. 2001: 1987-1998; Zedrosser et al. 2009: 1987-2006) in the study areas. In 8 of the 66 uteri with placental scars I found 2 sets of placental scars, i.e. overall I considered 74 scar-sets.

	Mean litter sizes		
		from literature	
Study area	from PSC	Swenson et al. (2001)	Zedrosser et al. (2009)
South	2.26±0.76 (38)	2.3±0.11 (55)	1.92±0.61 (27)[p]; 2.38±0.83 (109)[m]
Central	2.04±0.84 (25)	--	--
North	2.45±1.04 (11)	2.4±0.14 (33)	2.22±0.73 (18)[p]; 2.49±0.78 (57)[m]

[p] Primiparous females.

[m] Multiparous females.

Table 30 Female reproductive traits as evident from female uteri and their placental scars (PS), corroborative information from confirmed litters in the field or lactation. The complete uteri of 181 of 259 harvested female brown bears in Sweden (1986-2005) were analysed for the presence of placental scars. Supplementary, 106 complete and 8 incomplete uteri of the overall sample collection were examined for endometrial development.

Reproductive traits	Evidence from female uteri and corroborative information
Sexual maturity: 1st oestrus	Uterus development: bulges on the endometrium, thickened uterine wall by August
Primiparity	First presence of PS
1st successful litter production[b]	Old PS present, new PS absent
Prenatal mortality	Resorption-scars of the year[a] (no corresponding cubs-of-the-year)
Neonatal mortality	Number of confirmed cubs after emergency lower than number of new PS
Litter reduction	Number of confirmed cubs lower than number of new PS
Litter loss	New and old PS simultaneously present, or absence of milk in spite of new PS
Cub orphaning	Females harvested despite of evidence of lactation and new PS
Ovarian activity, no dependent offspring	Bulges on the endometrium, thickened uterine wall by August
Cubs-of-the-year, "if" no mortality	New PS (Category 1, Category 2), no bulges on the endometrium, uterine wall not apparently thickened by August
Cubs in the year before (yearlings)	Old PS (Category 3, Category 4), bulges on the endometrium, thickened uterine wall by August (likely if 2-year reproductive cycle)
Cubs 2 years before (2-year old young)	Very old PS

[a] I was not able to test for consistency.

[b] Alternatively females may have lost a litter and not have given birth in the subsequent year.

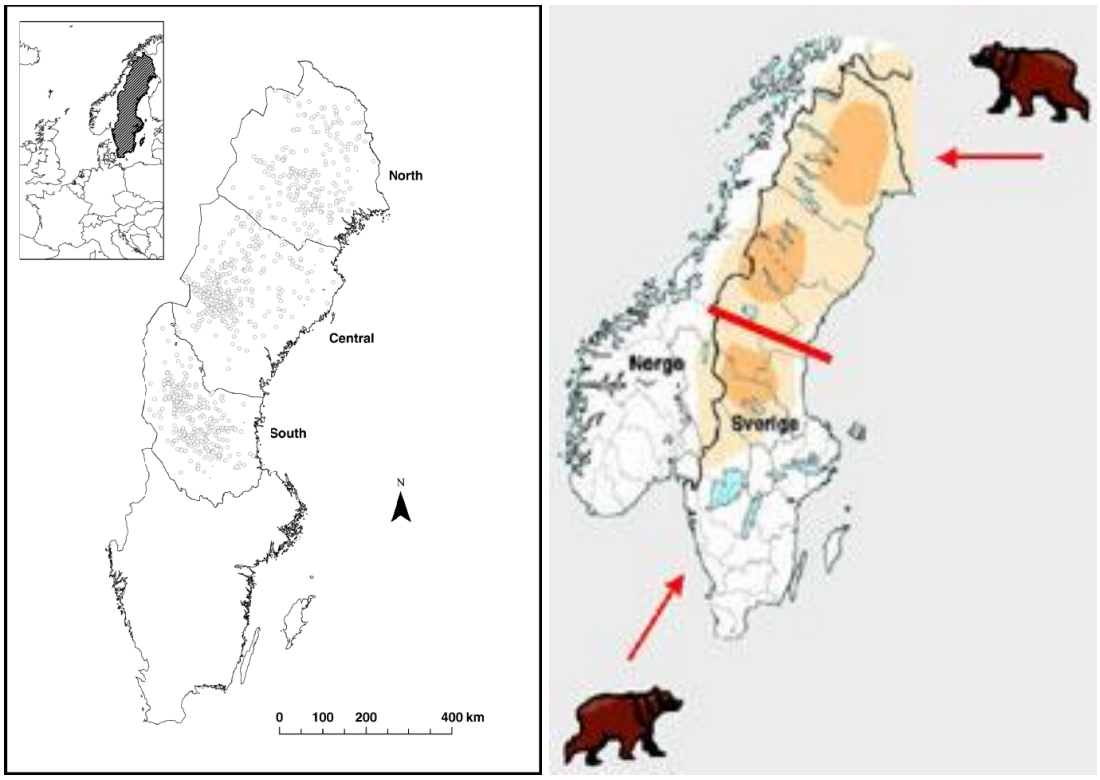


Figure 1 Left: Map of Sweden showing the spatial structure of the Scandinavian brown bear population, thereby outlining the distribution of the 3 subpopulations within the southern, central and northern study area (circles correspond to 883 harvest locations of female and male bears from 1981 through 2004: Bischof et al. 2008). Right: Map of Sweden showing the 3 core areas for reproduction. Brown bears colonised the southern area from the south, and the central and northern area from the east (SBBRP 2013) (Fig. by the SBBRP 2013).

Figure 2 Example picture showing the uterus morphology of a female brown bear, harvested in Sweden during 1986-2005, in concordance with the female reproductive state and the change of season: The adult female of unknown age had 3 new placental scars in her stained uterus, and was harvested in May. The thickness of the uterine wall was “regular”, and bulges on the endometrium were missing.

Figure 3 Example picture showing the uterus morphology of a female brown bear, harvested in Sweden during 1986-2005, in concordance with the female reproductive state and the change of season: The adult female was 4 years old, had 2 new placental scars in the unstained uterus, and was still lactating by the time of harvest in September. The thickness of the uterine wall was “regular”, and bulges on the endometrium were missing.

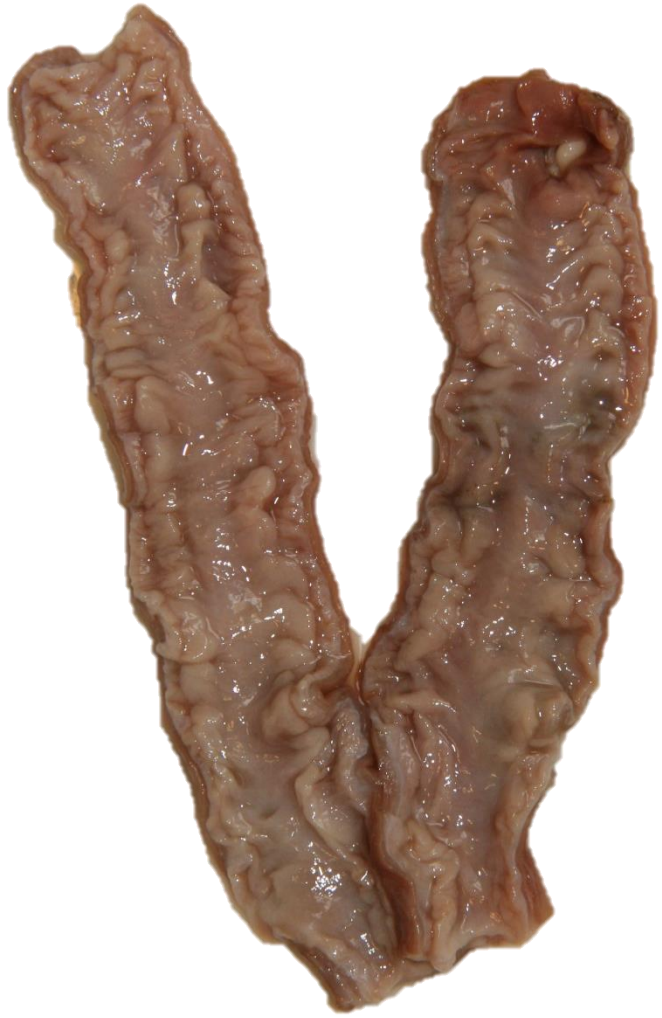


Figure 4 Example picture showing the uterus morphology of a female brown bear, harvested in Sweden during 1986-2005, in concordance with the reproductive state and the change of season: The adult female was 3 years old, had 1 new placental scar in the uterus, which was concealed before staining, and no milk by the time of harvest in September. The uterine wall was “thickened”, and bulges on the endometrium were present. The picture is showing the unstained uterus.

Figure 5 Example picture showing presumed “new” placental scars in the uterus of a female brown bear, harvested in Sweden during 1986-2005, prior to staining: The adult female with the sample-id 03AC08 had 3 new placental scars that could already be identified in the unstained uterus. Characteristic scar-features are the “half-moons” and the vibrant, black colouration.

Figure 6 Example picture showing presumed “new” placental scars in the uterus of a female brown bear, harvested in Sweden during 1986-2005, prior to staining: The adult female with the sample-id V0633/05 had 3 new placental scars that could already be identified in the unstained uterus. Characteristic scar-features are the bisected rings, which are filled with pigmentation to a lesser extent, the dark grey coloration and the lateral shadows.

Figure 7 Example picture showing a presumed “old” placental scar in the uterus of a female brown bear, harvested in Sweden during 1986-2005, prior to staining: The adult female with the sample-id 00ZZ03 had 1 old placental scar that could already be identified in the unstained uterus. Characteristic scar-features are the incomplete bisected rings with a less vibrant appearance and the absence of lateral shadows. The dimension of this old scar is smaller than the dimension of the new scars observed in female V0633/05, in Figure 6.

Figure 8 Example picture showing a presumed “old” placental scar in the uterus of a female brown bear, harvested in Sweden during 1986-2005, prior to staining: The adult female with the sample-id V1023/04 had 1 old placental scar that could already be identified in the unstained uterus. Further scars were presumed. Characteristic scar-features are the incomplete bisected rings with faded greyish coloration and the absence of lateral shadows.

Figure 9 Example picture showing artefacts in the uterus of a female brown bear, harvested in Sweden during 1986-2005, prior to staining: The adult female with the sample-id V0640/05 had dark and orange pigmented “marks” in the unstained uterus. These marks did not indicate 2-3 old placental scars, because they were confirmed as artefacts in the stained uterus.

Figure 10 Example picture showing “orange-coloured” placental scars in the uterus of a female brown bear, harvested in Sweden during 1986-2005, prior to staining: The adult female with the sample-id V0941/05 had 2 orange-brown placental scars that could already be identified in the unstained uterus. Characteristic features are the bisected rings that appear to some extent complete and the pale, faded-looking colouration.

Figure 11 (previous page) Pre- and post-staining pictures showing the uterus of a female brown bear, harvested in Sweden during 1986-2005, with an at first orange-coloured placental scar. An atypical, i.e. orange-coloured placental scar, was found cranial in the right uterine horn prior to staining (picture above), however, 3 new "category 1" placental scars were identified in the same uterus after staining (picture below).

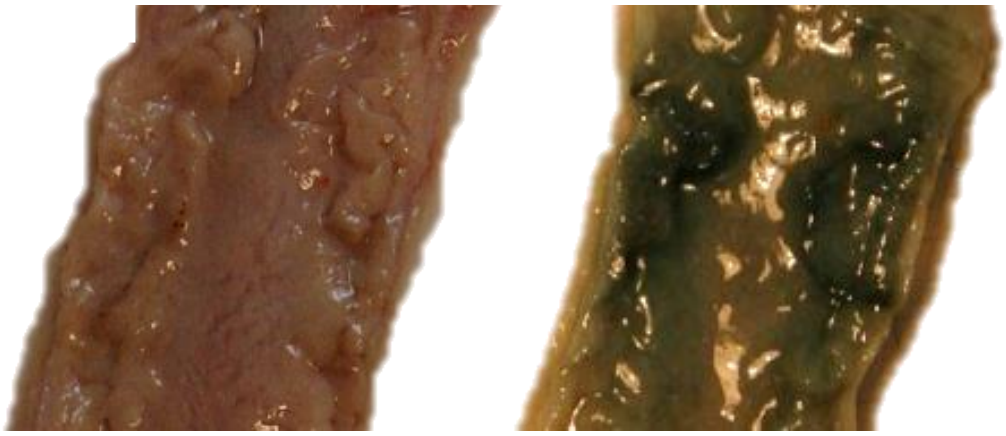


Figure 12 Pre- and post-staining pictures showing the uterus of a female brown bear, harvested in Sweden during 1986-2005, with an at first orange-coloured placental scar. A nearly concealed orange-coloured placental scar was found in the unstained uterus (left), however, a new “category 2” placental scar was idientified in the same uterus at the same location after staining (right).

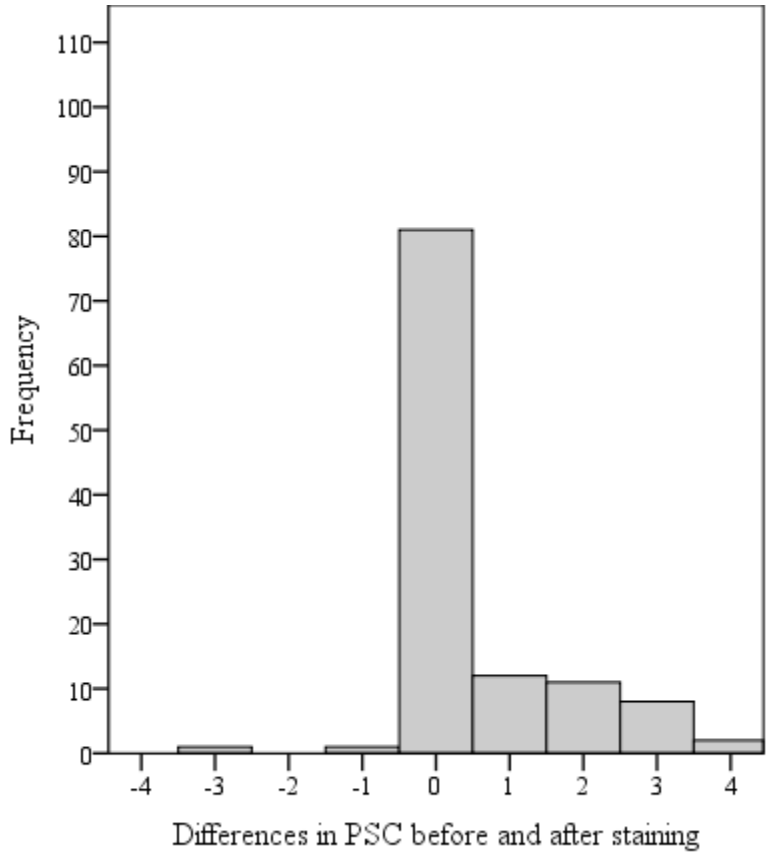


Figure 13 Differences in placental scar counts (PSC) in the uteri of 116 of 181 harvested female brown bears, in Sweden during 1992-2005, before and after staining. The Figure shows how often the number of 1-4 placental scars was overestimated (i.e. the frequencies of -1, -2, -3, -4 scar after staining), underestimated (i.e. the frequencies of +1, +2, +3, +4 scars after staining) and accurately estimated (i.e. the frequency of ±0 scars after staining) in the unstained uteri in comparison to the stained uteri. As indicated, litters of all sizes can go unrecognised prior to staining. The mean difference of placental scar counts was 0.53±0.10 (mean±SE; n=116).

Figure 14 Before and after pictures that show the remarkable benefits of staining for placental scar counts in the uterus of a female brown bear (with the sample-id 00WW99), harvested in Sweden during 1986-2005. The unstained uterus is shown left, the stained uterus right.

Figure 15 Before and after pictures that show the remarkable benefits of staining for placental scar counts in the uterus of a female brown bear (with the sample-id 02ZZ16), harvested in Sweden during 1986-2005. The unstained uterus is shown left, the stained uterus right.

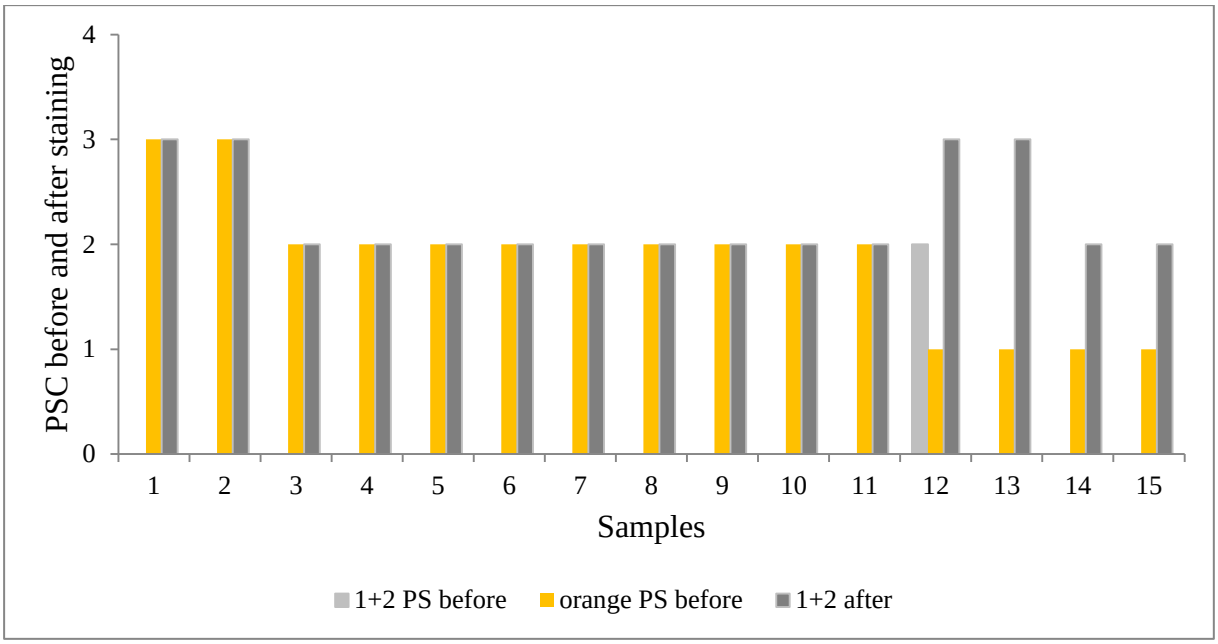


Figure 16 In the uteri of 15 of 181 harvested female brown bears, in Sweden during 1992-2005, I identified puzzling orange-coloured placental scars before staining. According to the "before and after staining scar counts" in these uteri, the unstained orange scars were integrated in the Scar-Category$_{1+2}$ after staining. Samples 13 to 15 showed an exceeding number of Category$_{1+2}$ scars after staining, which may be explained by the insufficient ability to detect orange scars before staining.

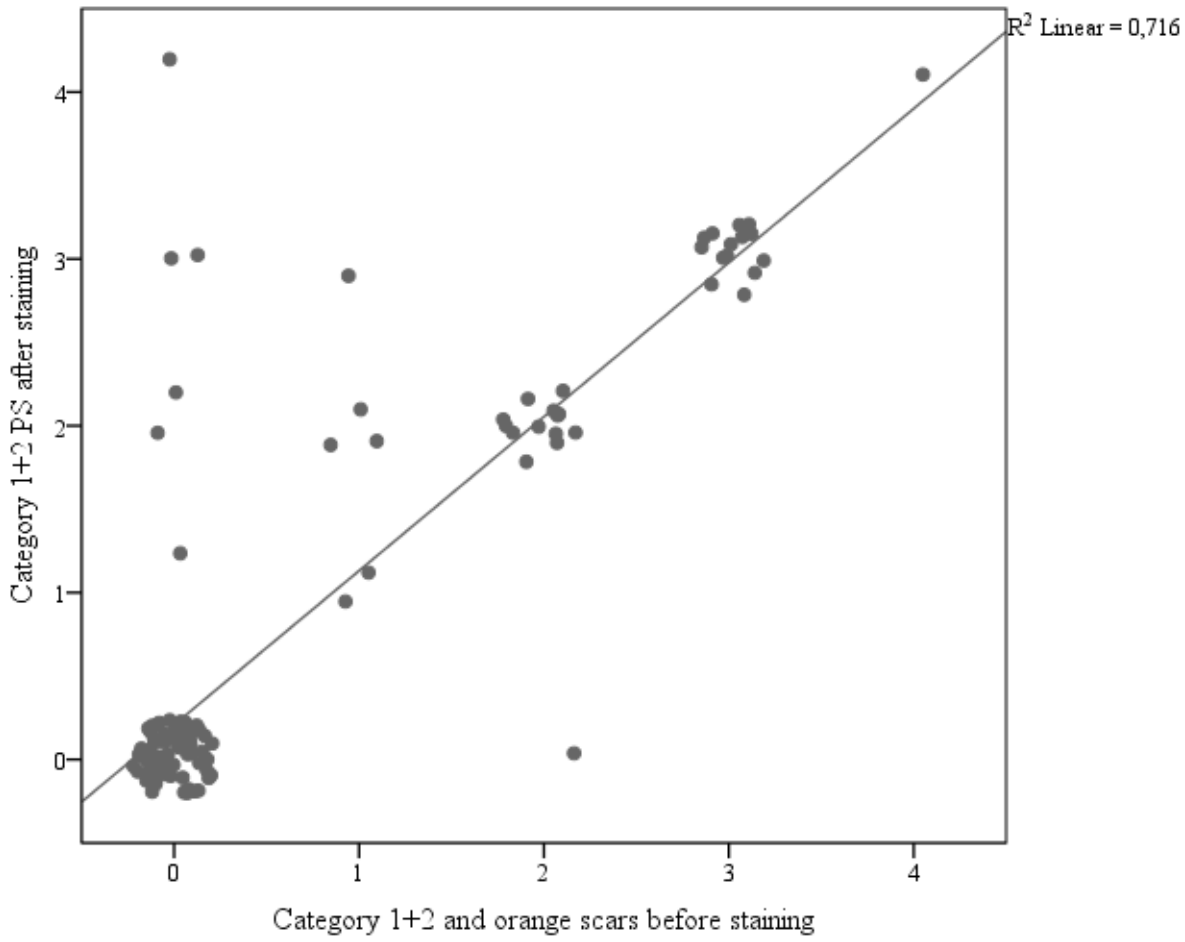


Figure 17 Linear regression for the relationship between $Category_{1+2}$ placental scar counts in the uteri of 116 of 181 harvested female brown bears, in Sweden during 1992-2005, before and after staining, when orange placental scars (PS) were added the $Category_{1+2}$ before staining.

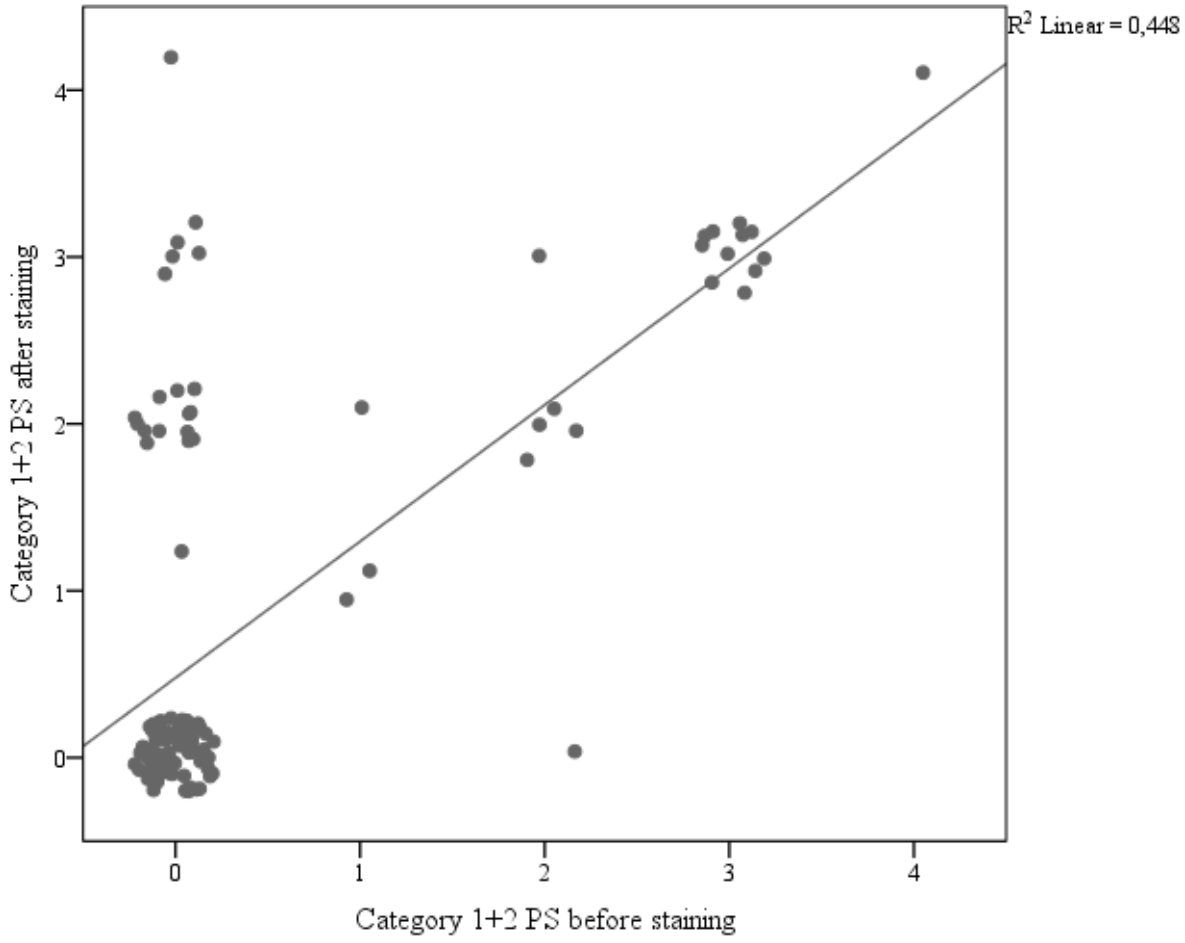


Figure 18 Linear regression for the relationship between $Category_{1+2}$ placental scar counts in the uteri of 116 of 181 harvested female brown bears, in Sweden during 1992-2005, before and after staining, when orange placental scars (PS) were not added to the $Category_{1+2}$ before staining.

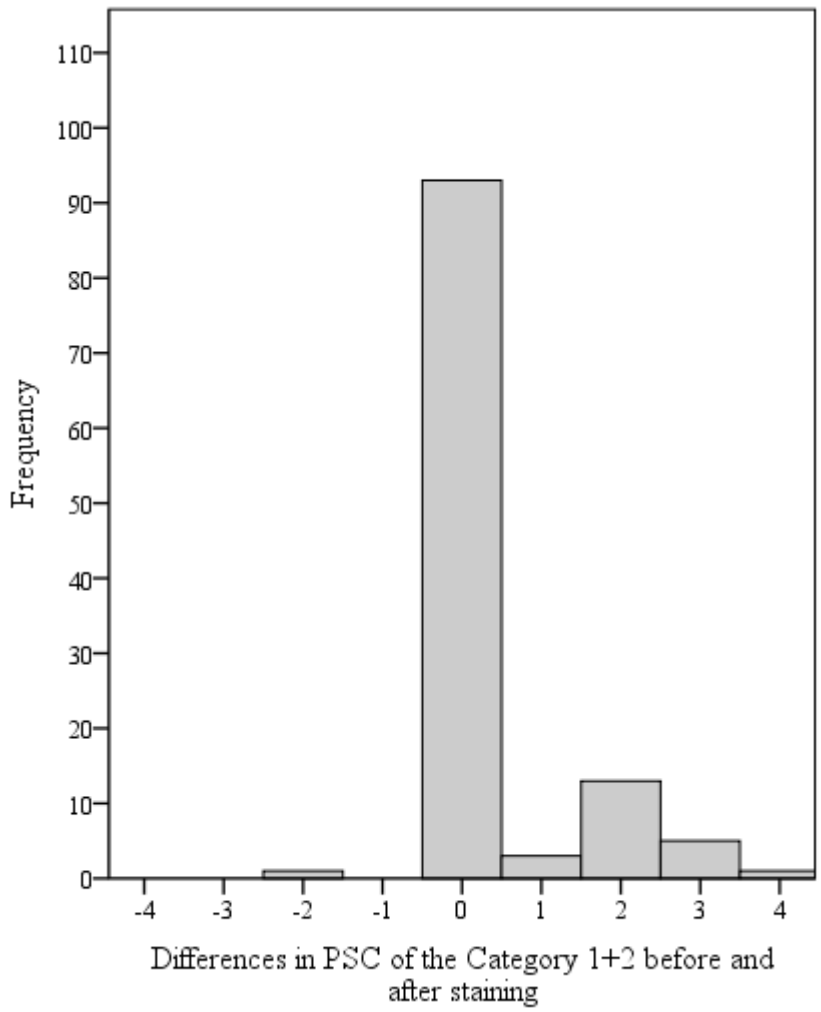


Figure 19 Differences in Category$_{1+2}$ placental scar counts (PSC) in the uteri of 116 of 181 harvested female brown bears, in Sweden during 1992-2005, before and after staining. The Figure shows how often the number of 1-4 placental scars was overestimated (i.e. the frequencies of -1, -2, -3, -4 scar after staining), underestimated (i.e. the frequencies of +1, +2, +3, +4 scars after staining) and accurately estimated (i.e. the frequency of ±0 scars after staining) in the unstained uteri in comparison to the stained uteri. As indicated, litters of all sizes can go unrecognised prior to staining. The mean difference of placental scar counts was 0.40±0.09 (n=116).

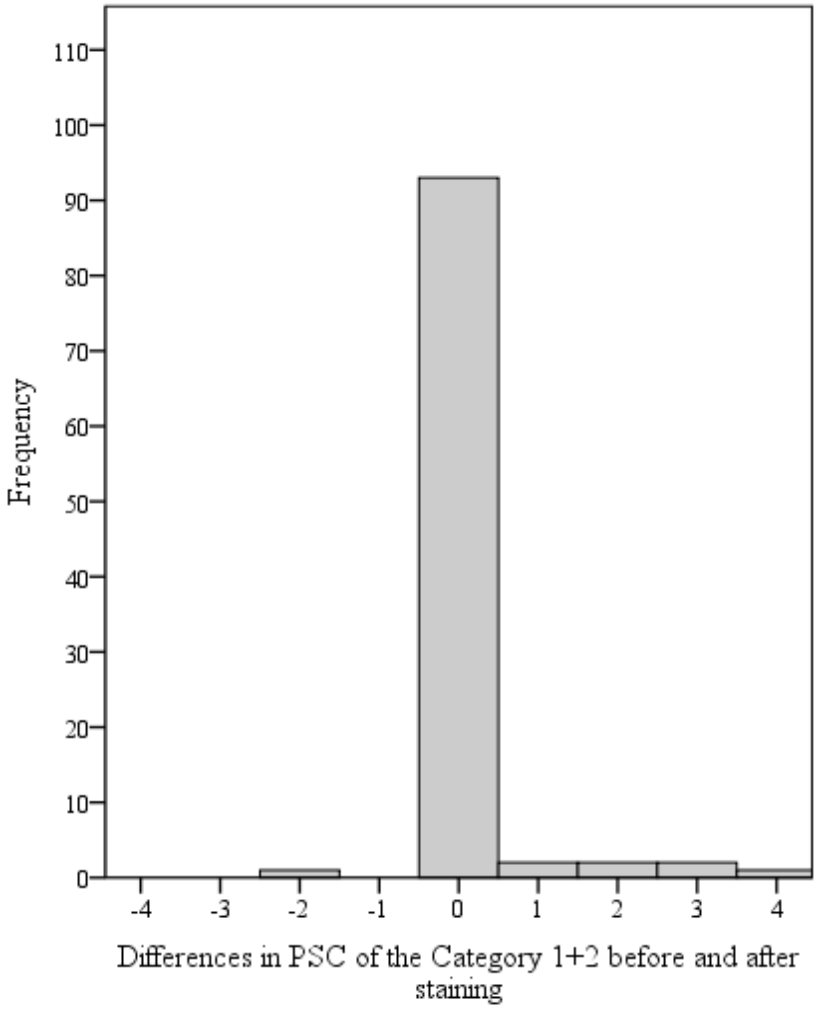


Figure 20 Differences in Category$_{1+2}$ placental scar counts (PSC) in the uteri of 101 of 181 harvested female brown bears, in Sweden during 1992-2005, before and after staining. The 15 uteri samples with orange-coloured placental scars were excluded from this analysis. The Figure shows how often the number of 1-4 placental scars was overestimated (i.e. the frequencies of -1, -2, -3, -4 scar after staining), underestimated (i.e. the frequencies of +1, +2, +3, +4 scars after staining) and accurately estimated (i.e. the frequency of ±0 scars after staining) in the unstained uteri in comparison to the stained uteri. As indicated, litters of all sizes can go unrecognised prior to staining. The mean difference of placental scar counts was 0.14±0.07 (n=101).

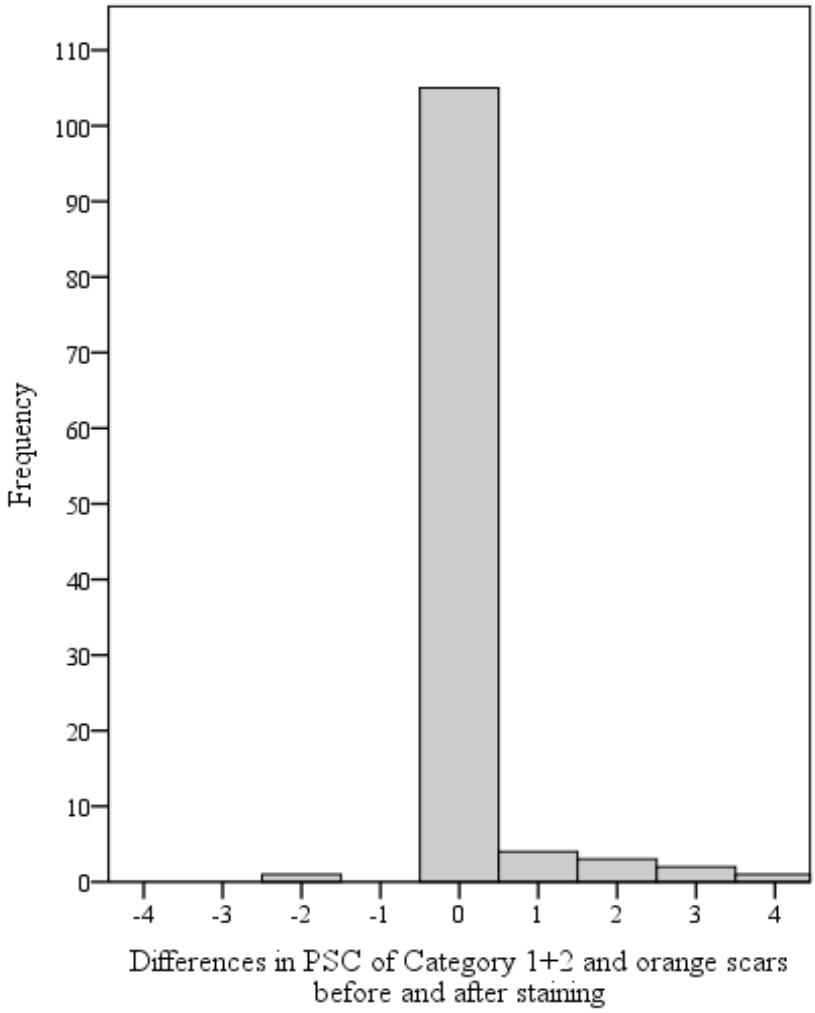


Figure 21 Differences between the sum of category 1, category 2 and orange-coloured placental scars before staining, and the number of $Category_{1+2}$ placental scars after staining in the uteri of 116 of 181 harvested female brown bears in Sweden (1992-2005). The Figure shows how often the number of 1-4 placental scars was overestimated (i.e. the frequencies of -1, -2, -3, -4 scar after staining), underestimated (i.e. the frequencies of +1, +2, +3, +4 scars after staining) and accurately estimated (i.e. the frequency of ±0 scars after staining) in the unstained uteri in comparison to the stained uteri. As indicated, litters of all sizes can go unrecognised prior to staining. The mean difference of placental scar counts was 0.16±0.06 (n=116).

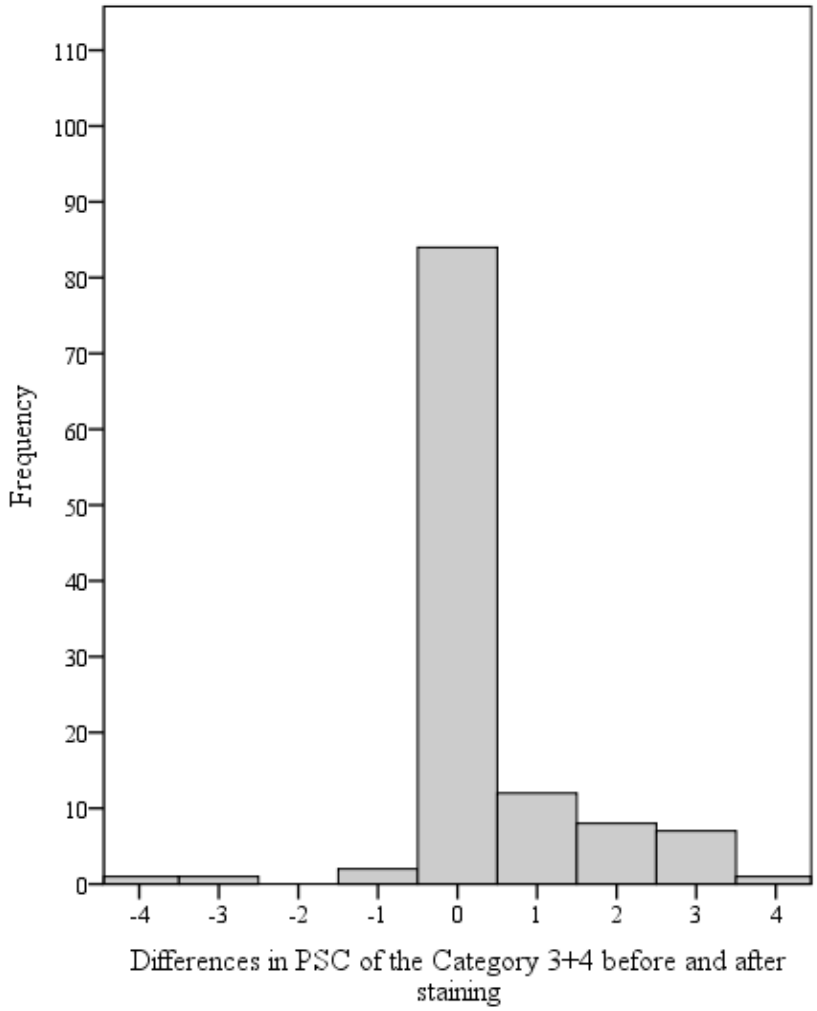


Figure 22 Differences in Category$_{3+4}$ placental scar counts (PSC) in the uteri of 116 of 181 harvested female brown bears, in Sweden during 1992-2005, before and after staining. The Figure shows how often the number of 1-4 placental scars was overestimated (i.e. the frequencies of -1, -2, -3, -4 scar after staining), underestimated (i.e. the frequencies of +1, +2, +3, +4 scars after staining) and accurately estimated (i.e. the frequency of ±0 scars after staining) in the unstained uteri in comparison to the stained uteri. As indicated, litters of all sizes can go unrecognised prior to staining. The mean difference of placental scar counts was 0.38±0.10 (n=116).

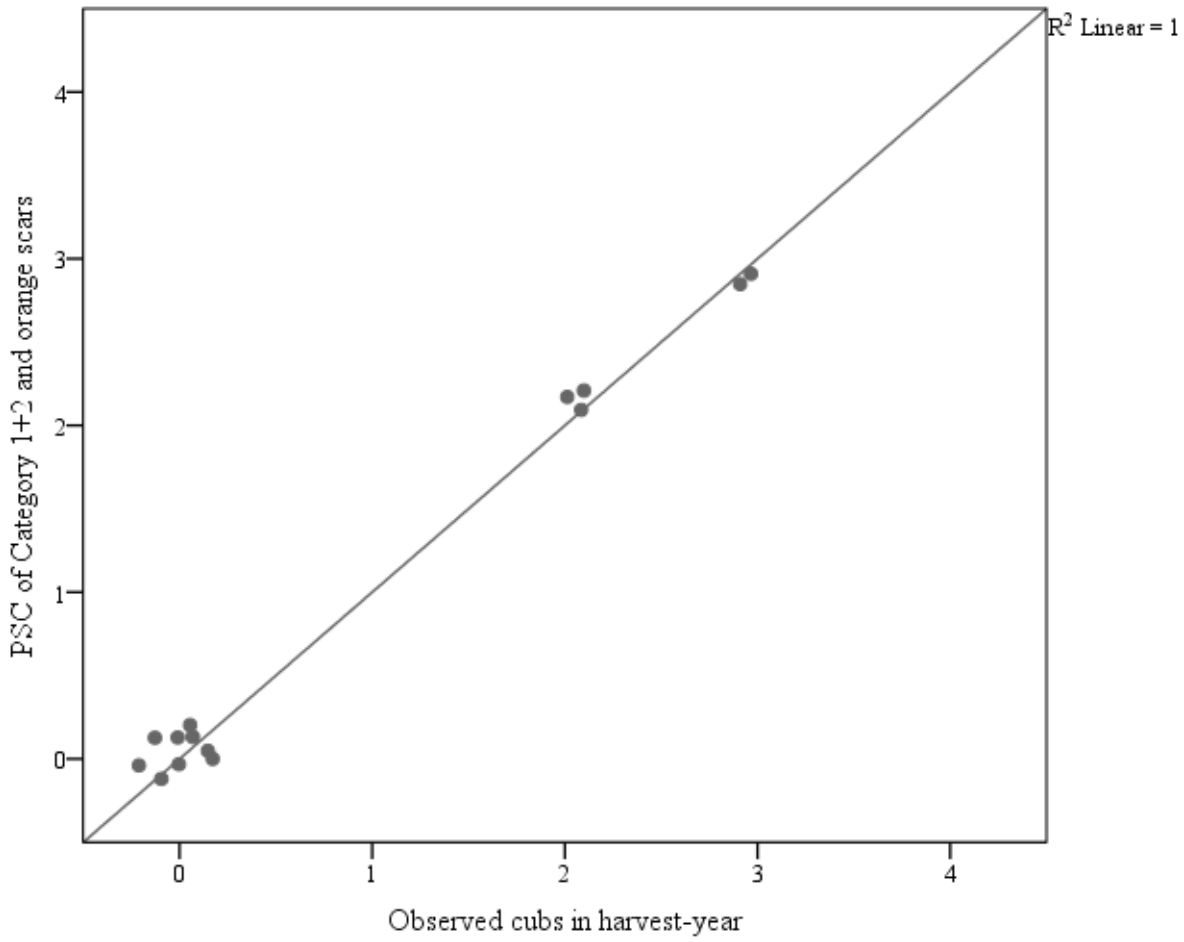


Figure 23 Correlation between the number of observed cubs in the harvest year and the number of Category1+2 and orange-coloured placental scars found in the uteri of 14 of 181 harvested female brown bears in Sweden (1992-2005) with known litter size. PSC are the placental scar counts.

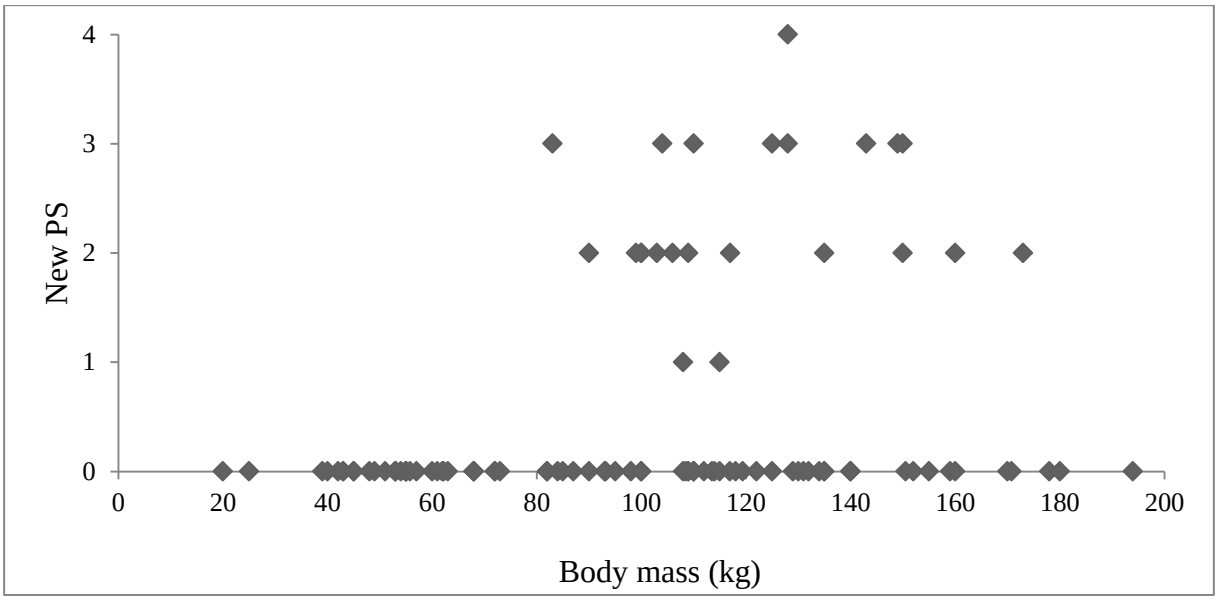


Figure 24 Number of new placental scars (PS) observed in the uterus and body mass of the autumn-harvested 103 of 181 harvested female brown bears in Sweden during 1992-2005. The autumn-harvested females were hunted from the 21st of August to the end of October.

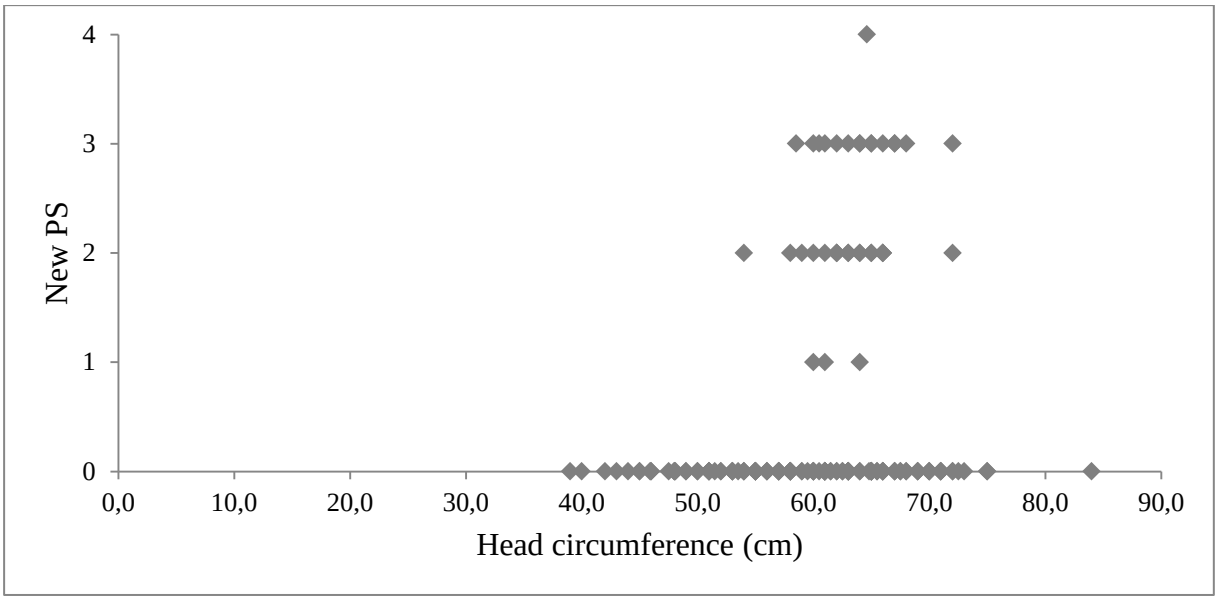


Figure 25 Number of new placental scars (PS) observed in the uterus and head circumference of 166 of 181 harvested female brown bears in Sweden during 1992-2005.

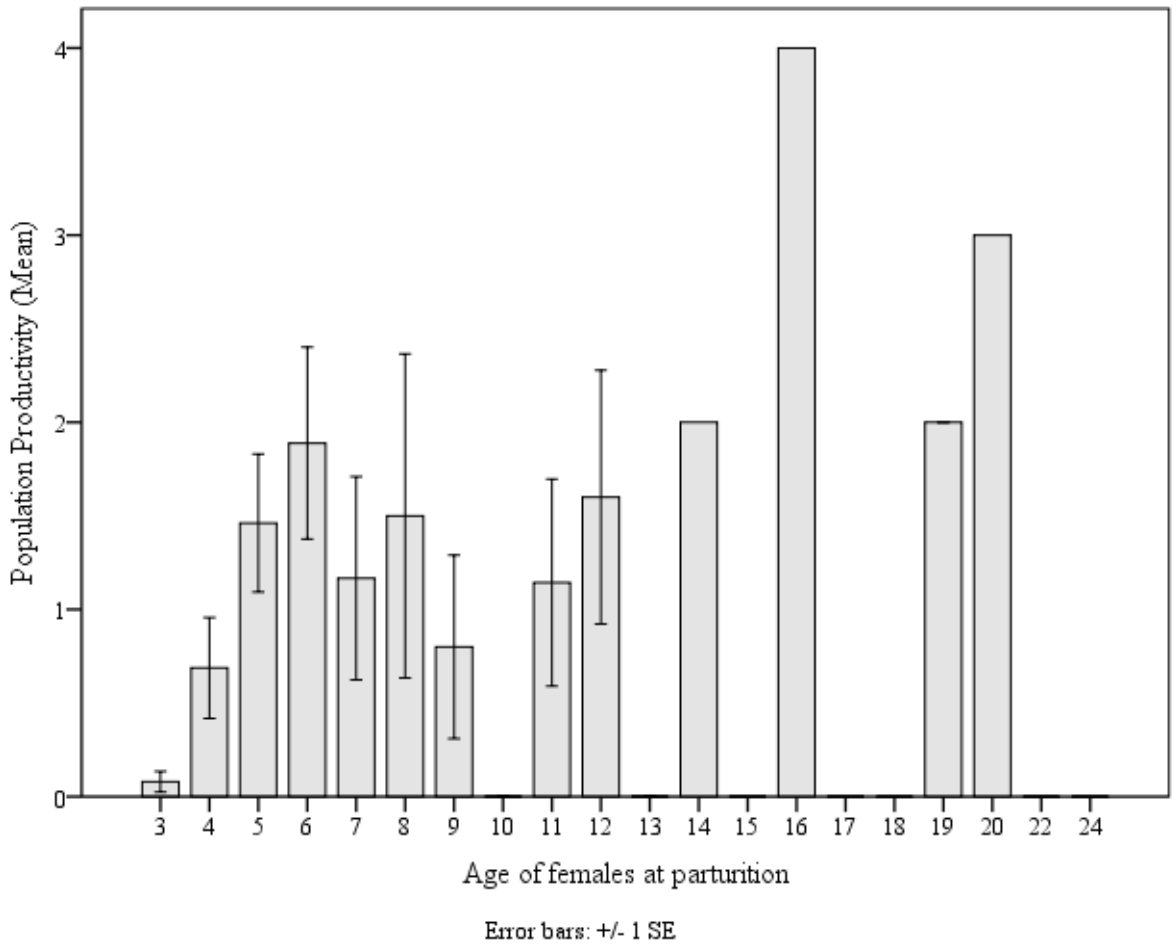


Figure 26 Estimation of the mean population productivity of 3 to 24-year-old female brown bears based on new placental scar counts in the uteri of 106 of 181 harvested females in Sweden during 1992-2005. Population productivity was calculated as the number of cubs, i.e. new scars (0-4), per potentially adult (≥3-year-old) female and year. The age of females at "parturition" corresponds to the age of females in the year of harvest.

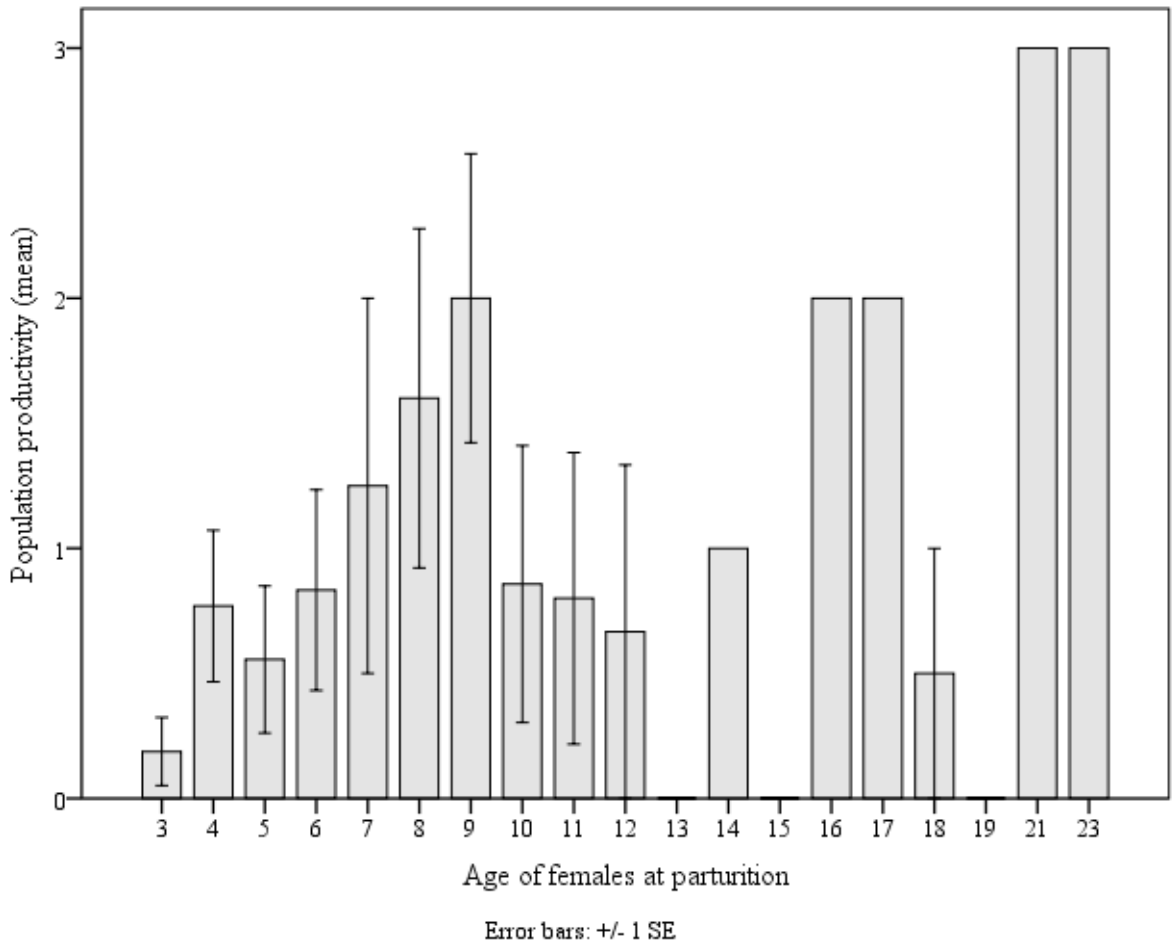


Figure 27 Estimation of the mean population productivity of 3 to 23-year-old female brown bears based on old placental scar counts in the uteri of 81 of 181 harvested females in Sweden during 1992-2005. Population productivity was calculated as the number of cubs, i.e. old scars (0-4), per potentially adult (≥3-year-old) female and year. The age of females at "parturition" corresponds to the age of females in the year prior to harvest.

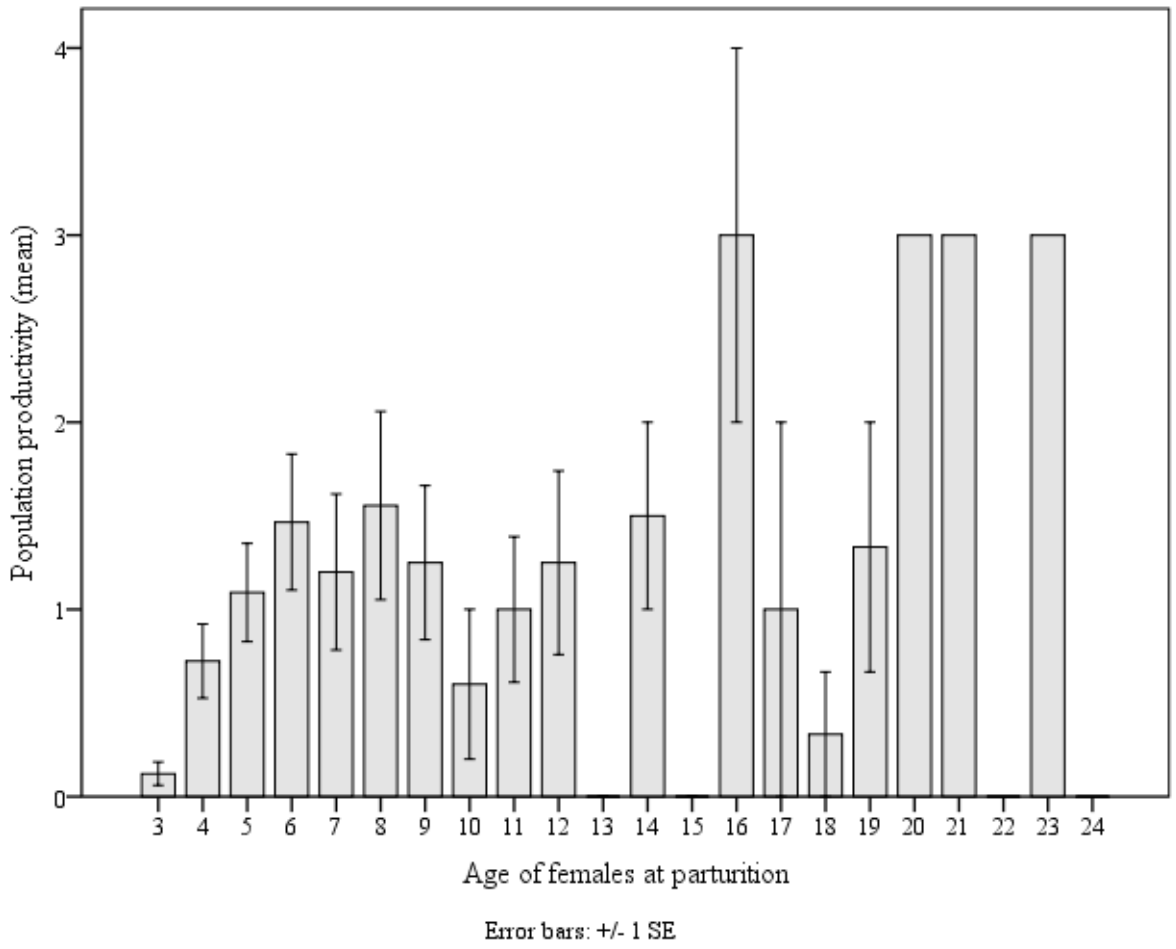


Figure 28 Estimation of the mean population productivity of 3 to 24-year-old female brown bears based on new and old placental scar counts in the uteri of 106 of 181 harvested females in Sweden during 1992-2005. Population productivity was calculated as the number of cubs per potentially adult (≥3-year-old) female and year. Overall I considered 187 either new or old scar-sets with 0 to 4 placental scars each, i.e. 106 scar-sets with new scars from the harvest year, and 81 scars-sets with old scars from the year before harvest. The age of females at "parturition" corresponds to the age of females in the year of harvest, in case of the new scar-sets, and to the age of females in the year prior to harvest, in case of old scar-sets.

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Education

Bachelor of Science (BSc) in Biology/Zoology at the University of Vienna, August 2010
Master studies of Zoology at the University of Vienna since October 2010
The Master thesis "Placental scar counts and the assessment of reproductive performance in female brown bears" was conducted at the Department of Integrative Biology and Biodiversity Research, Institute of Wildlife Biology and Game Management, University of Natural Resources and Life Sciences, Vienna, and was realised in the framework of the "Scandinavian Brown Bear Research Project", Orsa, Sweden.

The study and research interests comprise the disciplines behavioural physiology, behavioural endocrinology and behavioural ecology, as well as wildlife biology with a focus on reproductive biology; with study emphases on mammals (including humans), fish and arthropods.

Various

The academic and studies related activities include the participation in a study of the Clever Dog Lab; the assistance in biological field studies; the assistance in dog breeding; training of dogs, horses, eusocial insects; the completion of behavioural, behavioural physiology, reproductive biology, and evolutionary biology research projects during the Bachelor and Master programmes of Zoology; the tutoring of students in the course of the Master thesis; work experience in health care business.