Season controlled reproduction of undomesticated animals

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SUMMARY

The following article is a summary of research on the influence of season on the reproductive processes in undomesticated animals. The results presented below show: a/ an annual hormonal profile of domestic pig and wild boar crossbreed and the antioxidant blood system in the different seasons, b/ the possibility of gonadotropic hormone stimulation in chinchillas which are in diestrus or infertile, c/ the possibility of using bison’s semen (collected

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post mortem from the epididymis) for cryoconservation. Reproductive Biology 2006 6 Suppl. 1:137–149.

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**INTRODUCTION**

Seasonal reproduction in wild animals has been known for years. In our climate zone, termination of pregnancy appears in spring when environmental conditions (temperature and feed abundance) stimulate a successful lactation giving the offspring a better chance of survival. Depending on the length of pregnancy, the reproductive season of different species may take place at different times of the year. Natural photoperiod is a factor controlling synthesis of melatonin which plays an important role in regulating reproduction of long and short day breeders [7, 32]. In short-day breeders when the duration of melatonin secretion is sufficiently long, melatonin increases frequency of GnRH pulses which, in turn, results in increased amplitude of LH pulses and succeeding activation of gonads [32].

**SEASONAL CHANGES IN PLASMA HORMONE CONCENTRATIONS AND ANTIOXIDANT BLOOD ACTIVITY IN THE PIG/WILD BOAR CROSSBRED**

The pig/wild boar crossbred is characterized by distinct seasonal reproduction and thus, can be a perfect model for studying the mechanisms of reproductive processes which became less pronounced or lost in pigs due to domestication. The meat of crossbred animals is characterized by high nutrition level, taste qualities as well as low level of cholesterol. Providing a biotechnological basis for breeding can be meaningful for future economy.

Our experiment was carried out on 16 sexually mature gilts of pig and wild boar crossbreds during the four seasons (December, March, June,
September). Every month, four gilts were cannulated and jugular vein blood was collected for 30 days (3 times/day). At the end of blood sampling, fragments of ovaries and uteri were sampled for immunohistochemical detection of androgen as well as estrogen $\alpha$ (ER$\alpha$) and $\beta$ (ER$\beta$) receptors. Concentrations of steroid hormones [47, 51], triiodothyronine (T$_3$) and thyroxine (T$_4$; [Bio Source kit]), insulin-like growth factor-I (IGF-I; [DSL kit]) and leptin (Linco Research kit) were determined by respective RIAs. IGF-II concentration was measured by an EIA kit (DSL). The distribution of steroid receptors was also assayed [43, 54].

We found that season is an important factor influencing the length of the estrous cycle in the pig/wild boar crossbred. The cycle was the shortest in December (21-22 days) and the longest in June (28-30 days). The longest cycle appeared when domesticated pigs underwent a decline in fertility [33-35].

Characteristic seasonal fluctuation was observed in plasma hormone concentrations. The follicular phase had a higher plasma estradiol (E$_2$) level than the luteal phase in winter and spring, whereas no differences were observed in summer and autumn. Plasma progesterone (P$_4$) was always significantly higher during the luteal phase than the follicular phase as it is described in domesticated pigs [5, 8, 9]. Testosterone (T) and androstenedione (A$_4$) plasma levels were maximal in all examined seasons except summer. The lowest plasma estrogen concentration found at that time was due to the lack of androgen substrate for aromatization. Similar summer depression of estrogen synthesis and simultaneous decline in fertility is true in the domesticated pig [7, 29].

Concentrations of T$_3$ and T$_4$ did not significantly fluctuate during the follicular and luteal phases in all seasons. The plasma level of T$_3$ was significantly higher in the follicular phase in spring and winter while the T$_4$ level was higher only in winter [25]. This finding indicates the importance of T$_3$ and T$_4$ contribution to the thermogenesis process, and, to a lesser extent, their direct influence on reproduction.

Leptin is a hormone involved in the control of gonadotropin secretion [3, 46]. Peripheral plasma concentration of leptin was not affected by the phase of the cycle. The highest concentration was found in spring, a time...
of significant fat loss [28]. This may point to leptin’s role in the regulation of metabolism, and, although to a lesser extent, in reproductive processes. IGF-I and IGF-II are known factors modulating the regulation of ovarian steroidogenesis [12, 21]. In December, plasma concentrations of both growth factors were significantly higher during the follicular than luteal phase [24].

Blood antioxidant activity also underwent certain seasonal changes. The highest concentration of erythrocytal superoxide dismutase (SOD) was observed during the spring and autumn equinoxes [27]. The level of total antioxidant activity revealed the highest activity in summer and winter [26].

Endocrine regulatory processes of reproduction in mammals are multifactorial and multifunctional. They depend on local hormonal status and various hormone-receptor interactions. All these processes occur and disappear during specific time sequences controlled by the estrous cycle and also the season [37, 44, 50, 56, 57]. Satisfactorily visualized steroid receptor tissue localization will help us further to study hormone-receptor interactions involved in controlling reproductive functions of the ovary and uterus [23].

GONADOTROPIN-STIMULATED OVULATION IN DIESTROUS CHINCHILLAS IMPROVES FERTILITY

In natural conditions, chinchillas undergo seasonal reproduction, with its highest activity in spring [45]. After almost 100 years of farm breeding, chinchilla reproduction lost its seasonal quality due to uniform microclimatic breeding conditions. Farm bred chinchillas often manifest fertility irregularities like e.g. prolonged diestrus [41, 49].

The aim of our study was to define the efficiency of gonadotropin stimulation of diestrous females to induce ovulation in infertile chinchillas. In a preliminary experiment diestrous females were divided into five groups receiving: 1/ at the same time 200 iu of PMSG and 100 iu hCG (PG 600 Intervet; group 1); 2/ at the same time 100 iu PMSG and 50 iu hCG (PG
600 Intervet; group 2); 3/ 100 iu PMSG (Folligon Intervet; group 3); 4/ 200 iu PMSG (Biovet) and 72 hours later 200 iu hCG (Biomed; group 4); 5/ 0.2 ml \textit{aqua pro injectione} (Polfa; control group). The examined endpoints were the following: opening of vagina, amount and consistency of vaginal mucus, macroscopic evaluation of ovaries and uteri, weight of ovaries, progress in growth and maturation of ovarian follicles and oocytes, presence of mature follicles and corpora lutea (CL), litter size and histological observation of ovarian and uterine tissues.

We found that the mean ovarian weight was the lowest (42.9 mg) in the control group. The highest ovarian weight was recorded in group 1 (74.5 mg) and 4 (77.1 mg). Gonadotropin stimulation was most effective in groups 1 and 4 since in these groups a majority of ovaries contained mature follicles. In addition, ovaries of all females from group 4 contained CL.

In experiment 2, the most effective gonadotropin administration regime established in preliminary experiment was used to induce ovulation in low fertility and infertile females. The study was carried out on 90 female chinchillas with a low index of fertility. Selected females were divided into three groups and injected: 1/ at one time with 200 iu PMSG and 100 iu hCG per female (group 1); 2/ with 200 iu PMSG and 72 hours later with 200 iu of hCG (group 2), and 3/ with 0.2 ml \textit{aqua pro injectione} (control group). Eighteen out of 90 females became pregnant and delivered litters. After eight months, the remaining 72 females which did not deliver were sacrificed, and their ovaries and uteri were submitted to anatomical and histological examination. It was found that the most frequent cause of infertility in investigated chinchillas was pyometritis (19 females). Two females were diagnosed with follicular cysts. Ovarian atrophy (a very rare reproductive disorder in chinchillas) was diagnosed in two others. No macro- or microscopic impairments of ovarian and uterine morphology were found in the remaining females.

\textit{Post mortem} examination of chinchillas with defective pregnancies showed that 30% of females suffered from ovarian and uterine disorders. In remaining 70%, no pathological changes in the anatomy of reproductive organs were seen. Such a phenomenon may be caused by an impairment of the endocrine system, which has not yet been studied in this species.
Apart from anatomical defects, other recognized causes of infertility in female chinchillas include: inappropriate nutrition (monodiet, lack of fiber), hypovitaminoses A and E, inbreeding, unsuccessful mating, fetus resorption, overuse of females for breeding [20]. In addition, undernourished females deliver small litters. The reasons for unsuccessful mating may also include hormonal imbalance and receptor or steroidogenic enzyme deficiency. In this study, gonadotropin doses, selected in preliminary experiment, stimulated production of higher number of maturing follicles and CL. We demonstrated that gonadotropin treatment of low fertility chinchillas improved their fertility. Simultaneous injection of PMSG and hCG were also reported to improve ovulation rate in pigs [58]. Similarly, two-gonadotropin administration regime was more efficient than hCG alone in inducing ovulation in chinchillas [52, 53]. In ewes, a single PMSG dose appeared to be successful, but the quality of response depended on the dose used [31]. To stimulate ovulation in chinchillas, Jarosz [18, 19] recommended priming the females with progesterone followed by treatment with PMSG and hCG.

Administration of PMSG, however, may cause some disturbances in reproductive function. The disturbances may result from the fact that PMSG has a longer half-life than FSH which makes control of the reproductive cycle difficult [2]. Moreover, the subsequent increase in estrogen secretion changes ovarian, oviductal and uterine environment, which may impair sperm transport and result in oocyte fragmentation [17, 22]. Armstrong [1] emphasized the negative influence of PMSG on ovulation, luteinization, function of CL, abnormal sperm penetration of the oocyte, and formation of abnormal male pronucleus. Animals treated with PMSG at the time of follicle and oocyte maturation suffered from hormonal disorders including asynchronous maturation of oocyte nuclei, premature activation of meiosis and defective steroidogenesis [22].

The results of the present study demonstrated that 26-30% of females characterized by low fertility index were successfully mated after hormonal stimulation, especially when PMSG injection was followed by hCG. We conclude that the gonadotropin-stimulated ovulation led to successful pregnancy of low fertility chinchilla females without anatomical or cytological disturbances.
USE OF BISON’S (BISON BONASUS) SEMEN COLLECTED POST MORTEM FOR DETERMINATION OF ITS QUALITY

The bison is the largest European mammal and as an endangered species belongs to the group of strictly protected animals [16]. One of the most important threats to the future existence of the species is its very low genetic variability due to a very small number of founders. This problem is especially important for the Lowland line, Bison bonasus bonasus, which derived from only seven founders [4, 30, 38]. Low genetic variability results in decreased survival, fecundity as well as a low adaptive ability [39].

Literature on male bison fertility is scarce and incomplete. One possible reason is a lack of methods allowing collection of semen with a quality sufficient for its long storage. Electroejaculation commonly used in practice [6, 40, 42] frequently produces low quality semen and is difficult to perform due to the use of deep anesthesia. Alternatively, seminal fluids exhibiting characteristics similar to ejaculates can be obtained post-mortem [10, 13, 42]. The biological value of semen collected in this way may be investigated in selected wild bison males. Moreover, the semen can be used in biotechnology of bison reproduction. The aim of the present study was to obtain basic reproductive data from selected free living bison males. Post-mortem examination was performed of gross anatomy of the testes, epididymes and genital tracts as well as analysis of morphology and motility of epididymal spermatozoa.

To determine physiological characteristics of semen, reproductive organs from 50 bison males, living in the Borecka and the Bialowieska Primeval Forests, were harvested from November to March of 2000-2005. Sexually mature animals were divided into three groups: 1/ up to 3 years (n=9); 2/ 3-16 years (n=30); and 3/ 3-14 year-old bisons with diagnosed irregularities of reproduction system (n=11). In the latter group the adult bisons with limited reproductive abilities had testes irregular in shape or asymmetric, small volume of seminal fluid with low concentration or deficiency in sperm and cryptorchidism.

Testes (epididymes and ampullae) and vesicular glands (VG) were weighed and measured. Seminal fluids were collected from the caudae
epididymis (CE), ampullae (A) and VG after multiple incisions and by
gentle squeezing. The quantitative and qualitative evaluation of the seminal
fluids was carried out using methods applied for domestic animals.

In the group of young males, the weight of left and right testis ranged
from 2.7 to 92.3 g and from 2.9 to 74.6 g, respectively. The measurements
of right and left testes, i.e. the length measured with caput and caudae
epididymes, the width and the circumference, did not differ significantly.
The mean values of the parameters for the left testis were 6.6, 2.9 and 8.2
cm, respectively.

In the group of adult bisons, the mean weights of left and right testis were
186.3 g and 191.9 g, respectively. The obtained mean values corresponded
to the testicular mass characteristic for 6 year old males [30]. Testicular
weight data show that testicular asymmetry is evident in approximately
47% of males.

In the third group of bisons, the mean weight of testes (left: 107.3;
right: 101.3 g) was lower than in the fertile male group and ranged from
18.0 to 216.0 g. This group did not exclude males with cysts on caput,
corpus and/or caudae epididymis which occurred in 66% of the evaluated
male bisons. The occurrence of this defect was similar to that described by
Matuszewska and Sysa [36]. The location of cysts was especially common
on the epididymal caput but sometimes they were located between the
corpus and cauda epididymis.

The size of ampullae and vesicular glands can be an important indicator
of reproductive capacity of males. During post-mortem semen collection,
semen fluids from left and right epididymal caudae were sampled and they
provided similar volumes (0.25 vs. 0.26 ml) of fluid. Beside the vas deferens,
significant amounts of sperm were contained in ampullae which provided
0.19 ml fluid each. Contrary to the testes, the measurements of ampullae and
vesicular glands exhibited distinct symmetry. The mean length and width of
ampullae were 8.2 cm and 10.2 mm, respectively. The parallel measurements
of the VG were: 8.8 cm and 2.7 cm, respectively. The fluids of vesicular
glands (0.79 ml) composed of ejaculates and secretory products of these
glands are a good indicator of seasonal character of reproduction. In red deer
(Cervus elaphus), another short day breeder, during late breeding season, the
VG fluids undergo biochemical changes which are macroscopically visible as a grey fraction of ejaculate [13, 14, 48].

Sperm concentration, motility and morphology are the most important physiological properties of semen that indicate males’ capacity for reproduction. In the group of males with full reproductive capacity (group 2), sperm concentration in the fluids of the left and right CE was $1.75 \times 10^6$/mm$^3$ and $2.05 \times 10^6$/mm$^3$, respectively. Out of total spermatozoa count, 28.0% of the left and 31.4% of the right epididymis, examined ca 7 hrs after animals’ death, showed normal progressive motility. It should be stressed that evaluation of sperm motility was performed in PBS at 38°C. In recent trials, bison semen diluted with extender Triladyl and Biociphos showed an increase in sperm motility up to 20% of fresh semen [15].

The preliminary results of sperm morphological evaluation indicated that the major common defects were strongly folded or coiled tails, double forms and proximal droplets. Their frequency ranged from 13 to 68%. Among the minor defects the most frequent were simple bent or coiled tails and distal droplets occurring in 4 to 26% of spermatozoons counted. Particular frequency of the latter defect is typical of sperm sampled from CE and its number may exceed 60% [10, 13].

The peak of the mating season of European bison is in August/September and in this period bison’s semen has the highest biological value. Considering qualitative and quantitative parameters described, the semen sampled from November to March represented semen typical for the end of the reproductive season. However, it seems that over 50% of the males still met the requirements of the basic semen parameters such as sperm motility and concentration. According to Gill [11], during years 1951-1960, one hundred bison births were reported in July, 88 in August, 70 in September, 65 in October and 42 in November. Considering duration of pregnancy (265 days) the bison females were fertilized during the sampling period similar to that of our experiment. Thus it could be concluded that the seasonal reproduction of bison is less pronounced than in red deer, in which fertilization in February or March is almost impossible.
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