Reproductive Technologies Used in Female Neo-Tropical Hystricomorphic Rodents

Reproductive technologies aid in efficient reproduction, which is important in these species as they are hunted and valued for their meat. Knowledge of the anatomy and physiology would aid in assisted reproductive techniques, thus attention was given to these areas. Within this group of rodent species there were similar characteristics, some of which have been highlighted as well as any unique features. Some reproductive technologies used included colpocytology, ultrasonography, and hormonal analysis.

1. Introduction

Hystricomorphic rodents belong to the suborder of Hystricomorpha, which refers to one of the four types of rodent skulls based on the nature of their zygomasseteric system, whereby the anterior part of the masseter medialis runs from the medial side of the orbit through an enlarged infraorbital foramen and to the lateral surface of the rostrum and in extreme cases, such as in the capybara (*Hydrochoerus* spp.), its origin extends as far forward as the pre-maxilla [1][2].

Neo-tropical hystricomorphic rodents, such as the agouti (*Dasyprocta leporina*), capybara (*Hydrochoerus hydrochaeris*), and paca (*Cuniculus paca*), possess the potential for domestication and are of importance as they serve as game species, in addition to their ecological roles—such as scatter-hoarders [3][4][5][6][7]. Therefore, wildlife farming can help to create a captive-bred stock, which will create a gene pool, aid in food production, create employment, and aid in conservation [7][8].

2. Reproductive Technologies

One method of monitoring the estrous cycle was by vaginal cytology [9][10][11][12][13]. A moistened (saline solution) swab was introduced into the vagina, where it was used to rub against the vaginal wall before being placed on a glass slide. After drying at room temperature, the smears were stained with rapid panoptic and observed under a light microscope [11][13]. Other authors utilized a 10% saline solution vaginal wash, whereby 1 mL of the final wash was used to make a smear on the slide that was viewed under the microscope, confirming the presence of different cells according to the estrous cycle phase [14]. In proestrus and estrus, superficial cells predominated, while intermediate cells predominated during metestrus and parabasal cells in diestrus [10][11]. External estrus signs coincided with the predominance of superficial cells [11][13].

Another method of monitoring the estrous cycle was via ultrasonography [11][13]. Real time trans-abdominal ultrasound was used to monitor the ovaries, however, no differences in the ovaries could be noted during the different estrous cycle phases [15][11]. Peixoto et al. [13] noted that only one follicle (0.23 cm) in one female appeared round with a hypoechoic center. Similarly, while ovarian follicles were seen as round, hypoechoic structures (averaging 1 mm in diameter), the corpus luteum appeared hyperechoic, averaging 1.4 mm in diameter [11]. External estrus signs coincided with the development of ovarian follicles [11][13].

Estrus was induced using two different protocols, which also involved the use of anaesthesia for restraint (ketamine at 15 mg/kg, intramuscularly, and xylazine at 1 mg/kg, intramuscularly) [13]. The first protocol involved the administration of cloprostenol (5 µg) intraperitoneally, nine days apart. The second protocol was the intravulvar administration of a GnRH analogue (30 µg), followed seven days later by cloprostenol (5 µg) intraperitoneally and another GnRH analogue dose two days later. The first protocol resulted in only 40% of the animals being induced, with estrogen peaks at three and six days after administration of the second dose of cloprostenol [13]. The second protocol resulted in 40% of the animals showing estrus four days after the second dose of the GnRH analogue was administered, while 20% showed estrus ten days after

the second dose of the GnRH analogue was administered [13]. However, the authors stated that these protocols had limited efficiency in estrus induction of animals in the luteal phase of the estrous cycle and, even though it was induced, there was no synchronization [13].

The first ovarian tissue xenograft of fresh and vitrified fragments was successful in promoting the return of ovarian activity in SCID mice [16]. The authors used ovarian fragments taken from agoutis and placed some into SCID mice, while some were vitrified on a solid surface using a solution consisting of minimum essential media (MEM) plus fetal bovine serum, 0.25 M sucrose, an association of 3 M dimethylsulfoxide (DMSO) with 3 M ethylene glycol (EG), and finally, transplantation to the recipients. Resumption of ovarian activity occurred 21 days after xenografting (in 80% that received fresh ovarian tissue and 16% that received vitrified tissue) and was characterized by the presence of typical signs of proestrus and estrus.

Cryopreservation of ovarian tissue was explored using different cryoprotectant agents and their ability to preserve preantral follicles morphology and the ultrastructure was evaluated [17]. This was done by placing ovarian fragments (for 10 min) in a solution containing MEM plus fetal bovine serum and 1.5 M of one of the three cryoprotectants (DMSO, EG, or propanediol [PROH]), followed by cryopreservation in a programmable freezer. After exposure and/or thawing, the samples were fixed in Carnoy for histological analysis and in Karnovsky for ultrastructural analysis. Analysis of the results indicated that PROH was the best cryoprotectant agent of the three evaluated and thus was recommended. The use of DMSO and EG for solid-surface vitrification (SSV) was also proven effective in the preservation of ovarian tissue, with 3 M EG being the most effective [18].

Another study evaluated the different methods for ovarian tissue vitrification not only on the preservation of preantral follicles, but also on the microbiological load, whereby the fragments of the fresh control and vitrified culture groups were assessed for bacteria and fungi [19]. Ovaries were collected after slaughter and processed. Fragments from each pair of ovaries were allocated into different groups (fresh control, cultured control, SSV control, ovarian tissue cryosystem [OTC] control, cultured SSV, and cultured OTC groups). Ovarian tissue vitrification was done by placing fragments in a solution containing MEM plus fetal calf serum, 0.25 M sucrose, and 3.0 M EG for both SSV and OTC methods. After storage for two weeks and then re-warming, the fragments underwent in vitro culture for 24 h. Following analysis, it was concluded that the methods evaluated were efficient (70%) in preserving ovarian tissue, preantral follicle morphology, and DNA integrity, with the OTC method providing better conditions to prevent bacterial proliferation [19].

The ovarian slicing technique was conducted in order to obtain oocytes from *D. prymnolopha* [20]. Ovaries were obtained via ovariohysterectomies of anaesthetized animals. They were subsequently sliced and, using a stereomicroscope, cumulus-oocyte complexes were identified and quantified. Subsequently, they were placed in maintenance media and the morphology was assessed. This method of obtaining oocytes proved to be successful in the collection of a large number of cumulus-oocyte complexes with different degrees of quality [20].

2. Capybara (*Hydrochoerus hydrochaeris*)

2.1. Gross Anatomy of the Female Reproductive Tract

The ovaries, which were symmetrical and oval in shape, measured 2.6 cm (length) on average and had an ovarian bursa and the associated ovarian and utero-ovarian arteries and ligaments [21][22]. The paired fallopian tubes were supported by the mesosalpinx and consisted of the papilla, isthmus (right length–8.4 cm; left length–9.0 cm), ampulla (right length–4.1 cm; left length–4.2 cm), and infundibulum [22]. The muscular and mucosal layers were supplied by a plexus originating from the ovarian and cranial uterine arteries. Fimbriae surrounded the free margin of the infundibulum, while the inner surface contained wide folds that converged to form the abdominal ostium (a small opening), which led into the ampulla. The ampulla had the largest lumen and folded walls, which decreased in diameter and degree of folds as it moved along to form the longest and narrowest part, the isthmus. The opening of the isthmus into the uterine horns consisted of evaginated projections, the tubal papillae fimbriae [22].

Two broad uterine horns entered into a duplex uterus [21][22][23]. The right uterus measured 12.5 cm in length while the left measured 11.8 cm in length [22], which was similar to the findings by Kanashiro [23]. The uterine horns were supported by the mesometrium and intercorneal ligament [22][23]. Capybaras were said to have a relatively short (4.4 cm) double cervix that projected into the vaginal cavity (external uterine ostia). One was circular in shape, whereas the other was semi-lunar in shape, however both had longitudinal striations and they both secreted mucus [22].

The vagina was described as a tubular organ with an average length of 14.4 cm and hyperpigmentation unique to each individual was noted [22]. The external cervical ostia was located cranially, the vaginal vestibule caudally, the rectum

dorsally, the pelvic walls laterally, and the urinary vesicle and urethra ventrally. The vulva, anus, and two sinuses were found in a urogenital sac, with the clitoris located within a fossa in the ventral commissure of the vulva [22].

2.2. Histology of the Female Reproductive Tract

The ovary was covered by a simple cuboidal epithelium, except in the ovarian hilum area, which had a highly vascularized stroma that contained follicles at different developmental stages and the corpus luteum [22]. The infundibulum had a mucous layer, containing folds covered by a ciliated, pseudostratified epithelium [22]. Ciliated cylindrical cells, non-ciliated cylindrical cells, and cells containing clear cytoplasm and a small central nucleus were present within this epithelium. The ampulla had similar histological findings, with the exceptions being that some small areas of the epithelium were covered by a simple cylindrical epithelium and the serosa and muscular layers were thin [22]. The mucous layer of the isthmus contained only ciliated and non-ciliated cylindrical cells within the pseudostratified epithelium, which had few cilia and some areas covered by a simple cylindrical epithelium [22].

The uterus was composed of three tunics; mucosa, muscular, and serosa [23]. The endometrium was lined by a pseudostratified epithelium [22][23]. The lamina propria had diffuse lymphocytes and deeper areas had dense connective tissue with tubular glands, which were covered by a simple cylindrical epithelium [22]. The inner circular layer of the myometrium was thicker than the longitudinal layer and contained smooth muscle fibres and lymphocytes. The perimetrium was only made up of one or two rows of fibroblasts and mesothelium [22].

The double cervix contained longitudinal folds covered by a cylindrical stratified epithelium with cuboidal basal cells and superficial polymorphic cells that had a secretory role [22]. The lamina propria invaginated, forming structures that had lumens containing mucus and cell aggregates. The epithelial cells found in the vagina, via vaginal swabbing, were the parabasal, intermediate, nucleated superficial, and anucleated superficial cells [24].

2.3. Reproductive Physiology

Females in estrus were detected by behavioural changes in the males, including increased displays and vocalization [25]. The dominant male would keenly follow a female in estrus, sniffing her vulva. The pair would then enter the water, however the female would return to land and re-enter the water allowing copulation to take place, thus mating occurred in the water. Subordinate males were usually chased away by the dominant one [14].

Hormonal evaluation was done in conjunction with vaginal cytology to determine the relationship between the plasmatic estradiol to progesterone (E2/P4) ratio, the maturation index (MI), and the karyopyknotic index (KI); the latter two indices were based on vaginal cytological examination [26]. Blood was collected via heparinized vacutainers to obtain plasma and then estradiol and progesterone were assayed using solid-phase radioimmunoassay (RIA). The plasma results were then compared to the MI and KI. Median plasmatic values of estradiol and progesterone were 326.49 ± 202.61 pg/mL and 121.61 ± 59.17 ng/mL, respectively, and the intra-assay coefficient of variation was 11%. The author, however, did not obtain the expected results as no positive correlation was noted between the high estradiol levels and vaginal cytology [26]. Also, the placentation is characteristic of hystricomorphic rodents, i.e., contains a sub-placenta [27].

2.4. Reproductive Technologies

Colpocytology was performed in order to describe the vaginal cell types present during a 30-day period [24][26]. Vaginal swabbing was performed using a moistened swab (saline solution) introduced into the dorsal vaginal commissure. The slides were fixed and stained using Giemsa (2%), Methylene Blue (2%), or Shorr stains for analysis using light microscopy [24]. It was shown that Giemsa (2%) and Methylene Blue (2%) were more practical to use. Barbosa et al. [24] found four types of cells, as well as erythrocytes, leukocytes, and bacteria. However, de Miranda [26] classified cells, in order from smallest to largest, as basal, parabasal, small intermediate, medium intermediate, large intermediate, superficial, and anucleate. The author noted that an increase in cell size corresponded to an increase in acidity and decrease in the nucleus–cytoplasm ratio.

3. Paca (*Cuniculus paca*)

3.1. Gross Anatomy of the Female Reproductive Tract

The ovaries were yellowish and oval in appearance, with a smooth surface that had small transparent areas [28][29]. Feliciano et al. [29] also noted the presence of follicles within the ovaries based on echogenicity during ultrasonography. The ovaries were said to be supported by an incomplete bursa ovarica, which was attached by the mesovarium [30][31].

Continuous with the medial surface of the ovaries was the fallopian tube, a paired narrow, hollow organ [28][30]. Matamoros [28] found that it measured 5 cm in length, which was similar in comparison to the findings of Mayor et al. [31], who stated that the total length was 5.10 cm in non-pregnant females in the follicular phase. Non-pregnant females in the luteal phase had a total length of 4.27 cm, while it measured 5.29 cm in pregnant females. However, Reis et al. [30] found shorter fallopian tubes (right and left—3.69 cm) with diameters of 0.11 cm (right and left).

The uterine horns were interconnected by the intercornual ligament and attached to the abdominal wall via the mesometrium [30]. They were found to be longer in pregnant females, having an average length of 19.51 cm, as opposed to 12.70 cm in non-pregnant females in the follicular phase and 13.97 cm in non-pregnant females in the luteal phase [31]. A much shorter length of 9.63 cm, on average, with a diameter of 2.60 cm was found by other authors [30]. Reis et al. [30] noted that they converged caudally to form a septum, which created two cervical canals with two internal uterine ostia and one external uterine ostium.

The uterus was located in the sublumbar region, which is dorsal to the urinary bladder [30]. It was found to be bicornuate, with each horn measuring about 12 cm in length [28]. The false body was formed by an adjoining thin membrane. In contrast, Mayor et al. [31] described the uterus as being duplex, as both uteri had separate cervices that opened into the vagina. The cervix was found to be longer and wider in non-pregnant females in the follicular phase, having an average length of 2.08 cm and diameter of 2.12 cm, as compared to 1.43 cm (length) and 1.60 cm (diameter) in non-pregnant females in the luteal phase and 1.70 cm (length) and 1.89 cm (diameter) in pregnant females [31].

The vagina, measuring 14 cm in length, could be found in the pelvic region, dorsal to the urinary bladder and ventral to the rectum [28][30]. The external opening, the vaginal orifice, could be completely closed in some females [28]. Also present was the clitoris. Mayor et al. [31], however, found different lengths; 9.23 cm (non-pregnant females in the follicular phase), 10.78 cm (non-pregnant females in the luteal phase), and 9.84 cm (pregnant females). The urethra opened independent of the vagina, next to the clitoris [30]. The vulva was flat and located below a ventral anal depression, below which the clitoris was found [30]. The clitoris was conical in shape and covered by skin at the apex, which revealed two spines when retracted [32]. Females also had one pair of axillary and one pair of inguinal mammary glands [28].

3.2. Histology of the Female Reproductive Tract

The ovaries of a 2-month old female did not have a well-defined medulla, as the cortex predominated [28]. The outer part of the cortex contained numerous primary follicles, while the inner part had numerous growing follicles. The ovaries of a pregnant adult, however, differed in the number of follicles and corpora lutea [28]. The ovary of the pregnant horn had the main corpus luteum, three accessory corpus luteum, and Graafian follicles. The ovary of the non-pregnant horn contained six follicles and eighteen accessory corpus luteum. Pregnant females were found to have a greater number of accessory corpus luteum and antral follicles as compared to females in the luteal phase [31]. The mucosal surface of the oviduct contained numerous folds that were lined by a simple columnar epithelium [28].

The endometrium of the pregnant female contained developed uterine glands (in the non-pregnant body), which had a cylindrical epithelium, thin muscular layer, and a serous layer [28]. In the pregnant body, there was an embryo attached via a discoid (hemochorial-type) placenta. The two-month-old female also had numerous developed uterine glands. The muscular layer was thin, made up of an inner circular layer and outer longitudinal layer. The pregnant uterine horn was also larger in diameter, with a thinner endometrium and myometrium in the location of the placenta [31]. They also found the same type of placenta as Matamoros [28], with a sub-placenta located in only one part of the uterus.

The vagina of non-pregnant females in the follicular phase contained developed stratification and cornification; while non-pregnant females in the luteal phase showed non-developed stratification and pregnant females had columnar secretory cells apically [31]. The axillary mammary glands of pregnant animals contained developed alveoli, with lobules that were not well differentiated but contained fine lines of interlobular connective tissue [28]. The inguinal mammary glands of pregnant animals contained developed alveoli with lobules that were well differentiated and consisted of connective tissue and striated muscle fibres [28].

3.3. Reproductive Physiology

The courtship behaviour was similar to that of the agouti, except that the female pacas would attack by growling and made attempts to bite. The male pacas were also not as vocal nor did trembling of the front feet occur during courtship— as was the case in the male agouti [33]. Evaluation of fecal progesterone and estrogen, during the estrous cycle and pregnancy, was used to aid pregnancy diagnosis [34]. The results obtained showed that plastic beads were successful as fecal markers and that pregnancy diagnosis could be confirmed via fecal estrogen, but not fecal progesterone. In non-

pregnant females throughout the estrous cycle, fecal progesterone levels varied between 0.37–7.9 ng/g dry feces, while fecal estrogen levels varied between 5.08–37.72 ng/g dry feces, with no sustained progesterone peak characteristic of the luteal phaseor any cyclic estrogen peaks observed [34]. In pregnant females, fecal progesterone levels varied between 1.33–6.42 ng/g dry feces, while fecal estrogen levels varied between 8.97–1964 ng/g dry feces [34]. Similar to the other hystricomorphic rodents, the placenta was lobulated with a sub-placenta [35].

3.4. Reproductive Technologies

Four phases of the estrous cycle were identified using colpocytology [36][37]. Vaginal swabbing was done and smears were stained using the Harris–Shorr technique, followed by evaluation using a light microscope [37]. In proestrus, intermediate, parabasal, and superficial cells, along with leucocytes, were observed [37]. Similar findings were made by Guimarães et al. [36], who also noted a progressive increase in the number of nucleated superficial cells and a decrease in the other types of cells—with this phase lasting 7–12 days. During estrus, nucleated superficial cells were predominant, which aggregated and became anucleated superficial cells towards the end of the phase [37]. Contrary to other authors, estrus was predominated by anucleated superficial cells and no mucus was seen, even though clinically there was an increase in vaginal mucus and this phase lasted 1.05 days [36]. During metestrus, there were large amounts of intermediate, parabasal, metestrum, and foam cells, as well as leucocytes, and less superficial cells were observed [37]. These results agreed with Guimarães et al. [36], with this phase lasting 4–9 days. During anestrus, there was a prevalence of parabasal cells and debris and a small number of intermediate and metestrum cells and leucocytes [37]. Guimarães et al. [36] did not describe an anestrus phase, rather a diestrus phase, which lasted 7–20 days and was characterized by high numbers of parabasal and basal cells and less leucocytes, with intermediate and other cells in a degenerative process. The mean estrous cycle length was found to be 33.4 days [37], similar to that found by Guimarães et al. [36].

Colpocytology was used to identify the different phases of the estrous cycle by another author, however, despite cytological changes, it was not successful in detecting a complete estrous cycle and this was attributed to the handling of the animals [38]. In proestrus, polymorphonuclear intermediate cells and partially keratinized cells were seen. The presence of large, superficial keratinized cells indicated estrus, while intermediate cells and numerous neutrophils indicated diestrus. Also, no anestrus phase was observed [38].

Vaginal cytology, progesterone concentration, and fetal measurements were evaluated during different gestational periods [39]. Vaginal swabbing was done and smears were stained using Shorr and Periodic acid-Schiff techniques, followed by evaluation using a light microscope [39]. At day 30 of pregnancy, less than half the smears had surface cells with estrogenic features and all females had a vaginal discharge. At days 60 and 90, parabasal, intermediate, superficial, and navicular cells were present, with 70% of females at 60 days pregnancy having a vaginal discharge. Despite the gestational period, about half the females had an open vaginal vestibule [39]. Blood for plasma progesterone testing was obtained via venipuncture of the cephalic vein or lateral or medial saphenous veins and evaluated using the radioimmunoassay technique. However, these results were negligible as the majority of animals at days 60 and 90 of pregnancy had less than 1 ng/mL [39]. A 7.5 MHZ convex transducer was used to perform ultrasonography in order to measure the fetal biparietal diameter, with averages of 1.25 cm and 2.34 cm at 60- and 90-days gestation, respectively [39].

Estrus synchronization utilizing progestogen subcutaneous implants, in association with prostaglandin and equine chorionic gonadotropin (eCG) injections, was successfully achieved [40]. The average progesterone concentrations before progesterone implantation were 1.69 ng/mL in proestrus, 0.31 ng/mL in estrus, 1.62 ng/mL in metestrus, and 0.88 ng/mL in diestrus [40]. Two treatment groups were synchronized by implanting 1.5 mg norgestomet subcutaneously, followed seven days later by an intramuscular injection of 0.13 mg of prostaglandin. The implants were subsequently removed 24 h after and the animals were given 25 IU and 50 IU of eCG intramuscularly, respectively [40]. It was noted that only when the implant was removed did the animals go into estrus, with cytology showing intermediate cells with nucleoprotein pigments and intermediate cells of a pre-ovulatory type. The average progesterone value, when the implant was removed, was 1.35 ng/mL [40]. The groups undergoing the treatments, when compared to the control group, had higher pregnancy rates [40].

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